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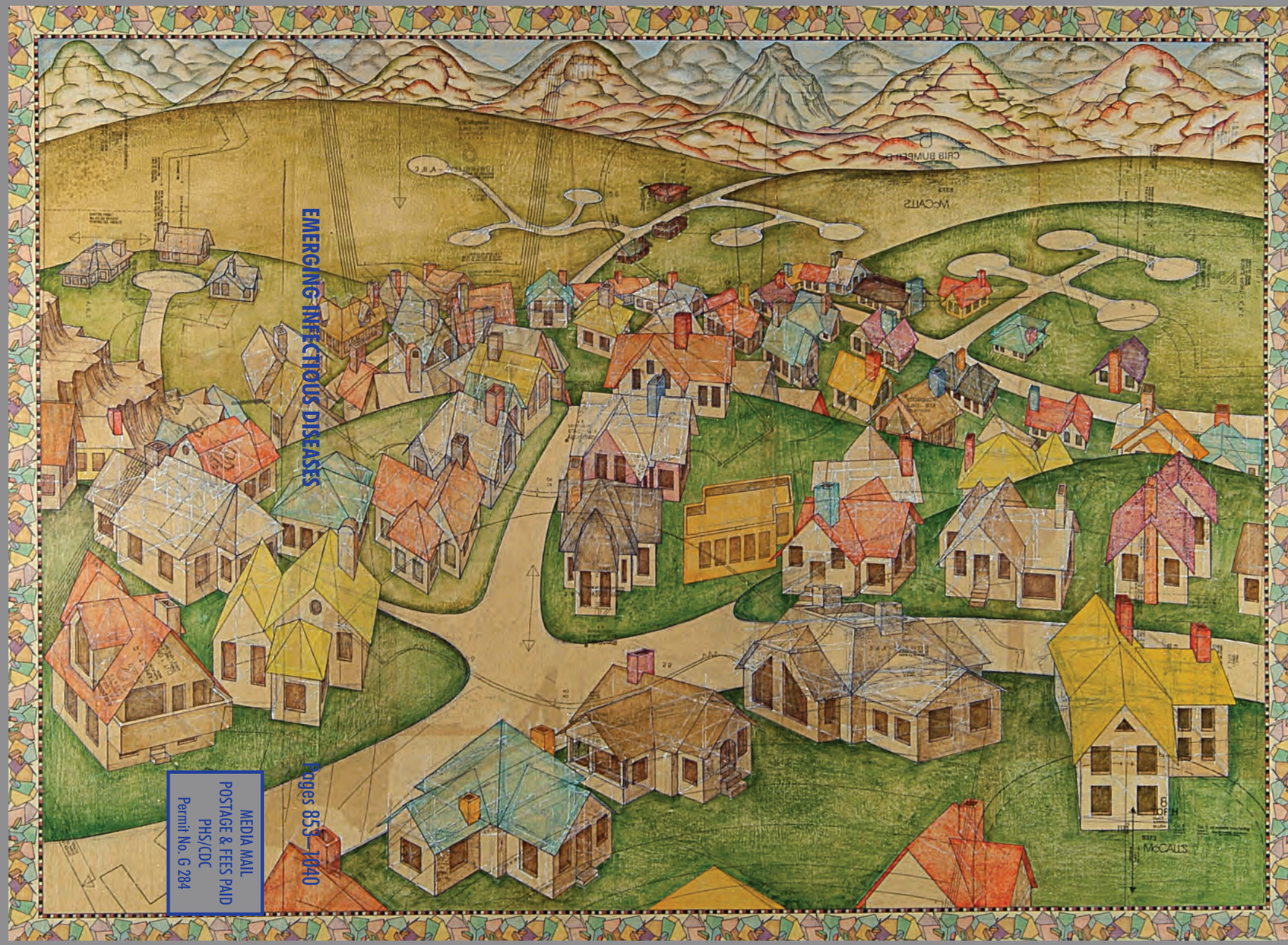
EMERGING INFECTIOUS DISEASES®

June 2013

The SARS Experience



James Barsness (b. 1954) *My Valley* (2003-2005) acrylic, ink on paper mounted on canvas (125.7 cm x 171.5 cm) Copyright courtesy of the artist and George Adams Gallery, New York

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EMERGING INFECTIOUS DISEASES

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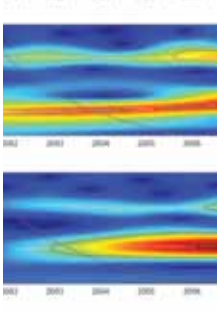

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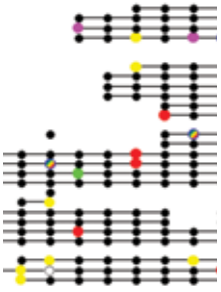

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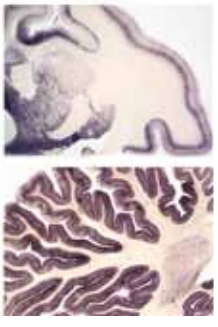

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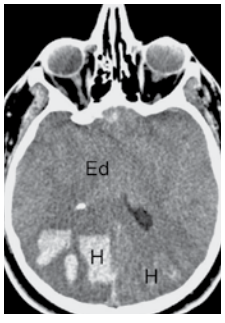

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Etymologia

Prospects for Emerging Infections in East and Southeast Asia 10 Years after Severe Acute Respiratory Syndrome

Peter W. Horby, Dirk Pfeiffer, and Hitoshi Oshitani

It is 10 years since severe acute respiratory syndrome (SARS) emerged, and East and Southeast Asia retain a reputation as a hot spot of emerging infectious diseases. The region is certainly a hot spot of socioeconomic and environmental change, and although some changes (e.g., urbanization and agricultural intensification) may reduce the probability of emerging infectious diseases, the effect of any individual emergence event may be increased by the greater concentration and connectivity of livestock, persons, and products. The region is now better able to detect and respond to emerging infectious diseases than it was a decade ago, but the tools and methods to produce sufficiently refined assessments of the risks of disease emergence are still lacking. Given the continued scale and pace of change in East and Southeast Asia, it is vital that capabilities for predicting, identifying, and controlling biologic threats do not stagnate as the memory of SARS fades.

It is a decade since severe acute respiratory syndrome (SARS) emerged and in a few dramatic months redefined perceptions of global vulnerability to emerging infectious diseases. It is believed that SARS originated from southern China, with the first cases identified in Guangdong Province, China, where sporadic cases and small outbreaks occurred between November 2002 and early January 2003. A larger outbreak, triggered by nosocomial transmissions in 2 hospitals, began during mid-January 2003 in Guangzhou city, the capital of Guangdong Province (*1*). On February 11, 2003, the international community, including the World Health Organization (WHO), became aware of this unusual cluster of severe pneumonia cases, which included many health care workers. Detailed information about the outbreak was not available to the international community, and when WHO issued a global alert on March 12, 2003, the virus had already spread to other countries and caused outbreaks in areas outside Guangdong, including Hong Kong, China; Hanoi, Vietnam; Singapore; and Toronto, Ontario, Canada.

The SARS epidemic provided a dramatic demonstration of the weaknesses in national and global capacities to detect and respond to emerging infectious diseases, and it was in many ways a watershed event that had a transformative effect on many of the clinical, public health, and other professionals involved. But has the response to SARS had any lasting effect on the probability of new infectious agents emerging, being detected at an early stage of emergence, and being effectively controlled?

More than 30% of the global population lives in East and Southeast Asia, and despite impressive improvements in health, infectious diseases remain a major problem in the region. In 2010, 47% of the estimated 2.1 million deaths among children <5 years of age in Southeast Asia were attributable to infectious diseases (e.g., pneumonia and acute diarrhea) (*2*). Alongside this existing pool of known human pathogens, a large and diverse population of mammalian wildlife species and domestic livestock reside in the region, acting as reservoirs or amplifying species from which new infectious diseases of humans might emerge (*3*,*4*). The reemergence of highly pathogenic avian influenza A(H5N1) virus in 2004, the isolation of novel bat-associated reoviruses from humans in Malaysia in 2006, and the discovery of a novel tick-borne bunyavirus associated with fever and thrombocytopenia in rural farmers in China in 2009 attest to the existence of a pool of potential zoonotic pathogens in East and Southeast Asia (Table) (*8,9,12*). We review how the conditions that drive the emergence of infectious diseases and the systems to detect and control them have changed in East and Southeast Asia in the decade since SARS.

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Table. Emerging and reemerging infectious disease events detected in East and Southeast Asia, 2004–2011*

Year, pathogen	Pathogen type	Driver of resistance	Location	Ref
2004				
Influenza A(H5N1)	Orthomyxovirus	Agricultural industry changes	East and Southeast Asia	(*5*)
2005				
Streptococcus suis serotype 2	Bacteria	Agricultural industry changes/co-infection with porcine reproductive and respiratory syndrome virus	China/Vietnam	(*6,7*)
2006				
Melaka virus	Reovirus	Improved detection	Malaysia	(*8*)
Kampar virus	Reovirus	Improved detection	Malaysia	(*9*)
2009				
Artemisinin-resistant malaria	Protozoa	Artemisinin or artesunate monotherapy	Cambodia	(*10*)
Reston Ebola virus†	Filovirus	Improved detection/co-infection with porcine reproductive and respiratory syndrome virus	Philippines	(*11*)
Severe fever with thrombocytopenia syndrome	Bunyavirus	Unknown	China	(*12*)

*Ref, reference.
†Detected in swine but not shown to cause human disease.

Altered Ecosystems

Over the past decade, East and Southeast Asia have been home to many of the top-performing world economies, and this macroeconomic success has resulted in large increases in the demand for natural resources. The demand for hardwood, firewood, wood pulp, agricultural and grazing land, living space, roads, minerals, and power has had an enormous effect on the ecosystems of the region. Deforestation occurred throughout the 1990s, but the last decade has seen net increases in forested areas of China, the Philippines, and Vietnam because of active afforestation (including new commercial plantations). Net forest losses continue, however, in Myanmar, Cambodia, Indonesia, and Papua New Guinea (Figure 1) (*13*).

The conversion of natural environments into agricultural or other commercially viable land (e.g., dams, mines) is usually associated with a decrease in biodiversity. A reduction in biodiversity can lead to increased disease transmission through a variety of mechanisms (e.g., reduced predation and competition) and cause an increase in the abundance of competent hosts and the loss of buffering species, leading to increased contact between amplifying host species and compatible pathogens (*14*). Although a reduction in biodiversity can lead to increased disease transmission, a large diversity of mammalian wildlife species is also associated with a large diversity of microbial species, which both increase toward the equator (*3*,*4*). Therefore, tropical areas (e.g., Myanmar, Cambodia, and parts of Indonesia) that have a rich pool of existing and potential pathogens but are experiencing ongoing ecosystem disruption and biodiversity loss may be at a particularly high risk for the emergence of zoonotic diseases.

Land-use changes are ongoing, but much of East and Southeast Asia already has very high pressures on productive land. The rate of land-use change in much of the region has probably peaked, and the region is now in an era of increasing intensification of land productivity. In fact, over the last decade, China has increased agricultural output despite a slight decrease in total agricultural land area (Figure 2) (*15*). This intensification is driven largely by demographic pressures, which are predicted to result in a 70% increase in food production by 2050; the consumption of grains is expected to decrease and demand for meats, fruits, and vegetables is expected to increase (*15*). The recent high and volatile prices for food commodities are a good indicator of the current vulnerability of agricultural production systems.

The environmental consequences of intensified agricultural production include the depletion and degradation of river and groundwater, reduced soil quality, and water and soil contamination with chemical fertilizers and pesticides. The loading of aquatic ecosystems with nitrogen and phosphorous (eutrophication) is a widespread environmental change with an as-yet unquantified effect on the risk for disease emergence. Eutrophication can result in potentially harmful blooms of cyanobacteria, but little is known about the effect on pathogens that cause disease in animals and humans. There is, however, evidence that eutrophication can alter ecosystems in such a way as to increase the transmission of parasitic diseases of amphibians, the concentration of *Vibrio cholerae*, and the abundance of mosquito vectors (*16*). Given the trend of increasing intensification of crop and animal production in East and Southeast Asia, much more attention should be given to the effect of the large-scale contamination of water and soil with nitrogen, phosphorous, and other chemicals on the functioning of ecosystems and on disease dynamics.

Livestock Production

Demand for livestock products in East and Southeast Asia has risen dramatically over the past 50 years: the per capita consumption of meat in developing countries has more than tripled since the early 1960s, and egg consumption has

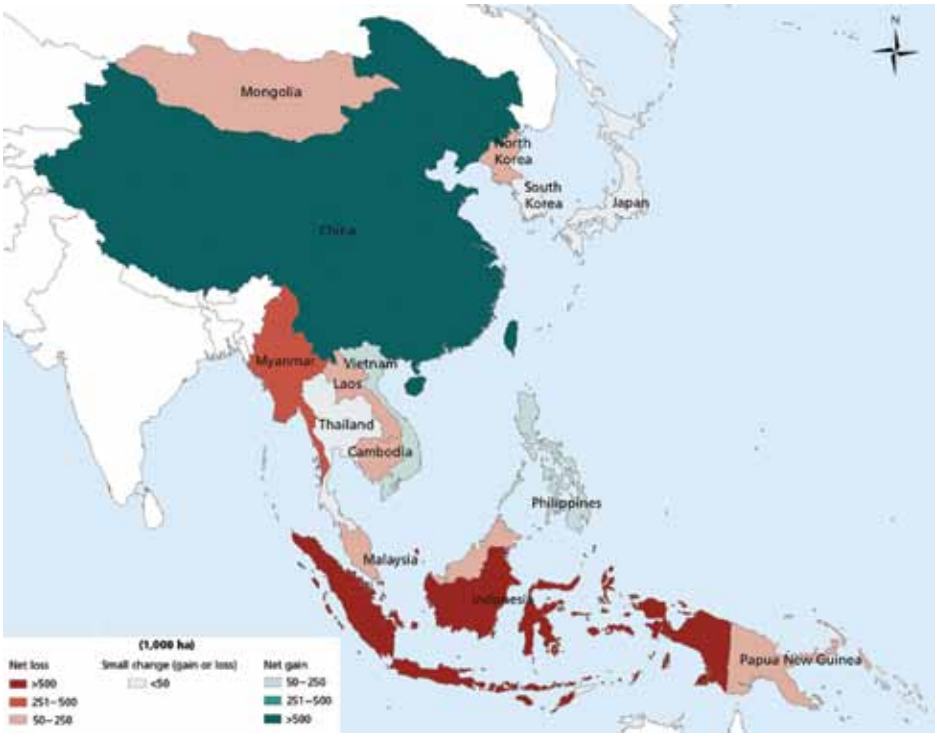

Figure 1. Annual change in forest area by country, 2005–2010. Source: Food and Agriculture Organization of the United Nations Global Forest Resources Assessment 2010 (www.fao.org/forestry/fra/fra2010/en/).

increased 5-fold (*17*). The increased demand for meat has been met by more intensive and geographically concentrated production of livestock, especially pigs and poultry. East and Southeast Asia are home to 569 million pigs (60% of the world pig population) and 9.2 billion poultry (43% of the world poultry population) (*18*). The Food and Agriculture Organization of the United Nations (FAO) has projected that pork consumption in China will increase by 55% between 2000 and 2030 (*19*), and Rabobank, an agricultural finance group, predicts a 45% increase in global meat demand over the next 20 years, with 70% of that occurring in Asia. The demand will be particularly strong for poultry because of its relatively low cost and short production cycle, and because there are fewer cultural restrictions regarding poultry than there are that concern pork and beef.

In addition, meat-producing companies will continue to consolidate at the global level. The intensification of livestock farming often results in more effective separation of domestic and wild animals, improved veterinary supervision and input, reduced movement of animals, and reduced species mixing, all of which may reduce the likelihood of disease emergence. However, higher densities of short production–cycle domestic animals, such as pigs and, in particular, poultry, introduce a vulnerability because such animals usually have limited genetic variation. Higher genetic diversity within a host species is often associated with differences in susceptibility to infection, thereby limiting the potential for infections to spread rapidly (*20*). Recent outbreaks of highly pathogenic porcine reproductive and respiratory syndrome virus throughout East and Southeast Asia, which at times co-occurred with outbreaks of *Streptococcus suis* infections, and the detection of Reston Ebola virus infection in pigs in the Philippines highlight the ongoing risk for disease emergence, amplification, and crossover from livestock to humans in East and Southeast Asia (*6*,*11*).

In East and Southeast Asia, antimicrobial drugs are used extensively in the livestock and aquaculture sectors to treat or prevent infections, and they are used non-therapeutically as growth promoters, which requires the prolonged administration of sub-therapeutic doses. This practice has a demonstrable effect on the emergence and prevalence of potentially clinically relevant resistant microorganisms in food animals. Furthermore, the subsequent excretion of antimicrobial drugs into the environment may subject environmental bacteria to antimicrobial selection pressures (*21*). It is clear that the continued use of non-therapeutic antimicrobial drugs in livestock and aquaculture industries that are increasing in scale and intensity poses a threat to human and animal health (*22*).

Wildlife and Farm Biosecurity

Reducing contact between domestic and wild animals, whether the wild animals remain wild or are captive in breeding farms or markets, is a key tactic recommended by the FAO for reducing risk to human health, and this reduced contact is part of the wider FAO strategy for biosecurity. Improving biosecurity in farms in East and Southeast

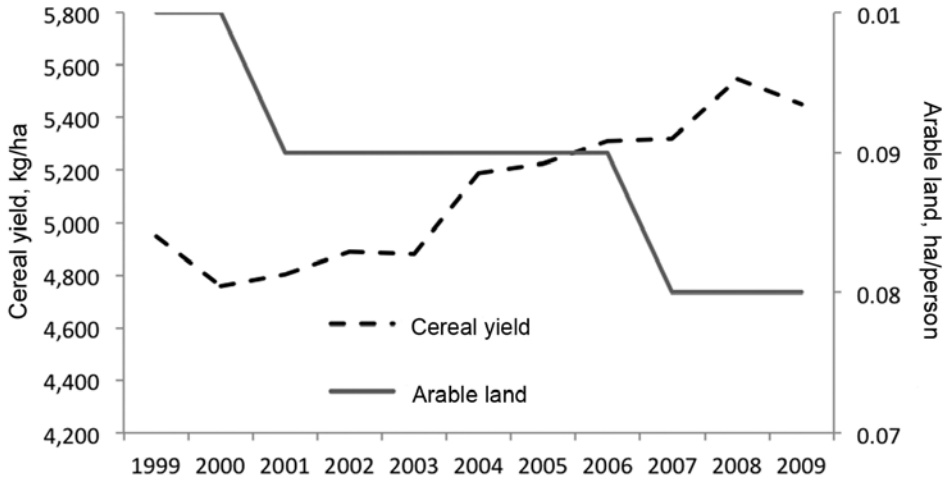

Figure 2. China's cereal production yield and arable land area, 1999–2009. Source: The World Bank Agriculture and Rural Development (http://data.worldbank.org/topic/agriculture-and-rural-development).

Asia is a major challenge because a large proportion of the farming is done in backyard and small- to medium-scale commercial farms, and there is often a mix of commercial and backyard farming in any 1 location (*23*). The longer-term vision is to restructure the livestock production sector toward a more integrated and controlled system in which controls benefit animal health and welfare, human health, and commercial profitability without adversely affecting the livelihood of poor persons.

In East and Southeast Asia, increasing intensification of animal husbandry may lead to healthier, better isolated animals and a subsequent lowered risk for emerging disease events. However, should an emerging infectious disease event occur, this intensification may result in greater amplification of disease in large, naive monocultures, as demonstrated in the Netherlands when they experienced major outbreaks of classical swine fever in 1997–1998 and avian influenza in 2003. The role of civet cats in the SARS pandemic and the smuggling of avian influenza A(H5N1) virus–infected birds of prey into Europe showed that the legal and illegal wildlife trade is an effective conduit for zoonotic pathogens to enter new niches (*24*,*25*). Wild animal products remain popular in East and Southeast Asia as traditional medicines, tonics, food delicacies, or symbols of wealth. Although all 10 countries in the Association of Southeast Asian Nations (ASEAN) are signatories to the Convention on International Trade in Endangered Species of Wild Fauna and Flora, Asia continues to host the largest illegal wildlife trade in the world (*26*).

Travel and Trade

The regional and global connectivity of East and Southeast Asia continues to increase; there is visa-free travel between ASEAN countries, relaxation of previously restrictive international travel policies in some countries, and a proliferation of budget airlines. Increased travel and trade between Africa and Asia is a particularly notable phenomenon: exports from Africa to Asia increased 20% from 2003 to 2008. This Africa–Asia traffic is a new corridor for the exchange of potential emerging infectious pathogens.

The ongoing development of a regional road transport network within East and Southeast Asia will also offer new opportunities for pathogen dispersal because, compared with air travel, roads offer a more egalitarian form of connectivity that includes animals as well as humans. Increases in domestic travel also continue; only 10% of the world is now classified as remote (i.e., >48 hours travel time to a big city), and an estimated 2.5 billion passenger trips were made during China's 2011 Lunar New Year celebrations—the greatest annual human migration on earth. Increased connectivity provides greater opportunities for pathogens to disperse beyond their traditional niches and presents a formidable challenge to the tracking and containment of outbreaks (*27*).

Urbanization, Human Demographics, and Behavior

Between 2011 and 2050, the global population is expected to increase from 3.6 billion to 6.3 billion (an increase of 2.6 billion [72%]), and this increase will be concentrated in cities (*28*). Most of the growth will occur in developing countries, particularly in Asia, which will experience an increase in its urban population of 1.4 billion persons. In many ways, this concentration of population growth in urban areas is a positive development, and the popularity of cities is a testament to the fact that cities generally provide better economic and education opportunities, better living and sanitation conditions, better nutrition, and therefore better health than in underdeveloped rural areas.

Cities are, however, key in the epidemiology of many infectious diseases because they can function as "pace-makers" that drive temporal and spatial transmission dynamics of local epidemiology (e.g., dengue), hubs for national and

global spread (e.g., SARS and HIV), or bridges between human and animal ecosystems (e.g., influenza A subtypes H5N1 and H7N9). East and Southeast Asia have made considerable progress in health and social welfare improvements: during 2000–2010, a total of 125 million persons in China and India moved out of slum conditions. However, urban poverty remains a concern. In 2010, an estimated 500 million persons in Asia lived in slums (*29*), and, at the end of 2008, there were an estimated 140 million rural migrant workers in China, many of whom lacked residency rights and had limited access to health care and other social supports (*30*). Circular migration between rural and urban settings is common and may facilitate the transfer of pathogens from wild or rural ecosystems to urban areas, with the potential for rapid amplification in settings with high concentrations of migrant workers.

Health Systems

Almost 35% of the emerging infectious diseases identified in Asia during 1940–2004 represent the emergence of a new pattern of antimicrobial drug resistance (*4*). The major driver of such resistance is drug pressure. In East and Southeast Asia, the sequential development of resistance by malaria parasites to chloroquine, sulfadoxine-pyrimethamine, mefloquine, and now artemisinin is a measure of both the adaptive capacity of the parasite and the failure of health systems to implement effective drug combination and cycling strategies to avoid resistance. Bacteria in East and Southeast Asia show high rates of resistance to antimicrobial agents; examples include multidrug-resistant *Acinetobacter baumannii*, *Salmonella enterica*, and *Enterobacteriaceae* (*31*). A high level of antimicrobial resistance is a marker of the failure to control access to antimicrobial drugs and to influence prescribing behaviors. Over-the-counter antimicrobial drugs are available without a prescription throughout much of East and Southeast Asia, even though antimicrobial drugs are officially prescription-only medicines in most countries. Left unchecked, the supply- and demand-side incentives for inappropriate antimicrobial drug use will lead to a region awash with antimicrobial drugs and with the potential for

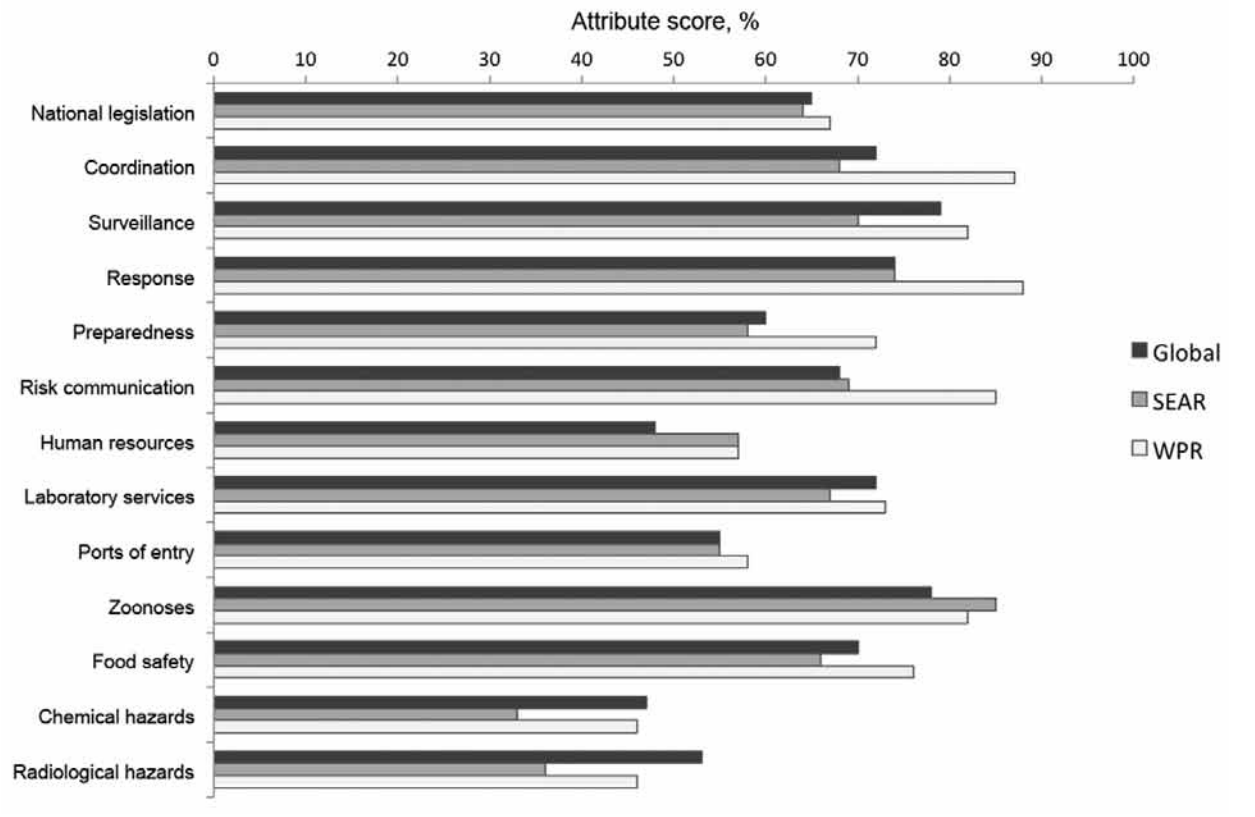

Figure 3. Self-reported global and regional average attribute scores for international health regulations core capacities, 2011. Source: World Health Organization, Summary of 2011 States Parties Report on International Health Regulations Core Capacity Implementation. (www.who.int/ihr/publications/WHO_HSE_GCR_2012.10eng/en/index.html). The attribute score is the percentage of attributes (a set of elements or functions that reflect the level of performance or achievement of an indicator) in which moderate or strong technical capacity has been attained in each core capacity area. SEAR results are the average for 11/11 eligible countries. WPR results are the average for 19/27 eligible countries (8 countries did not complete the questionnaire in 2011). SEAR, World Health Organization's (WHO) South-East Asia Region; WPR, WHO Western Pacific Region.

public health disasters, such as artemisinin resistance, which now threatens global malaria control.

Surveillance and Response

The SARS pandemic highlighted what had been apparent to some since the 1990s: few countries possessed the necessary surveillance and response capacities to rapidly detect and control emerging infectious diseases (*32*). The deficiencies of the 1969 International Health Regulations at the global level had long been recognized, and attempts to revise them were ongoing before 2003, but the SARS outbreak added new urgency and momentum for change. The International Health Regulations were successfully revised in 2005, and for the first time they defined a series of core capacities that each country is required to establish to detect, report, and control public health emergencies of international concern. The target for attaining these core capacities was set as June 2012, and an assessment undertaken in 2011 found that although these core capacities had not yet been fully achieved in several countries of East and Southeast Asia, considerable progress had been made (Figure 3) (*33*). For example, the influenza surveillance network in China expanded from 63 laboratories and 197 sentinel hospitals in 2005 to 441 laboratories and 556 sentinel hospitals in 2009 (*34*). Field epidemiology training programs have played a central role in strengthening epidemiology capacity in human and animal health, and new field epidemiology training programs were implemented in Laos, Mongolia, and Vietnam in 2009 and in Cambodia in 2011 (*35*). An analysis of the global capacity for detecting outbreaks showed improvements in the median time from outbreak start to outbreak discovery between 1996 (29.5 days) and 2009 (13.5 days) and from start to public communication (40 days in 1996 to 19 days in 2009); the WHO Western Pacific Region was the only WHO region that showed a statistically significant improvement in both areas (*36*).

Many of these improvements were facilitated by a large increase in political and financial support for emerging infectious disease surveillance and response from national governments and donor agencies after the outbreaks of SARS and influenza A(H5N1). Although data on the total expenditure for emerging infectious disease surveillance, preparedness, and response in East and Southeast Asia are not available, examples of international support include the first and second Asian Development Bank Greater Mekong Subregion Regional Communicable Diseases Control Projects ($38.75 million and $49 million, respectively), the Canada–Asia Regional Emerging Infectious Disease Project ($4.3 million), the US Government foreign assistance for disease control, research, and training (>$500 million in Asia during 2004–2011), and the US Agency for International Development's Pandemic and Emerging Threats Program. As a consequence, pandemic and epidemic preparedness planning has improved in most countries of East and Southeast Asia, but gaps between the plans and the ability to operationalize them remain in many countries (*37*,*38*).

Since 2003, international and national authorities have increasingly recognized the importance of more effective animal health surveillance. However, limited resources in most countries have meant that investments into improved surveillance capacity have occurred largely in those countries affected by major outbreaks, such as the case with an outbreak of Nipah virus infection in Malaysia and of influenza A(H5N1) virus infection in Thailand, China, Vietnam, and Indonesia. Where these types of investments did occur, they were often species- and threat-specific, rather than to facilitate strategic enhancement of generic surveillance efforts for dealing with emerging disease threats. Not too dissimilar from the situation in high income countries, institutional and administrative boundaries between the human and animal health sectors have largely prevented the development of integrated surveillance systems.

Regional and International Partnerships

It has been said that there have been more international meetings about influenza A(H5N1) virus than human cases of the disease. Whether this is true or not, a benefit of the many meetings held after the outbreaks of SARS and influenza A(H5N1) virus has been a strengthening of regional and international professional partnerships. Clinicians, epidemiologists, virologists, veterinarians, and public health officials in East and Southeast Asia are now better connected and familiar with their colleagues than they were before 2003: examples include the South East Asia Infectious Disease Clinical Research Network, the Mekong-Basin Disease Surveillance System, increasing membership of East and Southeast Asia countries in the Training Programs in Epidemiology and Public Health Interventions Network, and the establishment in 2003 of the ASEAN+3 Expert Working Group on Communicable Diseases (*39,40*). Networks of trusted colleagues are a powerful force for sharing expertise, clearing confusion, and bridging divides, and these newly formed partnerships are perhaps one of the greatest unquantified achievements of the last decade.

To coordinate and harmonize the diversity of initiatives spawned by SARS, the WHO South East Asia Office and Western Pacific Office jointly developed the Asia Pacific Strategy for Emerging Diseases in 2005. This plan provides a common framework for strengthening national and regional surveillance and response capacity for emerging infectious diseases in the 48 countries of the Asia Pacific Region; it was revised and re-endorsed by the WHO Regional Committees in 2010.

A more troublesome dimension of international partnerships since 2003 has been the dispute over the sovereignty and sharing of pathogen samples. Although these disputes have not benefited disease surveillance in the short term, they do have a legitimate basis, and it must be hoped that in the medium term, an airing and resolution of these issues will result in greater trust, improved surveillance, and a more equitable distribution of benefits. In this context, the ratification in 2011 of WHO's Pandemic Influenza Preparedness Framework for the Sharing of Influenza Viruses and Access to Vaccines and Other Benefits is a major step forward.

Conclusions

A major shift, from West to East, is underway in the global center of gravity: East and Southeast Asia are becoming the dominant force of economic, social, and environmental change. While rapid development has brought East and Southeast Asia many benefits, it has also resulted in widening health inequalities, environmental degradation, increased migration and urbanization, and a concentration of persons, food production, and economic activity. These changes might facilitate the emergence and transmission of new pathogens, but it would be simplistic and disingenuous to present the extensive changes in East and Southeast Asia as inevitably increasing the risk of emerging infectious diseases. It seems likely that the probability of new emerging infections may be reduced by many of these socioeconomic changes, such as urbanization and the industrialization and commercialization of agriculture and food production; however, the scale and effect of any individual emergence event may increase because of a greater concentration and connectivity of livestock and persons. Surveillance and response capacities have improved in the last decade, and East and Southeast Asia are far better prepared to detect and respond to emerging infectious diseases. However, we are still lacking the tools and methodologies to produce a sufficiently refined assessment of the distribution and profile of disease emergence risks that encompasses geographic heterogeneity; the interaction of different drivers of pathogen evolution, crossover, and dispersion; and dynamic systems and the uncertainty inherent in such assessments. Given the continued scale and pace of change in East and Southeast Asia, it is vital that the capacity to predict and identify biologic threats and to protect the public's health does not stagnate as the memory of SARS fades.

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Public Health Lessons from Severe Acute Respiratory Syndrome a Decade Later

Jeffrey P. Koplan, David Butler-Jones, Thomas Tsang, and Wang Yu

The outbreak of severe acute respiratory syndrome in 2002–2003 exacted considerable human and economic costs from countries involved. It also exposed major weaknesses in several of these countries in coping with an outbreak of a newly emerged infectious disease. In the 10 years since the outbreak, in addition to the increase in knowledge of the biology and epidemiology of this disease, a major lesson learned is the value of having a national public health institute that is prepared to control disease outbreaks and designed to coordinate a national response and assist localities in their responses.

After an index case and ongoing transmission in Guangzhou, People's Republic of China, in late fall 2002, the world experienced a widespread multicountry and multifocal outbreak of a new, virulent, transmissible respiratory illness in 2003. The causal agent of this disease was found to be a coronavirus, and the disease was named severe acute respiratory syndrome (SARS) (*1*). We are approaching the tenth anniversary of this dramatic event, which challenged public trust and government and health system capacity to address public health issues. What have we learned and what lessons have we acted upon during these years?

The science of SARS (biologic, ecologic, and epidemiologic) has been considerably elucidated (*1*). The coronavirus has been structurally described, associated with likely animal reservoirs (bat and civet cat), and demonstrated to be transmitted by droplets and possibly by aerosolization and contact with contaminated fomites. Transmission seems relatively easy because the basic reproduction number (R_0) is 2.2–3.7, but transmission is greatly enhanced by occasional supertransmitters. The number of deaths from SARS is considerable; the case-fatality rate is ≈10%. Masks and other personal protective gear, along with active surveillance, case detection, contact tracing, isolation, and quarantine, were effective measures in reducing transmission. Even a relatively limited outbreak cost cities and countries many billions of dollars in lost business and productivity in addition to the human and economic costs of the extraordinary health and public health measures that were needed to identify and contain the disease (*2*).

During and following the outbreak, 8 broader national and international policy, operational, and systems needs were identified by public health officials. The first need was stronger and more integrated coordination between animal and human public health. This need is currently being addressed in One Health efforts by the World Health Organization, the Food and Agriculture Organization, the World Organisation for Animal Health, the US Centers for Disease Control and Prevention (CDC), and the Public Health Agency of Canada (*3*). The second need was enhanced disease and symptom surveillance systems that would share information quickly within countries and across borders. The third need was capable and responsive public health laboratories whose crucial role in infectious disease outbreaks includes establishing the etiologic agent, confirming the diagnoses in clinically suspected cases, supporting surveillance activities, and providing insight into the most effective infection control practices. The fourth need was for infection control to be stressed constantly in all health care settings. The fifth need was for development of clear criteria for isolation and quarantine and evaluation of the effectiveness of such measures. Isolation and quarantine were applied inconsistently with varied and often vague criteria (travel from an infected country, fever, sitting in an airplane near a traveler suspected of having SARS). Several sites believed that their isolation and quarantine efforts were helpful in reducing disease spread (*4*,*5*). The sixth need was for making risk assessment and communication

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as necessary components of the public health skill set to be used continuously during a health threat to a population (*6*). The seventh need was a prompt and practiced public health response with broad geopolitical responsibility and authority, spanning and linking political jurisdictions from municipalities to states/provinces to national and global levels. This response is better performed when preparedness training and a national incident response system have been routinely incorporated into the public health system. The eighth need was for national public health institutes (NPHIs) that have value in preventing and controlling outbreaks and other health threats (*7*). Even in the absence of an NPHI, the success of such entities in time of health crisis argues for at least establishing a central focus for coordination and leadership with delegated responsibility and authority.

NPHIs are the linchpin of public health systems in >80 countries. These institutes are science-based organizations that lead and coordinate public health at the national level. In most instances, NPHIs are part of the government (usually under the Ministry of Health) or closely attached to it.

NPHIs vary greatly from multifunctional and multidisciplinary agencies such as the US CDC, the Robert Koch Institute (Berlin, Germany), the Chinese CDC (Beijing, China) the National Institute for Public Health and the Environment (RIVM) (Bilthoven, the Netherlands), Fundação Oswaldo Cruz (Rio de Janeiro, Brazil), and the Public Health Agency of Canada (Ottawa, Ontario, Canada) to more targeted ones such as the Health Protection Agency (London, UK) and the National Institute for Communicable Diseases (Johannesburg, South Africa). Yet, despite their differences in history, scope, and resources, NPHIs all provide core public health functions that improve the efforts of their countries to address health challenges within and beyond their borders. These functions include population health assessment, health protection (surveillance and response), disease and injury prevention, health promotion, and research (evidence to inform policies and programs). In many countries, the NPHI plays a major role in developing and supporting local capacity, at the municipal and state/provincial levels, through training, technical assistance, tools, guidelines, staff assignments, and financing.

Consolidating these functions—and the associated skills, disciplines, experience, and expertise—in an NPHI provides many benefits, 2 of which are particularly germane to acute public health threats. The first benefit is the ability to generate and share knowledge, data, and evidence to inform public health decisions and policies. The second benefit is increased capacity to mount a quick, decisive, and coordinated response during a public health emergency. More than 80 NPHIs are organizationally linked through the International Association of National Public Health Institutes (IANPHI) (*8*), which promotes creation of new NPHIs (in Mozambique, Guinea-Bissau, and El Salvador) and assists in expanding the breadth and depth of existing NPHIs (e.g., in Uganda, Nigeria, Togo, Morocco, Ghana, Côte d'Ivoire, Rwanda, Bangladesh, Ethiopia, Tanzania). The World Health Organization does not have a formal relationship with individual NPHIs but has developed a partnership with IANPHI, including frequent communication and joint programmatic activities.

SARS provided an opportunity to recognize the value of the effectiveness of NPHIs when present and the risk for added toll of illness when absent. Thus, several countries involved in the outbreak subsequently saw fit to establish an NPHI (Hong Kong and Canada) or greatly strengthen an existing one (China). Such institutes are a positive legacy of the 2003 outbreak.

Likewise, in the past 10 years, NPHIs have played a major role in the response to many other public health crises, varying from natural disasters to disease outbreaks to addressing chronic noncommunicable diseases (e.g., controlling a widespread *Escherichia coli* outbreak; Robert Koch Institute); evaluating the effects of radiation exposure (NPHI, Tokyo, Japan); identifying and controlling measles outbreaks (Institut de Veille Sanitaire, Saint-Maurice, France); detecting Ebola virus (Uganda Virus Research Institute, Entebbe, Uganda, and National Institute for Communicable Diseases, South Africa); addressing a new influenza (H1N1) subtype outbreak (Instituto Nacional de Salud Publica, Mexico City, Mexico, in collaboration with the Public Health Agency of Canada and the US CDC), solving a milk contamination puzzle and promoting antitobacco efforts (Chinese CDC); dealing with health issues related to flooding (National Institute of Health, Bangkok, Thailand), and describing the epidemiology of Nipah virus (Institute of Epidemiology, Disease Control and Research, Dhaka, Bangladesh).

These examples demonstrate the value added by NPHIs worldwide. In addition to their major national role, by grouping organizationally through IANPHI, NPHIs also have a bond for coordination of efforts and mutual assistance that promotes their overall performance and technical development. When emerging infectious diseases, such as SARS, which become local, national, or global threats, are considered, the value and need for developing and strengthening NPHIs could be one of the major lessons learned and applied from the SARS experience of 10 years ago.

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Progress in Global Surveillance and Response Capacity 10 Years after Severe Acute Respiratory Syndrome

Christopher R. Braden, Scott F. Dowell, Daniel B. Jernigan, and James M. Hughes

Ten years have elapsed since the World Health Organization issued its first global alert for an unexplained illness named severe acute respiratory syndrome (SARS). The anniversary provides an opportunity to reflect on the international response to this new global microbial threat. While global surveillance and response capacity for public health threats have been strengthened, critical gaps remain. Of 194 World Health Organization member states that signed on to the International Health Regulations (2005), <20% had achieved compliance with the core capacities required by the deadline in June 2012. Lessons learned from the global SARS outbreak highlight the need to avoid complacency, strengthen efforts to improve global capacity to address the next pandemic using all available 21st century tools, and support research to develop new treatment options, countermeasures, and insights while striving to address the global inequities that are the root cause of many of these challenges.

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Ten years have elapsed since the World Health Organization (WHO) issued its first global alert for an unexplained illness, which it named severe acute respiratory syndrome (SARS) (*1*). A few days later, the Institute of Medicine (IOM) released a report, Microbial Threats to Health, that highlighted many of the issues and challenges raised by SARS (*2*). This anniversary provides us with an opportunity to reflect on the international response led by WHO to this new global microbial threat, a response that resulted in control of the pandemic that resulted in >8,000 cases and nearly 800 deaths in >30 countries and had a large economic impact (*3*). The series of emerging and reemerging disease threats since 2003, from avian influenza

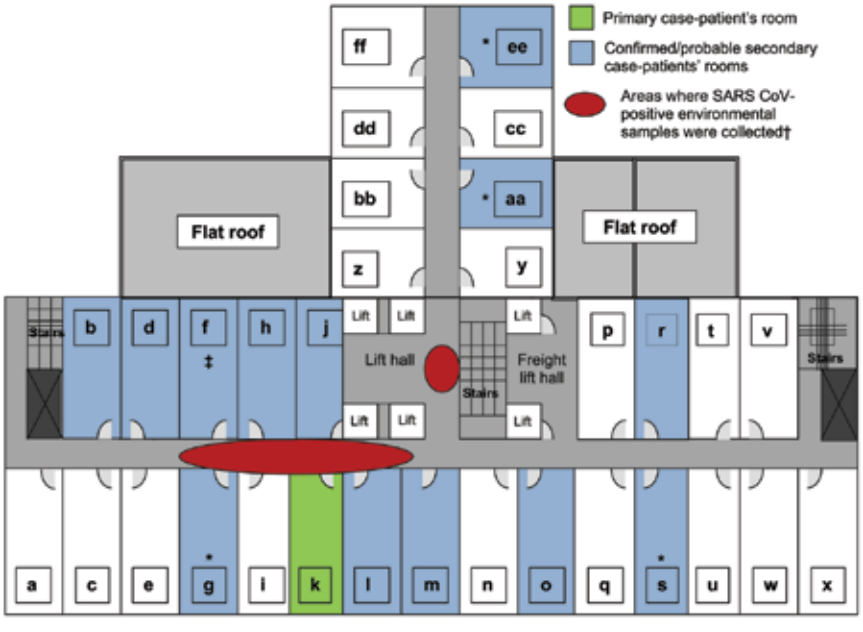

Figure 1. Layout of ninth floor of Hotel Metropole, where superspreading event of severe acute respiratory syndrome (SARS) occurred, Hong Kong, 2003. *2 cases in room; †see (*16*); ‡case-patient visited room. CoV, coronavirus.

(H5N1, H7N9) to extensively drug-resistant tuberculosis to the recently recognized novel coronavirus, reinforce the need to avoid the complacency that typically occurs in the aftermath of a successful response to a crisis resulting from an emerging microbial threat.

Lessons of SARS

Many features of the SARS epidemic and the public health response are worth recalling because they provide reminders of challenges posed by the emergence of a new disease that is transmissible from person to person. Some of these features include the initial lack of field investigative capacity, reference laboratory testing, and reporting transparency from southern China, which resulted in a 3-month delay in the reporting of the severe unexplained illness to WHO; the important role played by an alert clinician in Hanoi, Vietnam, in the initial recognition and response to the illness; the rapid spread of illness to >30 countries; and the effects on health care workers and family members, who were most at risk for person-to-person spread of the infection. Reviewing the events that occurred during the SARS epidemic is an opportunity to highlight the ultimate success of early patient isolation, contact tracing, quarantine, and infection control measures; the importance of rigorous attention to biosafety in laboratory settings; the effects of stigmatization of affected groups; the economic impact as a result of major disruptions in international travel and commerce; the identification of the mode and circumstances of cross-species transmission; and the role of "superspreaders" and superspreading events in the rapid dissemination of the illness.

In addition, during the epidemic, the leadership provided by WHO facilitated timely exchange of new information among clinicians, epidemiologists, and laboratory investigators around the world. These efforts included the formation of a global network (*4*) of virology and pathology laboratories that used modern diagnostic methods, which contributed to the rapid identification, characterization, and sequencing of the agent and the timely dissemination of critical information and guidance through agency reports, expedited peer-reviewed publications (*5*–*9*), lay media, and the Internet. These experiences exemplify the characteristic features of the global SARS outbreak.

Emergence in Guangdong Province

Details are sketchy about the earliest phase of SARS as it spread in southern China, but the best retrospective analyses show that the initial cases and clusters occurred in mid-November 2002; its spread to health care workers and family members was a critical aspect of the amplification of the epidemic during January 2003 (*10*). Initial investigations were conducted by provincial public health authorities who did not recognize or failed to report the potential global implications of the epidemic, and initial laboratory investigations incorrectly focused on a possible *Chlamydia* spp.–like organism as the etiologic agent (http://english.peopledaily.com.cn). Once the public health implications were recognized, however, the subsequent response to SARS by China was among the most aggressive and effective worldwide and included substantial improvements in epidemiologic training, laboratory capacity, and mandatory reporting, as detailed below.

Superspreading Events Linked to the Hotel Metropole

Several superspreading events contributed to the dissemination of the virus. Some of the most dramatic examples included those associated with the Hotel Metropole in Hong Kong (*11*), the Amoy Gardens apartment complex in Hong Kong (*12*), Air China flight 112 from Hong Kong to Beijing (*13*), and an acute care hospital in Toronto, Ontario, Canada (*14*). The episode at Hotel Metropole that contributed greatly to the initial cross-border spread of the disease was particularly noteworthy.

The cluster of SARS cases at Hotel Metropole in Hong Kong in 2003, the first superspreading event recognized outside mainland China, was responsible for the spread of the epidemic from Guangdong Province to Canada, Vietnam, Singapore, and Hong Kong itself. In addition to the first 13 cases originally associated with the Hotel Metropole (*11*), a follow-up cohort study of guests from Canada, Germany, England, and the United States who stayed at the hotel concurrent with the index case-patient, a physician from Guangdong, identified an additional 7 cases that met the probable (2) or confirmed (5) case definition for SARS coronavirus (CoV) infection (*15*). All 20 cases were associated with transmission of SARS CoV on the ninth floor of the hotel, where the index case-patient had stayed for 1 night before becoming critically ill and being admitted to a local hospital the next day. Three deaths occurred among hotel guests who had been identified as case-patients, resulting in a case-fatality ratio of 15%. Known secondary SARS cases were associated with at least 13 (42%) of 31 guest rooms on the ninth floor (Figure 1).

The high rate of infection among guests staying on the ninth floor at the Hotel Metropole is remarkable because they did not have direct contact with the index case-patient. For example, 1 resident of Hong Kong who visited a friend on the ninth floor (but was not a hotel guest) likely acquired his infection during his visit; this person subsequently infected 143 people at Prince of Wales Hospital in Hong Kong (*16*). Epidemiologic evidence suggested an environmental route of SARS CoV transmission. Indeed, environmental contamination with SARS CoV RNA was identified on the carpet in front of the index case-patient's

room and 3 nearby rooms (and on their door frames but not inside the rooms) and in the air intake vents near the centrally located elevators (*16*). Guest rooms had positive air pressure relative to the corridor, and there was no direct flow of air between rooms. The lack of air flow between rooms and the absence of SARS CoV RNA detected inside guest rooms suggest that secondary infections occurred not in guest rooms but in the common areas of the ninth floor, such as the corridor or elevator hall. These areas could have been contaminated through body fluids (e.g., vomitus, expectorated sputum), respiratory droplets, or suspended small-particle aerosols generated by the index case-patient; other guests were then infected by fomites or aerosols while passing through these same areas. Efficient spread of SARS CoV through small-particle aerosols was observed in several superspreading events in health care settings, during an airplane flight, and in an apartment complex (*12–14*,*16–19*). This process of environmental contamination that generated infectious aerosols likely best explains the pattern of disease transmission at the Hotel Metropole.

The compilation of data from multiple superspreading events in the SARS epidemic yields valuable findings that could be relevant for other respiratory infections of pandemic potential. These events underscore the potential for aerosol transmission in non–health care settings and the dramatic role such transmission can play in the global transmission of respiratory diseases.

Recognition and Reporting from Hanoi

One of the guests at the Hotel Metropole, a business traveler, was hospitalized at the French Hospital in Hanoi. Called to the investigation of the subsequent illnesses of health care workers at the hospital was Dr Carlo Urbani, a WHO physician specializing in parasitology who was known for having the mindset of an alert clinician and a strong dedication to the principles of public health. In a series of emails from Hanoi to his colleagues at WHO, Dr Urbani sent some of the first messages of alarm and detailed descriptions of the clinical features of what would come to be known as SARS. His reports would lead to an aggressive response by the government of Vietnam, which quarantined the hospital staff and ultimately contained the epidemic there (*20*). It also raised the alarm with colleagues at WHO and the US Centers for Disease Control and Prevention, who would work to characterize and contain the global epidemic. Dr Urbani himself became infected and was hospitalized in Bangkok, where he insisted on obtaining repeated samples from his own respiratory tract, which provided some of the first isolates of the novel CoV (*6*,*9*). Dr Urbani died on March 29, 2003 (*21*), one of many health care workers who responded to those in need, only to become victims themselves.

Health Care–associated Transmission in Toronto and Taiwan

Health care facilities played a substantial role throughout the SARS outbreak as sites of efficient transmission that led to acceleration of disease in communities. These facilities also served a critical role in stopping SARS through strict implementation of infection control practices. Important lessons regarding the epidemiology and control of SARS are evident in the spread of health care–associated SARS in Toronto and Taiwan (*22*). In both places, the spread of SARS was initiated by unrecognized transmission of the virus in health care facilities; however, the outbreaks progressed differently (Figure 2).

The first cases of SARS in Toronto occurred very early in the global outbreak. A 78-year-old woman who had stayed at the Hotel Metropole in Hong Kong in late February 2003 returned to Toronto before dying at home. However, her son had been infected and was subsequently admitted to a Toronto hospital, where nosocomial transmission led to >100 cases among patients, health care workers, and visitors. Prompt institution of practices to control airborne, contact, and droplet infection led to an apparent cessation of transmission, and on May 14, WHO declared that Toronto was no longer a SARS-affected area. Control recommendations were relaxed, and the crisis appeared to have ended; however, unrecognized infection continued among a small number of patients and visitors. Eventually, transmission to health care workers, patients, and visitors resurged, leading to an additional 79 cases, as evident in the bimodal shape of the epidemic curve. After strict infection control practices and vigilance for SARS were reinstituted, the last case was recognized in mid-June, and no other cases were recorded thereafter.

The experience in Taiwan was very different. Soon after the novel CoV was recognized in Hong Kong, officials in Taiwan instituted rigorous port entry screening and isolation among returning travelers who had suspected SARS and their contacts. Public health and academic medical officials focused exhaustive efforts on accurately diagnosing cases of SARS in travelers. This approach appeared to work well for 6 weeks, suggesting that SARS could be prevented from entering the island. However, despite these measures, unrecognized transmission of SARS began occurring in the community. SARS in a hospital laundry worker at the large urban Ho Ping Hospital in Taipei led to exposure of staff and patients and ignited an explosive outbreak that spread to other hospitals and the community. To contain the transmission, patients, staff, and visitors were quarantined in the facility, an action that had rarely been invoked in modern times. More than 1,000 persons were quarantined; some tossed soft drink bottles from windows with protest messages, others communicated the disarray within the facility through cell phone messages, and a few escaped. Public

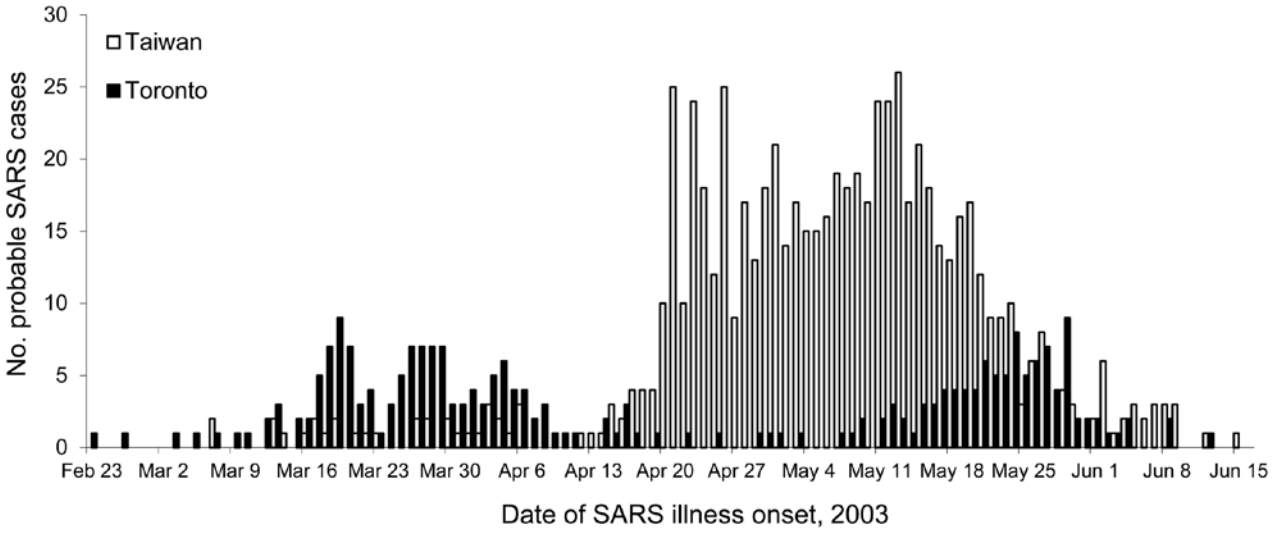

Figure 2. Probable cases of severe acute respiratory syndrome, by location and date of illness onset, Toronto, Ontario, Canada, and Taiwan, February 23–June 15, 2003.

health officials rapidly pivoted in control policies and community response actions to prevent a potential emerging infectious disease catastrophe. The epidemic curve for Taiwan reveals the very rapid rise in cases resulting from the hospital outbreak. Strict infection control practices were mandated in all health care settings. SARS evaluation centers ("fever clinics") were constructed outside hospital emergency departments. Community use of face masks, fever checks on entry to commercial establishments, and extensive community outreach and education were used to mitigate the effects of SARS. After 2 months of epidemic spread, leading to >600 cases, SARS was eventually contained, and no further cases were reported.

The Legacy of SARS

After the emergence of SARS, many after-action reports were written, many recommendations were made, and many steps were taken in response to lessons learned. SARS was frightening and had a dramatic effect on global travel and business. The outbreak showed how rapidly a new, fatal pathogen could spread and how disruptive the effects could be. The palpable impact of SARS was translated into action in the form of pandemic influenza planning and surveillance efforts, a greater focus on global health security, improved laboratory and surveillance networks, and most important, the revision of the International Health Regulations (IHR). These regulations had last been updated in 1969, and the experiences with SARS contributed to the urgency to finish the revision. The updates were completed in 2005, when 194 WHO member states approved the international treaty; IHR 2005 went into effect in 2007 (*23*).

The legacy of SARS is evident in many other efforts as well. New national public health agencies have been created in Canada (Public Health Agency of Canada) and the United Kingdom (Health Protection Agency). The WHO Global Outbreak Alert and Response Network has been strengthened (*24*). The Global Disease Detection Program was established at CDC, with the aim of strengthening countries' efforts in training, surveillance, and outbreak response and establishing 10 Regional Centers by 2012 (www.cdc.gov/globalhealth/gdder) in alignment with the directive for bilateral collaboration and assistance under article 44 of the IHR. With the support of the Bill and Melinda Gates Foundation, the International Association of National Public Health Institutes has been created and now has >75 members around the world (*25*). The Training Programs in Epidemiology and Public Health Interventions Network has expanded, and its regional partners (e.g., African Field Epidemiology Network, Eastern Mediterranean Public Health Network) have been strengthened (*26*).

Perhaps the most important legacy of SARS is the recognition of the critical need for a multilateral response, led by WHO, in the event of a rapidly moving but ultimately containable global epidemic. The central role of WHO in coordinating the laboratory network that identified the etiologic agent and shared reagents, the epidemiology network that characterized the spread and identified the most effective control measures, and the policy and communications network that incorporated rapidly changing knowledge into measured travel advisories was critical for the control of the epidemic and a credit to WHO.

As the importance of cross-species transmission in disease emergence has been increasingly recognized (*27*,*28*), the One Health movement, which emphasizes the importance of interdisciplinary collaboration to address issues at the interface of human health, animal (both domestic and wildlife) health, and environmental/ecosystem health, has gained momentum (*29*,*30*). The US Agency for International Development has supported the Emerging Pandemic Threats Program in an effort to strengthen prediction, detection, response, and amelioration programs in parts of the world shown to be at particular risk (e.g., areas of rainforest intrusion, environmental degradation, ecosystem disruption) for emergence of new diseases (*31*,*32*). The White House recently released the first National Strategy for Biosurveillance, which calls for an all-hazards approach, focusing on threats affecting humans, animals, or plants, to achieve early detection and situational awareness to enable better decision making (*33*).

Looking Forward

Although many disease detection and control improvements have been implemented in the past 10 years, important gaps in global capacity and coordination remain. One example is the need to greatly strengthen and monitor the national capacity required for full compliance with IHR 2005, including ensuring that adequate numbers of trained personnel are available to support the response to a public health emergency, that surveillance systems are capable of detecting public health emergencies, that access is adequate to laboratory diagnostic capabilities that can identify a range of emerging epidemic pathogens, and that countries have adequate rapid response capacity for public health emergencies (*34*). In addition, for state of the art, affordable countermeasures are needed (especially point-of-care diagnostics, the reinvigoration of the development pipeline for new antimicrobial drugs, and new and improved vaccines), and workable approaches must be determined for equitable distribution of countermeasures when emergencies arise. Finally, systems are necessary to facilitate the conduct of research to evaluate treatment options during public health emergencies, as are tools to assess the utility of social media in strengthening capacity for disease surveillance, event detection, and situational awareness.

Of 194 WHO member states that signed on to the IHR 2005, <20% had achieved compliance with the core capacities required by the deadline of June 2012 (*35*). Assessment of the 13 factors contributing to disease emergence and reemergence identified by IOM expert committees in consensus studies of emerging infections and microbial threats in 1992 (*36*) and 2003 (*2*) suggests that several of these factors contributed to the SARS pandemic (Table). Recent trends for most of these factors continue to operate in favor of the microbes, a finding that indicates a need to identify and respond to other microbial threats (e.g., avian influenza strains, novel CoVs, multidrug-resistant organisms) and emphasizes the necessity for all countries to continue to work on strengthening core capacities for surveillance and response and those for minimizing the risk of cross-border spread (*23*). As we reflect on the lessons learned from the global SARS outbreak, we need to avoid complacency; strengthen efforts to improve global capacity to address the next pandemic using all available 21st century tools; and support research to develop new options, countermeasures, and insights (*37*). At the same time, we must strive to address the global inequities that are the root cause of many of these challenges.

Table. Factors contributing to the emergence of infectious diseases according to IOM reports, 1992 and 2003

1992 IOM Report	2003 IOM Report
Human demographics and behavior	Human susceptibility to infection
Technology and industry	Climate and weather
Economic development and land use	**Changing ecosystems**
International travel and commerce	Poverty and social inequality
Microbial adaptation and change	War and famine
Breakdown of public health measures	**Lack of political will**
	Intent to harm

*__Boldface__ indicates factors that contributed to the emergence and spread of severe acute respiratory syndrome. IOM, Institute of Medicine.

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New Delhi Metallo-β-Lactamase–producing *Enterobacteriaceae*, United States

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We characterized 9 New Delhi metallo-β-lactamase–producing *Enterobacteriaceae* (5 *Klebsiella pneumoniae*, 2 *Escherichia coli*, 1 *Enterobacter cloacae*, 1 *Salmonella enterica* serovar Senftenberg) isolates identified in the United States and cultured from 8 patients in 5 states during April 2009–March 2011. Isolates were resistant to β-lactams, fluoroquinolones, and aminoglycosides, demonstrated MICs ≤1 μg/mL of colistin and polymyxin, and yielded positive metallo-β-lactamase screening results. Eight isolates had bla_{NDM-1}, and 1 isolate had a novel allele (bla_{NDM-6}). All 8 patients had recently been in India or Pakistan, where 6 received inpatient health care. Plasmids carrying bla_{NDM} frequently carried *AmpC* or extended spectrum β-lactamase genes. Two *K. pneumoniae* isolates and a *K. pneumoniae* isolate from Sweden shared incompatibility group A/C plasmids with indistinguishable restriction patterns and a common bla_{NDM} fragment; all 3 were multilocus sequence type 14. Restriction profiles of the remaining New Delhi metallo-β-lactamase plasmids, including 2 from the same patient, were diverse.

During the past decade, there has been an emergence of carbapenem-resistant *Enterobacteriaceae* that produce carbapenemases, enzymes that efficiently hydrolyze carbapenems, as well as most β-lactam drugs (*1*). The most common carbapenemase among *Enterobacteriaceae* in the United States is the Ambler class A *Klebsiella pneumoniae* carbapenemase (KPC), an enzyme that is found throughout the United States and globally (*2*,*3*). The emergence of another group of carbapenemases, the Ambler class B metallo-β-lactamases (MBLs), is of great concern worldwide (*4*). Until recently, MBLs were rarely identified in the United States and were found exclusively in *Pseudomonas aeruginosa* (*5*). However, recent reports of *K. pneumoniae* producing IMP– and VIM–type MBLs (*6*,*7*) have increased concerns over additional transmissible carbapenem resistance mechanisms in *Enterobacteriaceae*.

Among the most recent carbapenemases to appear in the United States is the newly described New Delhi MBL (NDM) (*8*–*12*). First reported in 2009, NDM-1 was initially identified in *K. pneumoniae* and *Escherichia coli* clinical isolates obtained from a Swedish patient who had been hospitalized in India (*13*). Drug-resistant gram-negative bacteria that produce NDM have been found in community and health care settings in India and Pakistan in a wide range of gram-negative genera containing diverse bla_{NDM}-harboring plasmids, and have been reported in >15 countries worldwide (*4*,*14*,*15*). The widespread dissemination of NDM-producing isolates and the apparent ease of mobility of bla_{NDM} is a major threat to public health on a global scale.

To complement reports of individual cases (*8*,*10*,*12*), we performed extensive laboratory characterization of 9 clinical isolates of NDM-producing *Enterobacteriaceae* collected from patients in the United States during April 2009–March 2011. Strain typing and plasmid restriction analysis were performed to identify common lineages. We also describe a novel NDM-encoding allele, designated bla_{NDM-6}.

Bacterial Strains

Nine clinical isolates (5 *K. pneumoniae*, 2 *E. coli*, 1 *Enterobacter cloacae*, and 1 *Salmonella enterica* serovar Senftenberg), were collected from 8 patients during April 2009–March 2011 and submitted to the Centers for

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Disease Control and Prevention (Atlanta, GA, USA) for reference susceptibility testing during January 2010–April 2011 (Table 1). Four patients were from California and 1 each was from Illinois, Maryland, Massachusetts, and Virginia. Species identification was confirmed with the Vitek 2 automated system (bioMérieux Vitek Systems Inc., Hazelwood, MO, USA). The *S. enterica* serovar Senftenberg isolate was further classified by serotyping (*12*). A previously identified NDM-1–producing *K. pneumoniae* isolate (0S-506) from Sweden (*13*) was used as a positive control for phenotypic and molecular characterization methods, including strain typing of *K. pneumoniae* isolates. As part of a public health intervention for each of these isolates, an epidemiologist from CDC contacted local health departments and providers to identify characteristics of patients from whom NDM-producing isolates were obtained.

Susceptibility to Selected Antimicrobial Agents

MICs of amikacin, aztreonam, cefotaxime, cefepime, ciprofloxacin, colistin, doripenem, ertapenem, gentamicin, imipenem, meropenem, polymyxin B, tetracycline, tigecycline, and trimethoprim/sulfamethoxazole were determined by using reference broth microdilution (BMD) with panels prepared in-house according to Clinical and Laboratory Standards Institute (Wayne, PA, USA) guidelines (*16*) and stored at -70°C until use. MICs of tigecycline were interpreted according to breakpoints established by the US Food and Drug Administration (Silver Spring, MD, USA) (www.rxlist.com/tygacil-drug.htm). *E. coli* ATCC 25922, *K. pneumoniae* ATCC BAA-2146, *P. aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used for quality control.

BMD Screening for Metallo-β-Lactamase

MICs in the absence and presence of a combination of 0.2 mmol/L EDTA (Sigma-Aldrich, St. Louis, MO, USA) and 0.02 mmol/L 1,10-phenanthroline (Acros Organics, Geel, Belgium) were determined as described (*17*) by using screening wells containing IMP at concentrations ranging from 0.25 μg/mL through 1,024 μg/mL. A ratio ≥4 in the IMP MIC compared with the IMP MIC in the presence of chelators (IMP + EP) was considered a positive result for MBL production. *K. pneumoniae* ATCC BAA-2146 and *P. aeruginosa* ATCC 27853 were used as positive and negative controls, respectively.

Modified Hodge Test

The Modified Hodge test (MHT), which is recommended by Clinical and Laboratory Standards Institute as a confirmatory test for carbapenemase production (*16*), was performed for each strain with 10-μg disks containing meropenem and ertapenem (Becton-Dickinson, Sparks, MD, USA). *K. pneumoniae* ATCC BAA-1705 and BAA-1706 were used as positive and negative controls, respectively.

Etest for Detection of MBLs

Detection of MBLs was performed with Etest MBL strips (AB bioMérieux, St. Louis, MO, USA) containing IMP (IP) and IMP + EDTA (IPI). Strips were used according to instructions provided by the manufacturer.

Detection of bla_{NDM} and bla_{KPC} by Real-Time PCR

A multiplexed Taqman-based real-time PCR for bla_{NDM} and bla_{KPC}, as well as the universal bacterial 16S rRNA–encoding gene (*18*) as an endogenous control for DNA amplification, was performed on the 7500 Fast

Table 1. Epidemiologic information for New Delhi metallo-β-lactamase–producing isolates, United States, April 2009–March 2011*

Patient no.	Isolate no.	Organism	Date of isolation	State	Isolation site	Patient age	Patient and travel history
1	1000654	*Enterobacter cloacae*	2009 Apr	MA	Urine	65 y	Hospitalized in India before coming to United States (*8*)
2	1000527	*Klebsiella pneumoniae*	2009 Dec	CA	Urine	73 y	Hospitalized in India before returning to United States (*8*)
3	1001728	*Escherichia coli*	2010 May	IL	Urine	41 y	Chronic medical problems; traveled to India 3–4 mo before positive culture. No known hospitalizations during travel (*8*)
4	1100192	*K. pneumoniae*	2010 Sep	CA	Resp.	13 mo	Hospitalized in Pakistan 5 months before admission in United States (*10*)
5	1100101	*E. coli*	2010 Oct	VA	Resp.	67 y	Received medical care in India but not hospitalized
6	1100770	*K. pneumoniae*	2010 Dec	CA	Urine	70 y	Hospitalized for 1 mo in India before transfer to US hospital
7	1100975	*K. pneumoniae*	2011 Jan	MD	Resp.	60 y	Hospitalized in India before transfer to US hospital (*12*)
	1101168	*Salmonella enterica* serovar Senftenberg	2011 Feb	MD	Feces		
8	1101459	*K. pneumoniae*	2011 Mar	CA	Blood	57 y	Hospitalized in India; subsequently hospitalized in United States

*Resp., respiratory sample.

system (Applied Biosystems, Carlsbad, CA, USA). Cell lysates were prepared as described (*19*). Each PCR (20-μL volume) included 1× QuantiFast Probe PCR Master Mix (QIAGEN, Valencia, CA, USA), a combined primer/probe solution with final concentrations of 500 nmol/L for each primer and 250 nmol/L for each probe (Table 2), and 2 μL of template. Included in each assay were a bla_{NDM}-positive control (*K. pneumoniae* ATCC BAA-2146), a bla_{KPC}-positive control (*K. pneumoniae* ATCC BAA-1705), a carbapenemase-negative control (*K. pneumoniae* ATCC BAA-1706), and a no template control. Cycling conditions were a 3-min enzyme activation step at 95°C, followed by 40 cycles for 3 s at 95°C and 30 s at 60°C.

Reactions with 16S cycle threshold (C_t) values of 10–30 were considered valid, those with NDM or KPC C_t values of 10–30 were considered NDM positive or KPC positive, and those with NDM or KPC C_t values ≥40 were considered NDM negative or KPC negative (www.cdc.gov/HAI/settings/lab/kpc-ndm1-lab-protocol.html).

DNA Sequence Analysis of bla_{NDM}

Forward and reverse primers outside the bla_{NDM} coding region (Table 2) were used to amplify a 1,013-bp product. Bidirectional DNA sequencing of bla_{NDM} was determined from independent products with primers used for amplification, as well as bla_{NDM} internal primers (Table 2).

Plasmid Isolation and Transformation

Plasmid DNA was isolated from 50-mL overnight cultures by using a Plasmid Midi Kit (QIAGEN) according to the manufacturer's protocol. To enhance the yield of large, low-copy plasmids, DNA was eluted with elution buffer prewarmed to 65°C. *E. coli* DH10BT1 competent cells (Invitrogen, Carlsbad, CA, USA) were transformed with plasmid DNA by electroporation (Gene Pulser Xcell; Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's instructions. Transformants were selected on Luria-Bertani agar containing 1 μg/mL of meropenem and were screened by real-time PCR for bla_{NDM}. Transformant plasmid DNA was evaluated by electrophoresis, and a representative transformant containing a single NDM-encoding plasmid was chosen for further study (designated by TF suffix). *E. coli* NCTC50192, which contained 4 plasmids (≈154, 66, 38, and 7 kb) (*20*), and *E. coli* V517, which contained 8 plasmids ranging from ≈56.4 kb to 2.2 kb (*21*), were used as plasmid size standards.

Characterization of bla_{NDM}-bearing Plasmids

Plasmid DNA from each transformant was digested with *Xmn*I (New England Biolabs, Ipswich, MA, USA), separated by electrophoresis, transferred to a nylon membrane (Zeta-Probe; Bio-Rad Laboratories), and hybridized with an 808-bp digoxigenin (DIG)–labeled bla_{NDM} probe (Table 2) by using the PCR DIG Probe Synthesis Kit (Roche Applied Science, Mannheim, Germany). Hybridization at 42°C, washes, and detection by using a DIG Luminescent Detection Kit (Roche Applied Science) were performed according to the manufacturer's instructions.

NDM-encoding plasmids were assigned to an incompatibility group by using PCR replicon typing as described (*22*). Additional β-lactamases that were co-transferred with each bla_{NDM}-carrying plasmid were identified by using the Check-MDR CT101 Microarray Assay (Check-Points BV, Wageningen, the Netherlands), which detects genes encoding extended-spectrum β-lactamases (ESBLs) (TEM, SHV, and CTX-M), plasmid-mediated AmpCs (CMY, DHA, FOX, MOX, ACC, MIR, and ACT), as well as KPC and NDM (*23*). PCR was used to screen for *armA* and *rmtC* 16S rRNA methylase genes that confer resistance to aminoglycosides (*24*).

Table 2. Sequences of primers and probes used for identification of NDM–producing isolates, United States, April 2009–March 2011*

Assay	Primers and probes	Sequence, 5′ → 3′
Real-time PCR:NDM/KPC screen	NDM, forward primer	GAC CGC CCA GAT CCT CAA
	NDM, Reverse primer	CGC GAC CGG CAG GTT
	NDM, probe (HEX)†	TG GAT CAA GCA GGA GAT
	KPC, forward primer	GGC CGC CGT GCA ATA C
	KPC, reverse primer	GCC GCC CAA CTC CTT CA
	KPC, probe (FAM)†	TG ATA ACG CCG CCG CCA ATT TGT
	16S, forward primer	TGG AGC ATG TGG TTT AAT TCG A
	16S, reverse primer	TGC GGG ACT TAA CCC AAC A
	16S, probe (CY5)†	CA CGA GCT GAC GAC AR‡C CAT GCA
DNA sequence analysis§	NDM-1 forward	ACT CGT CGC AAA GCC CAG
	NDM-1 reverse	CTC ATG TTT GAA TTC GCC C
Internal DNA sequencing primers	NDM-2F	ACA AGA TGG GCG GTA TGG A
	NDM-2R	CGT CCA TAC CGC CCA TCT
DIG-labeled probe synthesis	NDM-F1	GAA TTG CCC AAT ATT ATG CAC C
	NDM-R1	AGC GCA GCT TGT CGG CCA TG

*NDM, New Delhi metallo-β-lactamase; KPC, *Klebsiella pneumoniae* carbapenemase; DIG, digoxigenin.
†NDM probes were labeled with HEX, KPC probes were labeled with FAM, and 16S probes were labeled with CY5 at their 5′ ends. Each contained a black hole quencher at the 3′ end.
‡R, A or G (International Union of Biochemistry codes for DNA bases).
§Amplification using primers NDM-1 forward and NDM-1 reverse, both located outside the coding region of bla_{NDM}, results in a 1,013-bp product.

Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) was performed by using the CHEF mapper electrophoresis system (Bio-Rad Laboratories) with *Xba*I-digested *K. pneumoniae* and *E. coli* chromosomal DNA, as described for *E. coli* (www.cdc.gov/pulsenet/protocols.htm). PFGE patterns were compared by using the Dice coefficient and clustering by using the unweighted-pair group method with average linkages (Bionumerics version 5.10; Applied Maths Inc., Austin, TX, USA).

Multilocus Sequence Typing

Multilocus sequence typing (MLST) was used to classify *K. pneumoniae* and *E. coli* isolates. This procedure was performed and results were interpreted according to protocols on the Institut Pasteur MLST database website (www.pasteur.fr/recherche/genopole/PF8/mlst) (*25*,*26*).

Clinical and Epidemiologic Information

We identified bla_{NDM} by real-time PCR and DNA sequence analysis for 9 clinical isolates received at the Centers for Disease Control and Prevention during January 2010–April 2011 from 8 patients (Table 1) in 5 states. Two isolates, *K. pneumoniae* 1100975 and *S. enterica* serovar Senftenberg 1101168, were isolated from the same patient 1 month apart from a clinical specimen and a surveillance specimen, respectively (*12*). NDM-producing *Enterobacteriaceae* were isolated from a variety of specimen sources, including urine (4/9), respiratory samples (3/9), feces (1/9), and blood (1/9) (Table 1), and mostly represented colonization. All 8 patients (age range 13 months–73 years, median 62.5 years) had a recent travel history (within 4 months) that included India or Pakistan, during which 6 patients received inpatient medical care, and 1 received outpatient medical care. One patient was a citizen of India who traveled frequently between the United States and India. All medical exposures abroad resulted from medical problems that occurred in that country; none of the patients had traveled for the purpose of obtaining medical care (i.e., medical tourism).

Antimicrobial Drug Susceptibility Patterns

All 9 NDM-producing isolates from the United States and *K. pneumoniae* 0S-506 from Sweden were resistant to all β-lactams tested (including carbapenems and aztreonam), ciprofloxacin, amikacin, and gentamicin, and

Table 3. Antimicrobial drug susceptibility profiles of NDM-producing isolates collected and *Escherichia coli* transformants, United States, April 2009–March 2011*

Isolate no.	Organism	MIC, μg/mL									Broth microdilution MBL screen result			Modified Hodge test result
		TGC	SXT	CTX	FEP	ATM	DOR	ETP	MER	IMP	IMP+EP†	Ratio	MBL	ETP/MER
0S-506	*Klebsiella pneumoniae*	≤0.5	>8	>64	>32	>64	>8	>4	>8	>32	1	≥64	+	–/–
1100770	*K. pneumoniae*	2	>8	>64	>32	>64	>8	>4	>8	32	0.5	64	+	+/–
1100975	*K. pneumoniae*	2	>8	>64	>32	>64	>8	>4	>8	32	1	32	+	+/+
1100192	*K. pneumoniae*	1	>8	>64	>32	>64	>8	>4	>8	8	≤0.5	≥16	+	+/–
1000527	*K. pneumoniae*	>4	>8	>64	>32	>64	>8	>4	>8	>32	≤0.5	>64	+	+/+
1101459	*K. pneumoniae*	2	>8	>64	>32	>64	>8	>4	8	16	≤0.5	≥32	+	+/+
1101168	*Salmonella enterica* serovar Senftenberg	1	0.5	>64	>32	>64	8	>4	8	4	≤0.5	≥8	+	+/+
1100101	*E. coli*	≤0.5	>8	>64	>32	>64	>8	>4	>8	16	1	16	+	+/–
1001728	*E. coli*	≤0.5	>8	>64	>32	16	>8	>4	>8	8	≤0.5	≥16	+	+/+
1000654	*Enterobacter cloacae*	>4	>8	>64	>32	>64	>8	>4	>8	>32	4	>8	+	+/+
0S-506	TF	≤0.5	≤0.25	>64	16	64	4	4	4	4	≤0.5	≥8	+	+/+
1100770	TF	≤0.5	≤0.25	>64	32	32	4	8	4	4	≤0.5	≥8	+	+/–
1100975	TF	≤0.5	≤0.25	>64	16	32	4	4	4	2	≤0.5	≥4	+	+/–
1100192	TF	≤0.5	≤0.25	>64	16	≤2	2	2	1	2	≤0.5	≥4	+	+/+
1000527	TF	≤0.5	≤0.25	>64	32	8	8	8	8	4	≤0.5	≥8	+	+/+
1101459	TF	≤0.5	≤0.25	>64	16	≤2	4	4	4	4	≤0.5	≥8	+	+/+
1101168	TF	≤0.5	≤0.25	>64	16	≤2	8	>8	8	8	≤0.5	≥16	+	+/+
1100101	TF	≤0.5	≤0.25	>64	>32	>64	>8	>8	16	8	≤0.5	≥16	+	+/+
1001728	TF	≤0.5	≤0.25	>64	32	32	8	8	8	8	≤0.5	≥16	+	–/+
1000654	TF	≤0.5	>8	>64	>32	>64	>8	>8	32	32	≤0.5	≥64	+	+/+
Recipient	*E. coli* DH-10BT1	≤0.5	≤0.25	≤0.12	≤0.5	≤2	≤0.12	≤0.12	≤0.12	≤0.5	≤0.5	≤1	–	–/–

*NDM, New Delhi metallo-β-lactamase; MBL, Ambler class B metallo-β-lactamase; TGC, tigecycline; SXT, trimethoprim/sulfamethoxazole; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; DOR, doripenem; ETP, ertapenem; MER, meropenem; IMP, imipenem; EP, chelation; TF, transformant.
†IMP + EDTA + 1,10-phenanthroline, μg/mL.

demonstrated MICs ≤1 μg/mL for colistin and polymyxin B; 7/9 were susceptible (MIC ≤2 μg/mL) to tigecycline. Only 2 isolates were susceptible to tetracycline, and only the *S. enterica* serovar Senftenberg isolate was susceptible to trimethoprim/sulfamethoxazole (Table 3).

Detection of NDM Producers

Although each of the 9 isolates showed resistance to carbapenems, detection of carbapenemase activity by using the MHT was variable (Table 3). Six of 9 isolates had a positive MHT result for meropenem and ertapenem, and 3 were positive for ertapenem but negative for meropenem. *K. pneumoniae* 0S-506 was MHT negative for both carbapenems. The Etest MBL result was positive for *K. pneumoniae* 0S-506 and for 6/9 isolates from the United States (IP:IPI ratio ≥12). The remaining 3 isolates showed either a phantom zone or deformed ellipse, which are also indicative of an MBL according to the AB Biodisk information, although deformation of the ellipse can be difficult to recognize (*10*). The BMD MBL screen provided the most conclusive results for MBL detection: all 9 isolates and *K. pneumoniae* 0S-506 demonstrated an MIC IMP:IMP+EP ratio ≥8, which is indicative of MBL production (Table 3).

Sequencing of the bla_{NDM} Gene

DNA sequencing of bla_{NDM} from each of the 9 isolates showed that 8 encoded NDM-1, but the coding sequence in *E. coli* 1100101 and its transformant differed from that of bla_{NDM-1} (GenBank accession no. FN396876) by a C→T modification at nucleotide position 698, resulting in an alanine→valine substitution at aa 233 in the inferred protein. This novel NDM variant has been designated NDM-6 (G.A. Jacoby and K. Bush, www.lahey.org/Studies) and its sequence has been deposited in GenBank under accession no. JN967644.

Antimicrobial Drug Resistance Transferred on the bla_{NDM} Plasmid

Because the NDM-producing isolates from the United States and *K. pneumoniae* 0S-506 from Sweden contained multiple plasmids (range 3–6 plasmids), the bla_{NDM}-bearing plasmid from each isolate was transferred to a plasmid-negative *E. coli* by electroporation. A transformant carrying only the bla_{NDM}-bearing plasmid was chosen for further study.

Eight transformants were either resistant or intermediate to cefepime, cefotaxime, and all carbapenems tested, but transformant 1100192-TF was susceptible to meropenem (Table 3). Five transformants remained resistant to aztreonam, indicating that an additional resistance mechanism (e.g., AmpC or ESBL) was also carried on the NDM-encoding plasmid because MBLs do not independently hydrolyze aztreonam. Resistance to amikacin and gentamicin was co-transferred in 7 instances, but 1100192-TF remained susceptible to both drugs, and 1100101-TF was resistant only to amikacin. All transformants remained susceptible to ciprofloxacin and tetracycline, and only 1000654-TF showed resistance to trimethoprim/sulfamethoxazole.

The MHT identified carbapenemase activity in all transformant strains, although 3 were positive with only 1 of 2 carbapenems (Table 3). Only transformant 1000654-TF showed an Etest MBL IP:IPI ratio ≥8; the remainder had either a phantom zone or an ellipse deformation. The BMD MBL screening method detected MBL production in all transformants (Table 3).

Additional β-lactamase genes carried in parental strains and transformants were identified by using the Check-MDR CT101 microarray assay (*23*) (Table 4). Three transformants that remained susceptible to aztreonam had only the NDM β-lactamase. Transformants with aztreonam resistance carried either a CMY-II-type AmpC (n = 4) or a CTX-M-1-type ESBL (n = 2), in addition to bla_{NDM}.

Table 4. Antimicrobial drug resistance determinants detected in clinical isolates and transformants, and incompatibility group assignment of bla_{NDM}–bearing plasmids, United States, April 2009–March 2011*

Determinant	*Kp* 0S-506		*Kp* 1100770		*Kp* 1100975		*Kp* 1100192		*Kp* 1000527		*Kp* 1101459		*Sal* 1101168		*E. coli* 1100101		*E. coli* 1001728		*E. clo* 1000654		*E. coli* R
β-lactam resistance genes†	I	TF	I	TF	I	TF	I	TF	I	TF	I	TF	I	TF	I	TF	I	TF	I	TF	
$bla_{CTX-M-1-Type}$	+	–	+	–	+	–	+	–	+	–	+	–	–	–	+	+	–	–	+	+	–
$bla_{CMY-II-Type}$	+	+	+	+	+	+	+	–	+	+	–	–	+	–	+	–	+	+	–	–	–
bla_{NDM}	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–
Aminoglycoside resistance‡																					
armA	+	+	+	+	+	+	ND	ND	–	–	–	–	+	+	–	–	–	–	+	+	–
rmtC	–	–	–	–	–	–	ND	ND	+	+	–	–	–	–	–	–	+	+	–	–	–
Plasmid replicon‡		A/C		A/C		A/C		UT		A/C		UT		L/M		FII		A/C		FII	

*_Kp_, _Klebsiella pneumoniae_; _Sal_, _Salmonella enterica_ serovar Senftenberg; _E. coli_, _Escherichia coli_; _E. Clo_, _Enterobacter cloacae_; R, recipient; I, isolate; TF, _E. coli_ DH-10BT1 transformant containing a single New Delhi metallo-β-lactamase–encoding plasmid; +, target detected by the assay; –, target not detected by the assay; ND, not done (aminoglycoside resistance was not transferred); UT, untypeable by PCR replicon typing (*22*).
†Detected by using the Check-MDR CT101 microarray assay (*23*).
‡ Detected by PCR.

Four of 8 transformants resistant to amikacin and gentamicin contained *armA*, and 2 contained *rmtC* (Table 4), both of which are 16S rRNA methylase genes that confer high-level resistance to nearly all aminoglycosides (*24*). The mechanism conferring aminoglycoside resistance in the remaining 2 transformants was not caused by *armA* or *rmtC* (Table 4) and was not characterized further.

bla$_{NDM}$-bearing Plasmids

The incompatibility groups of NDM-encoding plasmids were primarily A/C (n = 4), but also included FII (n = 2), L/M (n = 1) and 2 plasmids that were untypeable (Table 4). Eight *Xmn*I restriction patterns were observed among the NDM-encoding plasmids isolated from transformants of the isolates from the United States and isolate 0S-506 from Sweden (Figure 1). Plasmid restriction profiles from *K. pneumoniae* 0S-506 and 2 *K. pneumoniae* isolates (1100770 and 1100975) were indistinguishable. Each isolate carried *bla*$_{NDM}$ on an *Xmn*I fragment of ≈6 kb (Figure 1) and had similar transferred antimicrobial susceptibility profiles (Table 3); carried the same ESBL and *AmpC* genes; and had plasmid replicon type A/C (Table 4). Other *bla*$_{NDM}$-bearing plasmids were diverse, including those isolated from the same patient (*K. pneumoniae* 1100975 and *S. enterica* serovar Senftenberg 1101168) (Figure 1).

Strain Typing

The *K. pneumoniae* isolates with indistinguishable *bla*$_{NDM}$ plasmid profiles were closely related by PFGE, and all were classified as sequence type (ST)14 by MLST (Figure 2) (*13*). The remaining *K. pneumoniae* (Figure 2) and *E. coli* (data not shown) isolates showed more diverse PFGE patterns and MLST types, including ST37, ST11, and ST147. *E. coli* isolates were identified as ST500 and ST43. For most isolates, ST43 corresponds to ST131 in the MLST scheme of Wirth et al. (*27*) (S. Brisse, pers. comm.).

Conclusions

The 9 NDM-producing isolates described were resistant to all β-lactams, including aztreonam, as well as all commonly used aminoglycosides and fluoroquinolones. In addition to NDM, each isolate carried ≥1 other β-lactamase, including CMY-II-type AmpCs and/or CTX-M-1-type ESBLs (which co-transferred with NDM for all but 3 isolates). Most *bla*$_{NDM}$-bearing plasmids also carried *armA or rmtC* 16S rRNA methylase genes, which confer high-level resistance to most aminoglycosides and are often associated with these plasmids (*24*,*28*). Although resistance to ciprofloxacin and tetracycline did not transfer with the *bla*$_{NDM}$-bearing plasmid, trimethoprim/sulfamethoxazole resistance was conferred to 1 transformant. For several strains, the transformant displayed decreased carbapenem resistance compared with a parental strain

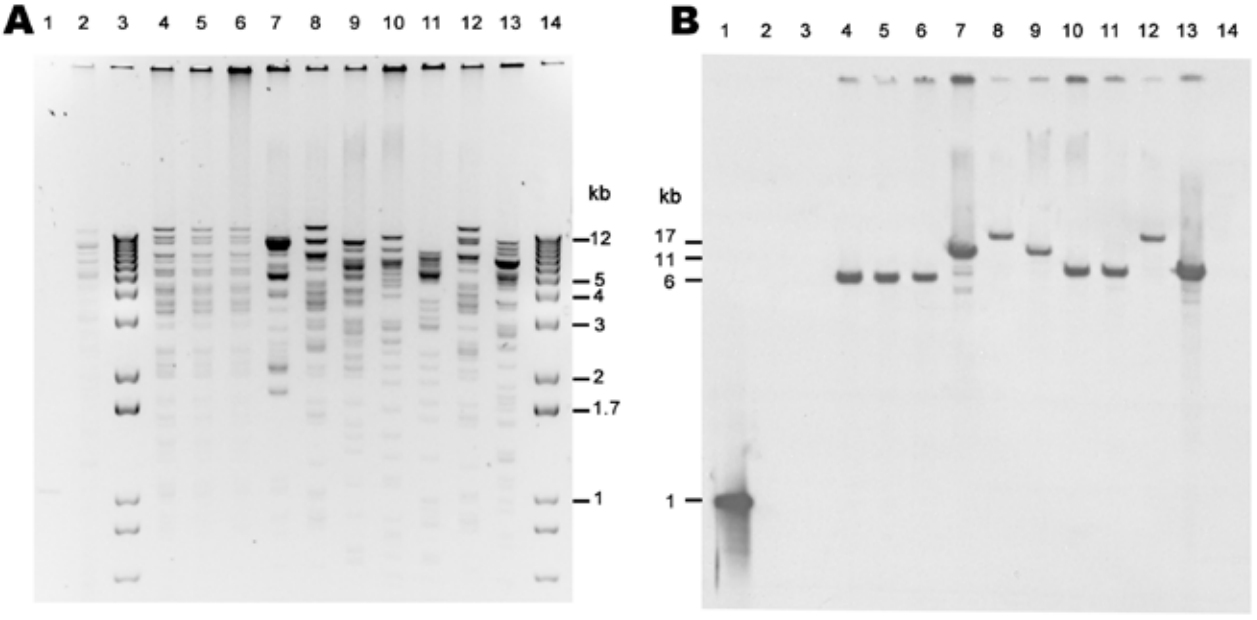

Figure 1. *Xmn*I restriction analysis of New Delhi metallo-β-lactamase (NDM)–encoding plasmids, United States, April 2009–March 2011, from transformants (A) and subsequent Southern blot analysis with digoxigenin-labeled *bla*$_{NDM}$ probe hybridized to a blot of same gel (B). Lane 1, NDM PCR product, positive control; lane 2, NDM-negative plasmid (ATCC-1705); lanes 3 and 14, 1-kb plus marker; lane 4, TF 0S-506; lane 5, TF 1100770; lane 6, TF 1100975; lane 7, TF1100192; lane 8, TF 1000527; lane 9, TF 1101459; lane 10, TF 1101168; lane 11, TF 1100101; lane 12, TF 1001728; lane 13, TF 1000654.

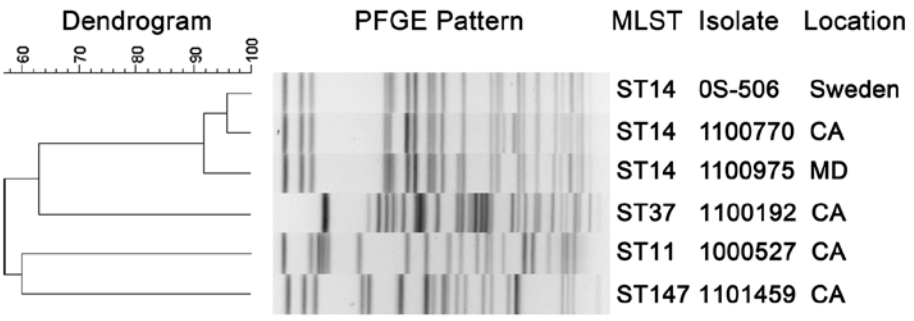

Figure 2. Dendrogram showing pulsed-field gel electrophoresis (PFGE) analysis and multilocus sequence typing (MLST) results for New Delhi metallo-β-lactamase–producing *Klebsiella pneumoniae* isolates, United States, April 2009–March 2011. Scale bar indicates % similarity. CA, California; MD, Maryland.

(e.g., 1100192-TF), suggesting that additional mechanisms (e.g., AmpC and ESBL) present in the parental strain and not carried on the NDM-encoding plasmid may have contributed to the initial carbapenem-resistant *Enterobacteriaceae* phenotype observed. These findings emphasize the diversity of resistance mechanisms carried on NDM-encoding plasmids, as reported (*28*).

We used 3 screening methods for phenotypic detection of MBL activity: MHT, Etest MBL, and BMD MBL. The MHT was not sensitive for detection of NDM activity; 3 isolates were positive only for 1 carbapenem tested, and *K. pneumoniae* 0S-506, the first characterized NDM-producing isolate (*13*), was negative for both carbapenems. Etest MBL definitively identified 6 parental isolates and 1 NDM-producing transformant as MBL producers, but 3 parental strains and 8 transformants displayed a phantom zone or slight deformation of the IP or IPI ellipse. The Etest MBL package insert states that these findings are indicative of an MBL, but the ellipse deformation we observed was subtle and less dramatic than the example provided. The BMD MBL screen provided the most unambiguous results, and yielded IMP to IMP + EP MIC ratios ≥8 for all NDM-producing parental and transformant strains. In an earlier validation study, this BMD MBL screen had a sensitivity of 95% and a specificity of 100% (*29*).

We reliably detected bla_{NDM} with a novel multiplexed real-time PCR designed to detect the bla_{NDM} and bla_{KPC} genes. DNA sequence analysis confirmed the PCR results and identified the bla_{NDM} allele in each isolate. One isolate contained a variant allele designated bla_{NDM-6}. An NDM-6–producing *E. coli* strain was also recently identified in a patient in New Zealand who had received medical care in India (*30*).

Plasmids carrying bla_{NDM} have been reported to range in size from 50 through 400 kb (*14*,*15*). Because all isolates in this report carried multiple plasmids, it was necessary to transfer the NDM-encoding plasmid to a plasmid-negative recipient for analysis. Three *K. pneumoniae* isolates, including the original NDM-producer from Sweden (*13*), contained an ≈170-kb bla_{NDM}–bearing plasmid, and each isolate was indistinguishable by restriction analysis and Southern blot. These strains were also closely related by PFGE and MLST (ST14). Furthermore, the antimicrobial drug susceptibility profiles of their parental and transformant isolates were similar. In contrast, the remaining isolates contained different bla_{NDM}-bearing plasmids ranging in size from 100 kb through 200 kb, carried bla_{NDM} on different restriction fragments, and were not related by PFGE or MLST. Most of the bla_{NDM}-bearing plasmids belonged to incompatibility groups A/C or L/M, both broad host range plasmids, and FII, a narrow host range plasmid (*31*). All 3 replicon types have been found to be associated with a variety of β-lactam resistance mechanisms in *Enterobacteriaceae* (*32*). These findings were consistent with reports of extensive diversity among bla_{NDM}-bearing plasmids in *Enterobacteriaceae* (*14*).

The MLST types identified among NDM-producing *K. pneumoniae* described here have been associated with various resistant strains of *K. pneumoniae* worldwide (*28*,*33*). ST11, ST147, and ST15, a single locus variant of ST14, have been identified as epidemic clones of CTX-M-15–producing *K. pneumoniae* in Hungary (*34*). In addition, ST11 is the dominant KPC-producing strain in China (*35*) and is a single locus variant of ST258, the dominant KPC-producing strain in the United States (*2*). ST11 NDM-producing *K. pneumoniae* strains were among the first NDM-producing *Enterobacteriaceae* reported in New Zealand (*30*), and ST147 NDM-producing *K. pneumoniae* isolates have been reported in Switzerland (*28*), Canada (*36*), Australia (*37*), and in an Iraqi patient transferred to a hospital in France (*28*). ST14 has been identified among KPC-producing strains in the United States (*38*), and is associated with NDM-producing *K. pneumoniae* isolates in Kenya and the Sultanate of Oman (*28*), and as the most frequently encountered ST in a recent study of NDM-1–producing *K. pneumoniae* from 3 countries (*33*). We also report a ST43/ST131 NDM-producing *E. coli* strain in our study. This clone is most notably associated with the global dissemination of the CTX-M-15 ESBL (*39*).

Each patient associated with the isolates described here had recently been in India or Pakistan, and most had received inpatient medical care in those countries. The link between NDM acquisition and health care exposure abroad has been extensively described (*4*,*15*,*40*). However, 1 patient only had outpatient health care during travel, and another had no documented health care, although the second patient had several active medical problems, including the presence of an invasive medical device during travel. In contrast to the early NDM case-patients reported in the United Kingdom (*15*), none of the patients in our study had traveled specifically for the purpose of obtaining medical care.

Several factors contribute to the global dissemination of bla_{NDM} as it spreads through a variety of plasmids and bacterial strains. The environmental and epidemiologic factors driving this spread and the molecular mechanisms by which it disseminates are not well understood. However, in the 3 years since its initial description, NDM has spread rapidly worldwide and has now been described in ≥15 countries in 5 continents (*4*,*8*). Since the completion of this study, numerous additional NDM-producing *Enterobacteriaceae* have been identified in the United States. The relative ease with which this resistance mechanism appears to move within and between different bacterial genera, as well as mobility of humans infected or colonized with NDM producers, serves to highlight the need for reliable and rapid means of detecting drug-resistant organisms to implement infection control measures to prevent further dissemination.

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Pandemic Influenza Planning, United States, 1978–2008

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During the past century, 4 influenza pandemics occurred. After the emergence of a novel influenza virus of swine origin in 1976, national, state, and local US public health authorities began planning efforts to respond to future pandemics. Several events have since stimulated progress in public health emergency planning: the 1997 avian influenza A(H5N1) outbreak in Hong Kong, China; the 2001 anthrax attacks in the United States; the 2003 outbreak of severe acute respiratory syndrome; and the 2003 reemergence of influenza A(H5N1) virus infection in humans. We outline the evolution of US pandemic planning since the late 1970s, summarize planning accomplishments, and explain their ongoing importance. The public health community's response to the 2009 influenza A(H1N1)pdm09 pandemic demonstrated the value of planning and provided insights into improving future plans and response efforts. Preparedness planning will enhance the collective, multilevel response to future public health crises.

Historical Background

Influenza pandemics occur when an animal influenza virus to which humans have no or limited immunity acquires the ability, through genetic reassortment or mutation, to cause sustained human-to-human transmission leading to community-wide outbreaks (*1*). The existence of a pandemic is currently determined by the extent of disease spread, not by the lethality of the disease caused by the novel virus (*2*). During the twentieth century, influenza pandemics occurred in 1918, 1957, and 1968. The 1918 pandemic, known as the "Spanish flu" pandemic, was unique in that the highest number of deaths was among young, healthy persons. Excess mortality in the United States during the 1918 pandemic was estimated at 546,000 deaths (*3*). The pandemics in 1957 and 1968, although associated with death rates greater than those for seasonal influenza epidemics (*3*), were far less devastating than the 1918 pandemic.

Before 1976, public health planning for pandemics primarily occurred in response to detection of a novel influenza virus. This reactive mode continued despite the framework outlined in 1960 by US Surgeon General L.E. Burney for responding to the next pandemic. That framework involved recognition of the pandemic (i.e., surveillance), manufacture and distribution of vaccine, and identification of research needs (*4*). Large-scale infectious disease response planning may have been hampered by the tacit assumption that the government's public health resources were better directed to other priorities.

In January 1976, a novel swine-origin influenza virus emerged among soldiers at Fort Dix, New Jersey (*5*); 1 soldier died, and an estimated 230 were infected. The emergence of influenza virus of swine origin at Fort Dix led to the decision to mount a national immunization program (*6*). The following events occurred subsequent to this decision: Congress funded vaccine production and liability indemnification of manufacturers, vaccine was produced, a mass immunization campaign commenced, and 45.65 million persons were vaccinated in the United States (*7*). Initial fears that the virus would cause a pandemic did not materialize: sustained transmission did not occur outside of Fort Dix. The vaccination campaign began in October 1976 and was halted in December because of initial reports of a rare association between the so-called "swine flu" vaccine and Guillain-Barré syndrome; the association was later confirmed (*7*). An influential policy review of the "swine flu affair" (i.e., the campaign to immunize the US population against a possible epidemic) identified several critical needs for future planning: 1) a more cautious approach to interpreting limited data and communicating risk to the public, 2) greater investment in research and preparedness, 3) clearer operational responsibilities within the federal

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government, 4) clear communication between planners at all levels of government, 5) strengthened local capacity for plan implementation, and 6) improved mechanisms for program evaluation (*8*).

In November 1977, separate from the Fort Dix outbreak, a strain of human influenza A(H1N1) virus reemerged in the former Soviet Union, northeastern China, and Hong Kong, China, even though the virus had not circulated since 1957. This strain primarily affected young persons, and caused mild illness (*9*). The virus was found to be closely related to a 1950 A(H1N1) strain but dissimilar to the 1957 strain, suggesting that this 1977 outbreak strain had been preserved since 1950 (*9*).

The confluence of fears of a possible pandemic in 1976 followed by the reemergence of a new strain of circulating seasonal influenza virus in 1977 led to focused pandemic planning efforts in the United States. The primary purpose of this article is to describe US pandemic planning during 1978–2008, just before the onset of the influenza A(H1N1) pdm09 pandemic in April 2009. We believe that understanding the historical and policy context within which the A(H1N1)pdm09 pandemic occurred is helpful in assessing the implications of pandemic planning for responses to future pandemics and for ongoing infectious disease preparedness efforts.

Sources

We conducted searches of the medical literature and key websites (e.g., www.pandemicflu.gov) for peer-reviewed manuscripts and published governmental plans relevant to pandemic planning during 1978–2008. We also consulted authors' personal files and the Internet for records of speeches, national and international conference proceedings, and other unpublished original source documents. In addition to published survey data concerning local and state response planning (*10*,*11*), we sought unpublished data from the Association of State and Territorial Health Officials, the National Association of County and City Health Officials (NACCHO), and the Council of State and Territorial Epidemiologists (CSTE).

Chronology of US Pandemic Planning

A historical overview of key milestones in US pandemic planning is provided in the Table. In 1977, a federal interagency working group on influenza was formed at the request of the deputy assistant secretary for health in the Department of Health, Education, and Welfare, partly in recognition of the need for greater cooperation across government "silos." The interagency group included representatives from the Center for Disease Control (CDC; renamed Centers for Disease Control and Prevention in 1992), the National Institutes of Health, the Food and Drug Administration (FDA), and the Department of Defense. Under CDC leadership, the work group drafted the first US pandemic plan, which was released in 1978 and included recommendations for annual influenza immunization of persons at high risk, strengthening of surveillance, expanding research, and establishing a planning and policy mechanism (*12*).

The plan was revised in 1983 to include a new recommendation to develop means to distribute and use influenza antiviral drugs (R.A. Strikas, pers. comm.). Even before completion of the pandemic plan, participants of a 1977 conference on influenza, held by the secretary of the Department of Health, Education, and Welfare, recommended continued federal support for influenza vaccination, particularly to increase vaccination levels of persons at high risk, to improve pandemic preparedness. In addition, CDC implemented a federally funded seasonal influenza immunization program, which purchased 3.4 and 2.4 million vaccine doses for the 1978–79 and 1979–80 influenza seasons, respectively, of which ≈1 million and >1.4 million doses, respectively, were administered. Initial plans were to purchase 8–9 million doses of vaccine. However, budget constraints limited vaccine purchases and ended the program after 1980 (*13*,*14*).

The next major event leading to further US pandemic planning was 1986 legislation creating the National Vaccine Program Office (NVPO), which was given a mandate to coordinate federal vaccine-related activities. At the Options for the Control of Influenza II meeting held in 1992, a consensus report identified the core components of pandemic preparedness: surveillance, vaccines, antiviral drugs, nonmedical/personal hygiene measures, communications, and enhanced annual seasonal influenza vaccination programs (*15*). In 1993, NVPO formed the federal interagency Group on Influenza Pandemic Preparedness and Emergency Response (GrIPPE). The group, which included nonfederal consultants and representatives from CDC, FDA, the National Institutes of Health, and the Department of Defense, drafted a pandemic planning framework that was published in 1997 (*16*) and updated by federal staff in 2002 (*17*). The GrIPPE-initiated planning documents emphasized the need for enhancements to influenza surveillance, vaccine production and distribution, antiviral drugs, influenza research, and emergency preparedness. Perhaps the most consequential outcome of GrIPPE was the creation of a core group of public health experts dedicated to pandemic planning.

Global events helped accelerate interest in pandemic planning. In 1997, Hong Kong recorded the first outbreak of avian influenza A(H5N1) virus infections in humans. Virus was transmitted from infected chickens directly to humans, and 6 of 18 persons with confirmed infection died. In late 1997, >1.5 million chickens were culled throughout Hong Kong as part of successful efforts to stem the

Table. Timeline of selected key events in pandemic planning, United States, 1978–2008

Year	Event	Outcome or follow-up
1978	First US pandemic plan, drafted by Federal Interagency Working Group on Influenza	Planning workgroup and its process assisted in strategy for addressing 1977–78 influenza A(H1N1) outbreak.
1983	Revision of 1978 US pandemic plan	The revised plan laid groundwork for subsequent planning documents.
1988	Institute of Medicine report, The Future of Public Health*	The report recognized the need to improve public health surveillance and response.
1992	Options for the Control of Influenza II meeting, Courchevel, France	The meeting led to formation of the US Federal interagency Group on Influenza Pandemic Preparedness and Emergency Response in 1993.
1997	Publication of elements of the US pandemic preparedness plan in Journal of Infectious Disease	The report updated the action plan, and a further update was published in 2002 in Clinical Infectious Diseases.
1998	CDC emerging infectious disease strategic plan update†	Pandemic influenza was noted as an emerging infection.
1999	Council of State and Territorial Epidemiologists survey data published	Enhanced influenza surveillance was recognized as a cornerstone of pandemic preparedness.
1999	World Health Organization Guidelines for Regional and National Planning	The World Health Organization strongly recommended all countries establish National Pandemic Planning Committees.
2001	Anthrax-related bioterrorism in the United States	The federal response increased state/local preparedness funding.
2003	Severe acute respiratory syndrome outbreaks worldwide	The outbreak led to a globally coordinated response to emerging respiratory pathogens.
2003	Initial detection of human avian influenza A(H5N1) cases in China and Vietnam	The outbreak enhanced attention to pandemic preparedness by Department of Health and Human Services and the US government, accompanied by additional funding.
2005	Department of Health and Human Services pandemic strategic plan	The plan engendered multiple subsequent high-level policy documents and plans from the US government.
2006	Implementation plan for the national strategy for pandemic influenza	This plan led to action steps and a timeline for all pandemic planning pillar areas.
2007	Pandemic influenza vaccine allocation guidance‡	This document preceded the 2009 influenza A(H1N1)pdm09 vaccine recommendations

*http://iom.edu/Reports/1988/The-Future-of-Public-Health.aspx
†www.cdc.gov/mmwr/PDF/rr/rr4715.pdf
‡www.flu.gov/images/reports/pi_vaccine_allocation_guidance.pdf

outbreak (*18*). This event, combined with the 2003 re-emergence of A(H5N1) virus, led to concerns that the next pandemic would be caused by spread of A(H5N1) virus through Asia into Africa and Europe.

In the United States, despite the crucial role of state and local authorities in implementing pandemic plans, a 1995 CSTE survey indicated that <60% of state health departments perceived the need for a state-specific plan (*10*). Through a cooperative agreement between CDC and CSTE, a state and local planning effort was begun in the fall of 1995. The state Project Steering Committee included the GrIPPE co-chairs and representatives from CDC, NVPO, CSTE, and the Association of Public Health Laboratories.

A meeting of >40 state and local health officials convened in September 1996 in Atlanta and identified 4 "pillars" deemed most critical for state and local pandemic preparedness efforts: 1) surveillance, 2) vaccine delivery, 3) communication and coordination, and 4) emergency response. From this meeting and subsequent subgroup meetings dedicated to the 4 pillar areas, critical elements of draft state and local guidelines were developed by January 1997. Four states (Connecticut, Missouri, New Mexico, and New York) and 1 local area (East Windsor Township, New Jersey) were selected by the state Project Steering Committee—primarily on the basis of the identification of a key project leader within each jurisdiction—and funded to pilot test the draft guidelines; 1 additional state, Maine, volunteered to test the draft guidelines without CSTE support. These 5 states conducted pilot tests during February and March 1998 and submitted results to CSTE. Findings were discussed on April 7–8, 1998, at a meeting in Atlanta. The major outcomes from pilot testing were the following recommendations: 1) a fifth pillar area, guidance for use of antiviral drugs, should be added to the guide; 2) the format of the guidelines should be more in concert with the national plan (*18*); and 3) all states should receive the revised guidelines to enable development of state-specific plans (R.A. Strikas, pers. comm.). These 3 issues were discussed at the Association of State and Territorial Health Officials/NACCHO annual meeting in September 1998 and incorporated into the state and local pandemic influenza planning guidelines (R.A. Strikas, pers. comm.), which were then further revised. California, Maryland, Minnesota, and South Carolina were funded through CSTE to develop state plans and submitted their model plans in April 2000.

A national pandemic influenza steering committee was subsequently formed; it was comprised of immunization

program managers, emergency preparedness personnel, and representatives from CDC, CSTE, NACCHO, and the Association of Public Health Laboratories (*19*). A national steering committee was a logical extension as the planning process moved from a federal to a national effort.

In 2000, federal funding increased the number of states engaged in pandemic plan development. Florida, Indiana, Massachusetts, New Hampshire, and New Jersey were funded to complete plans by March 2001. In January 2001, Kansas, Washington, Nebraska, Connecticut, and New York were funded to develop plans by March 2002 (*11*). Throughout this process, all states received the same nominal level of funding support, which was typically used to convene a statewide stakeholders meeting. Elements critical to the planning process included technical support provided by the national steering committee and the identification of a key public health professional within each state who assumed responsibility for leading and coordinating planning efforts. Arkansas, Arizona, and Oregon concurrently developed plans of their own accord; West Virginia, Tennessee (1999), and Pennsylvania (1999) had already developed plans. Ultimately, funds were sought for every state to develop a plan.

At this early stage in the planning process, the importance of disseminating information to the broader public health community was recognized. On February 25, 1999, and July 13, 2000, CDC presented satellite videoconferences on influenza pandemic preparedness for states and local areas, which were viewed by >7,000 and ≈6,000 participants, respectively. State and local public health staff engaged in development of pandemic plans participated in the broadcasts. At a meeting of state and local planners sponsored by CSTE and CDC in Atlanta on September 12–13, 2000, detailed discussions were held regarding 1) a scenario of how an influenza pandemic might affect states in 2001; 2) how states should enhance surveillance; 3) how vaccination priorities should be determined, and 4) other national and federal pandemic planning issues, such as infection control, patient triage, and antiviral drug usage (R.A. Strikas, pers. comm.).

After the September 11, 2001, terrorist attacks on the United States, public health preparedness emerged as a priority of the federal government. In 2001, bioterrorism emergency funding support was provided to all states to assist in the nation's response to the anthrax attacks. The 2003 reemergence of avian influenza A(H5N1) infections in humans fundamentally altered the scale of pandemic preparedness. As the A(H5N1) virus spread to more countries in East and Southeast Asia during 2004–2005, concern grew among senior policymakers and public health experts that the world was on the verge of an influenza pandemic. A(H5N1) infection in humans primarily resulted from exposure to ill poultry and had a case–fatality rate of ≈60%. Substantial federal funding was provided for federal-level planning, procurement of countermeasures (e.g., vaccines and antiviral drugs), development of countermeasures, and state and local pandemic preparedness efforts (*20*). State health departments eventually received $550 million to prepare for an influenza pandemic. Additional high-level policy engagement by the US federal government included the National Strategy for Pandemic Influenza, which was announced in November 2005 (*21*), and the White House's National Implementation Plan, which was published in May 2006 and addressed federal planning and response strategies: international transport and border control; protection of human and animal health; and security and continuity of operations issues (*22*).

In 2006, the Biomedical Advanced Research and Development Authority (BARDA) was established within the Department of Health and Human Services in response to the growing need for a centralized effort to coordinate research, development, and procurement of countermeasures against potential natural or intentional public health emergencies (*23*). BARDA preparations for a possible A(H5N1) pandemic included development of a stockpile of influenza vaccines produced by using strains circulating in poultry and wild birds in Asia (*24*). In addition, the US government began to purchase influenza antiviral medications for the Strategic National Stockpile sufficient to treat 25% of the US population. Additional investments were initiated to procure ventilators and personal protective equipment, such as respirators.

The US government also initiated an advanced development agenda for vaccines, therapeutics, and diagnostics. BARDA co-invested with industry to modernize vaccine production methods, with the 5-year aim of creating the capacity to produce sufficient vaccine to protect the entire US population within 6 months of the onset of an influenza pandemic (*22*). The US government invested in modernizing diagnostic technologies for public health laboratories. In September 2008, FDA approved specific PCR tests for a panel of influenza diagnostics to be used in CDC reference laboratories in the United States and Department of Defense laboratories around the world. This diagnostic test panel will detect and identify A(H5N1) infections and distinguish novel influenza virus infection from infection with seasonal A, B, and A(H1) and A(H3) influenza viruses. BARDA and CDC awarded contracts in November 2006 for development and evaluation of clinical point-of-care rapid diagnostics to identify seasonal influenza viruses and A(H5N1) viruses (*25*).

Beginning with its first published pandemic plan in 1999 (*26*), the World Health Organization globally promoted pandemic planning among member states, with continued planning efforts thereafter (*27*). The International Partnership on Avian and Pandemic Influenza was formed to coordinate support for developing countries' efforts to control the spread of A(H5N1) virus and to prepare for an

influenza pandemic. This international body convened a series of meetings beginning in January 2006; these efforts generated hundreds of millions of dollars in pledges to support global pandemic preparedness and promoted a level of visibility and readiness that would not otherwise have been possible. In addition to direct financial assistance, the US government provided technical assistance to help countries develop capacities for rapid response, laboratory diagnosis, and surveillance.

The federal government recognized that the foundation for domestic pandemic response rests with state and local governments; thus, the 2005 Department of Health and Human Services strategy and the White House strategy and implementation plan called for major efforts in planning, exercising, and refining state and local preparedness. The 2006 Pandemic and All-Hazards Preparedness Act called for a review of comprehensive state pandemic preparedness plans. The federal government reviewed and scored the plans and released the results to the public in January 2009 (*28*); preparedness levels varied across states and across the domains that were scored. In 2008, as part of its local health profile survey, NACCHO queried local health departments about emergency preparedness and planning activities they had undertaken during the past year (*29*): 89% of 2,332 responding health departments said they had developed or updated pandemic influenza preparedness plans, and 86% said they had participated in tabletop drills or exercises. In addition, 76% had updated their written response plan on the basis of a postexercise after-action report, 72% had participated in a functional drill, and 49% had participated in a full-scale drill or exercise. A total of 68% of local health departments had reviewed existing state legal authorities for isolation and quarantine, and 46% had assessed the emergency preparedness competencies of staff. Only 1% of local health departments did none of the above.

Evolution of the Pillars of Pandemic Preparedness

The 4 pandemic planning pillars—surveillance, vaccine and antiviral drug delivery, emergency response, and communication—are a solid foundation for pandemic preparation. Although state pandemic plans may have different structures, reliance on these pillars has remained more or less constant across jurisdictions and over time. The major contemporary developments in these core areas are summarized below.

Surveillance, including rapid detection of human infection with novel influenza viruses, remains a cornerstone of pandemic response. This need has been recognized since the early stage of state- and local-based planning (*10*). Improvements in diagnostic technology have enabled confirmation of infection with novel influenza viruses within hours rather than weeks. Human infection with a novel influenza virus became a nationally notifiable disease in 2007, and since then, an increased number of infections have been detected (*30*). Virologic surveillance is also used to determine which seasonal viruses are circulating and thus provides information for seasonal vaccine strain selection. Systems to measure the effect of seasonal influenza (i.e., pediatric deaths, hospitalizations, and syndromic surveillance) have also been enhanced. These systems have been further adapted to measure the effect of pandemic influenza (*31*). The need to maintain ongoing surveillance for novel influenza viruses (e.g., viruses of swine or avian origin) in humans and animals exemplifies the One Health concept (*32*).

In recognition that vaccine might be in short supply during the early phase of a pandemic, federal vaccine allocation guidelines were published in 2008 (*33*). These guidelines laid the groundwork for the pandemic vaccine priority-group recommendations put forth during the 2009 A(H1N1)pdm09 pandemic (*34*). Antiviral medications are critical to a pandemic response, particularly in the interval between recognition of the pandemic and the availability of vaccine. Plans for using these countermeasures have stressed the need for early treatment of affected persons and assumed that the drugs would be scarce.

It was recognized at the 1996 CSTE meeting that close coordination between emergency response staff and public health authorities is needed to develop and implement effective state and local influenza response plans. This recognition has strengthened over time. Although, states were initially not allowed to use bioterrorism funds awarded in 2001 to support pandemic planning, key emergency management concepts, including the all-hazards approach and unified incident command, were eventually integrated into planning efforts (*35*).

Communication, more than ever, is a fundamental component of any response effort. Timely, transparent, and proactive communication is critical, particularly in the early stages of a confirmed or suspected outbreak, when factual information is limited and the public demand for information and guidance is high. Continuous media coverage and the evolving role of social media (*36*) must be used to enhance communication to and from the public, particularly concerning new or evolving recommendations for disease control.

Conclusions

Pandemic planning since 2005 had a direct and obvious effect on the response to the 2009 influenza A(H1N1) pdm09 pandemic; however, pandemic preparedness has been a feature of public health since the late 1970s. Coordinated state and federal planning processes have been a consistent feature of that planning. The pillars of pandemic planning response have remained conceptually

constant: surveillance; vaccination and delivery of other medical countermeasures; emergency response coordination; and communications.

Although the 2009 A(H1N1)pdm09 pandemic spread globally within a matter of weeks, a 1918-like pandemic did not materialize. Nonetheless, this most recent pandemic resulted in ≈12,500 deaths in the United States, ≈90% of which occurred in persons <65 years of age (*37*). In the wake of this pandemic, the challenge in preparedness is to sustain the interest of private and public sectors in planning for a large-scale outbreak that may have a much more severe effect at a time that cannot be predicted.

Recent assessments of state level epidemiology capacity revealed potentially critical gaps in personnel and training needed for a rapid response to an epidemic (*38*). There will be a need for continued commitments to support state, local, and national planning for the next infectious disease emergency. A comprehensive, coordinated, and effective response cannot be built at the time of a crisis. For future planning and response efforts, sufficient resources are required to sustain the public health response infrastructure developed during the past decade.

An effective response to a pandemic requires at least 4 distinct elements. First, material resources, such as vaccines, antiviral drugs, and personal protective equipment are essential. Second, a commitment to planning, exercising, and refining plans is necessary. Third, a sufficiently large and robustly trained workforce is the basis of any response. Fourth, a commitment to improvement is crucial. This concept extends from continuously improving plans and training to ensuring that scientific advances are incorporated into procurement and planning. One of the main lessons from the history of influenza is to expect the unexpected. Plans and training should be flexible and designed to respond to various levels of disease severity or newly identified pathogens. Benefits from pandemic preparedness will enhance our collective public health response to the next infectious disease crisis.

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Cell Culture and Electron Microscopy for Identifying Viruses in Diseases of Unknown Cause

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During outbreaks of infectious diseases or in cases of severely ill patients, it is imperative to identify the causative agent. This report describes several events in which virus isolation and identification by electron microscopy were critical to initial recognition of the etiologic agent, which was further analyzed by additional laboratory diagnostic assays. Examples include severe acute respiratory syndrome coronavirus, and Nipah, lymphocytic choriomeningitis, West Nile, Cache Valley, and Heartland viruses. These cases illustrate the importance of the techniques of cell culture and electron microscopy in pathogen identification and recognition of emerging diseases.

Thin section and negative stain electron microscopy (EM) examination of viruses grown in cultured cells have been instrumental in determining an etiologic agent in numerous disease outbreaks caused by previously unknown viruses. Many examples have been reported. In 1976, EM of cell culture isolates identified the causative virus of an outbreak of hemorrhagic fever in Zaire as a member of the family *Filoviridae*, now known as Zaire ebolavirus (*1*–*3*). Reston ebolavirus was another previously unrecognized virus that was detected by cell culture and EM in 1989; it was isolated from cynomolgus monkeys imported into the United States from the Philippines (*4*). In Australia in 1994, during an outbreak of fatal respiratory disease in horses and influenza-like illness in humans, a previously unknown virus, Hendra virus, was isolated in culture and recognized as a member of the family *Paramyxoviridae* by EM (*5*,*6*). An outbreak of an unidentified rash illness in humans, associated with sick prairie dogs, occurred in the upper midwestern United States in 2003, and EM detected a poxvirus from a cell culture isolate, which was later characterized as monkeypox virus (*7*,*8*). Recently, the etiologic agent of severe fever with thrombocytopenia syndrome in China was isolated and identified by EM as a member of the family *Bunyaviridae* (*9*).

Inoculation of patient specimens onto cultured cells or into laboratory animals enables biologic amplification of virus particles to levels where they can be detected by EM and identified to a virus family because, with a few exceptions (*10*), the morphologic features of all viruses within a given family are the same. Once recognized by EM, the findings can be confirmed by other techniques, including serologic testing, immunohistochemical (IHC) and indirect fluorescence antibody (IFA) assays, and molecular methods that can further characterize the virus to species and strain.

Cell culture methods are relatively unbiased, restricted only by the ability of the virus to grow in a particular cell line. Vero E6 cells, considered one of the most permissive of all cell lines, provide an extremely versatile medium for recovery of unknown pathogens. EM is also an unbiased assay in that there is no need for specific immunologic probes, and has the added advantage of being able to detect and classify the unknown agent. EM observations of cell culture isolates can provide the first clue of an etiologic agent and guide subsequent laboratory and epidemiologic investigations. Detection of a pathogen is critical during outbreaks because identification of an etiologic agent enables public health officials to mount a timely response

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and limit further spread of the agent involved. In addition, pathogen identification is invaluable in individual cases of severe illness in which an infection is caused by an undetermined agent. We report several cases where cell culture and EM at the Centers for Disease Control and Prevention (CDC) enabled initial recognition and identification of a cause of the viral illness.

Tissue Culture

Of the variety of tissue culture cells available, many are maintained in minimal essential medium at 37°C. Once cells have become confluent, they can be inoculated with suspensions of ground tissues (e.g., lung, liver), incubated for 1 hour, and grown until there is an ≈3+ cytopathic effect. Cells are then removed from the growth vessel by scraping or with glass beads, rinsed with 0.1 mol/L phosphate buffer, centrifuged, and fixed in buffered 2.5% glutaraldehyde for 1 hour.

Electron Microscopy

Specimens are postfixed in 1% osmium tetroxide, en bloc stained with 4% uranyl acetate, dehydrated through a graded series of alcohol and propylene oxide, and embedded in a mixture of Epon substitute and Araldite. Thin sections are stained with 4% uranyl acetate and Reynolds's lead citrate.

Family *Coronaviridae*

Beginning in late 2002, an outbreak of severe pneumonia associated with human deaths occurred in Guangdong Province, China, which escalated to a global pandemic of respiratory illness in early 2003. The World Health Organization reported 8,098 probable cases in 29 countries, and the disease killed 774 persons worldwide (*11*). Patients had an influenza-like illness with fever, cough, dyspnea, headache, and consolidation shown on chest radiographs, and the disease became known as severe acute respiratory syndrome (SARS). Isolation of a virus was achieved in several laboratories around the world by inoculating respiratory specimens onto cell culture, and thin section EM first identified the isolate as a coronavirus (*12*). This finding was quickly corroborated by negative stain EM, IHC assay, serologic testing, and molecular assays. Thus, once the isolate was identified as a coronavirus by EM, the focus of the laboratory investigation shifted toward verification of this finding. The natural reservoir for the progenitor of SARS coronavirus is most likely the Chinese horseshoe bat (*Rhinolophus sinicus*) because SARS corona-like viruses were identified and characterized in these animals (*13*,*14*).

Coronavirus particles are mostly spherical, sometimes pleomorphic, and have an average diameter of ≈80 nm (Figure 1, panel A). Nascent particles are formed when the helical nucleocapsids align along cytoplasmic membranes of the budding compartment (the membrane region between the rough endoplasmic reticulum and the Golgi complex), and virions obtain their membranes by budding into the cisternal lumen. Also found in some infected cells are accumulations of the viral nucleocapsids, and double-membrane vesicles that are believed to be the replication complex for this virus. Particles accumulate in cytoplasmic vesicles, which migrate toward the cell surface and fuse with the cell membrane, releasing the virus particles. Many virions remain adsorbed to the cell membrane, which gives infected cells a characteristic appearance of an outer layer of particles (Figure 1, panel B) (*15*).

Family *Paramyxoviridae*

In Peninsular Malaysia and Singapore during 1998–1999, an outbreak of viral encephalitis with a high mortality rate occurred among men who had been exposed to pigs. The illness was characterized by fever and headache, followed by a rapid deterioration in consciousness; >100 deaths were reported. Concurrently, respiratory illness increased among pigs in the same region. A virus was isolated in Malaysia from cerebrospinal fluid of a patient and was identified by EM as a member of the family *Paramyxoviridae* (*16*,*17*). It was shown to be the etiologic agent for human and swine diseases and is now known as Nipah virus. Serologic and PCR findings for the isolate indicated that Nipah virus was closely related to Hendra virus, a novel paramyxovirus which had been isolated in Australia in 1994 (*5*). The natural reservoir for Nipah virus was found to be flying foxes (*Pteropus hypomelanus* and *P. vampyrus*) (*18*).

Nipah virus particles are pleomorphic and vary greatly in size. Particles are composed of a tangle of nucleocapsids enclosed within the viral envelope, which contains surface projections 12 nm in length (Figure 1, panel C). Negative stain EM showed that the helical nucleocapsids have a herringbone appearance and an average diameter of 21 nm. The nucleocapsids can aggregate in the cytoplasm to form inclusions or migrate to the cell surface where they become tightly apposed with the cell membrane as the virus buds. (Figure 1, panel D) (*19*).

Family *Arenaviridae*

Organ and tissue transplantation have become relatively common surgical procedures, and on rare occasions, transplant recipients can become infected when a pathogen is transmitted from the donor. The immunocompromised status of the organ recipients enables amplification of the pathogen, which may lead to illness and death. In recent years, there have been several unexpected donor-derived clusters of infection, including reports of transmission of rabies virus, West Nile virus, and *Trypanosoma cruzi*, the etiologic agent of Chagas disease

(*20–23*). In 2 clusters of organ transplantation, 1 in 2003 and 1 in 2005, symptoms such as unexplained fever, graft dysfunction, and altered mental status developed in recipients; 7 of the 8 recipients died. An etiologic agent was isolated from cerebrospinal fluid of a patient in the 2003 cluster, and identified by EM as belonging to the family *Arenaviridae*. IFA assay and PCR showed that the agent was lymphocytic choriomeningitis virus (LCMV), an arenavirus transmitted by rodents (*24*). In immunocompetent humans, this virus typically causes a subclinical infection that rarely results in death.

Arenaviruses are mostly spherical, although there can be pleomorphic forms (Figure 2, panel A). Most notable is the inclusion of ribosomes inside the virus particles. Virions have a mean diameter of 110–130 nm but can vary in size. The virus particles bud at the cell membrane and have a dense outer envelope with small surface projections, and the appearance of the interior of the particles ranges from slightly granular to lucent.

Family *Flaviviridae*

Cell culture isolation and EM examination were also instrumental in the diagnosis of an etiologic agent in a patient with an unusual clinical manifestation. A 59-year-old man in Florida, USA, had hemorrhagic symptoms, including fever, hypotension, rash, loose bloody stools, and acute renal failure; he died within 1 week. The patient reported having recent arthropod bites, and a rickettsial disease was suspected. A serum sample was obtained from the patient, but it was negative by IFA assay for several rickettsial agents. A punch biopsy specimen of a skin lesion was obtained and homogenized and inoculated onto cell culture. A cytopathic effect was subsequently noted, the culture was examined by EM, and the isolate was recognized as belonging to the family *Flaviviridae*. PCR showed that the isolate was West Nile virus (*25*), which is only rarely known to cause a hemorrhagic-like disease.

Flavivirus-infected cells show a proliferation of the endoplasmic reticulum membranes with viral particles found within the lumen. Virions are ≈40 nm in diameter and have a dense core of 25–30 nm (Figure 2, panel B). Surrounding the core is an electron-lucent halo enclosed by the viral envelope. Smooth membrane structures having a mostly clear interior with a slight web-like arrangement are also found in the lumen of the endoplasmic reticulum and have been shown to be the replication complexes for this virus (*26*). Accumulations of dense convoluted membranes are also found in infected cells (*27*).

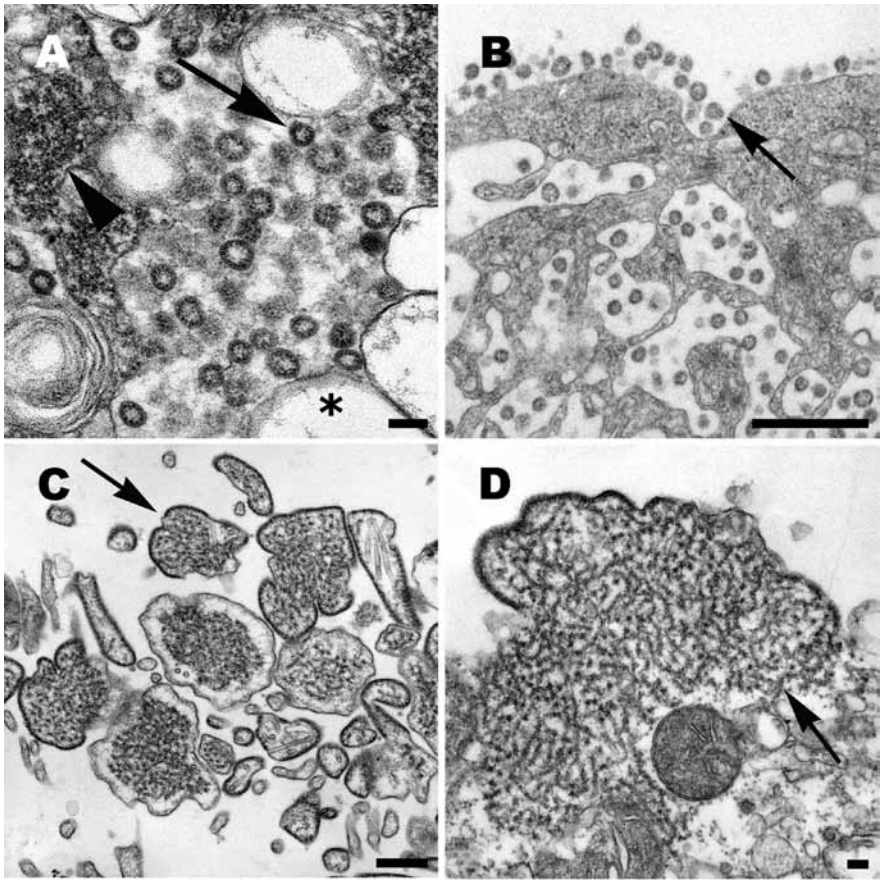

Figure 1. A) Cell culture isolate of severe acute respiratory syndrome coronavirus, in which virions are seen in the cisternae of the budding compartment (arrow). Also present are an inclusion of viral nucleocapsids (arrowhead) and double-membrane vesicles (asterisk). Scale bar = 100 nm. B) Coronavirus particles in cytoplasmic vesicles that appear to migrate to the cell surface. Virions are seen lining the cell membrane (arrow), a characteristic feature of this virus. Scale bar = 500 nm. C) Large, pleomorphic, extracellular Nipah virus particles (arrow), in which the viral envelope encloses the nucleocapsids. Scale bar = 500 nm. D) Nipah virus nucleocapsids (arrow) aggregate in the cytoplasm and become tightly apposed to the cell membrane as the virus begins the process of budding. Scale bar = 100 nm.

Family *Bunyaviridae*

There were 2 instances at CDC in which a bunyavirus was isolated from patients and identified by EM. In the first instance, a 28-year-old man in North Carolina, USA, had myalgias, fever, chills, and headache; his illness progressed to severe encephalitis and multiorgan failure, and resulted in death. An isolate was obtained at Duke University Medical Center (Durham, NC, USA) and at CDC from blood, serum, and cerebrospinal fluid, and EM examination recognized a virus belonging to the family *Bunyaviridae*. The virus was found to belong to the Bunyamwera serogroup (genus *Orthobunyavirus*) by ELISA and identified as Cache Valley virus by PCR and sequencing (*28*). Cache Valley virus was originally isolated from mosquitos in 1956, and the patient reported receiving many mosquito bites 2 weeks before the onset of illness. This finding was the first reported isolation of Cache Valley virus from a human case-patient.

In a recent second instance, a bunyavirus was isolated from 2 patients in northwestern Missouri, USA, who had a history of tick bites. Both patients had fever, fatigue, diarrhea, thrombocytopenia, and leukopenia, but recovered from their illnesses and were released after 10–12 days of hospitalization. An *Ehrlichia* sp. was initially suspected, and leukocytes from the patients were inoculated onto DH82 cells, a canine monocyte cell line. When cytologic changes were seen in the culture, cells were processed for EM examination. Rather than a bacteria, a bunyavirus was recognized. It was identified as a new member of the genus *Phlebovirus* by deep sequencing and is now known as Heartland virus (*29*).

Virus particles in bunyavirus-infected cells bud upon smooth cytoplasmic membranes and are found in vesicles and also extracellularly (Figure 2, panels C, D). The spherical, enveloped virions have surface projections visible on the surface of some particles. Virions have a granular interior with varying densities.

Conclusions

For decades, the combination of the classical techniques of virus isolation in tissue culture and examination by EM has been critical in detection of previously unrecognized viruses. Cell culture is a fundamental procedure that can be accomplished in most hospital microbiology laboratories and should be considered if an infectious viral agent is suspected. Although some examples provided in this report were handled in biosafety level 3 laboratories at CDC, other examples were

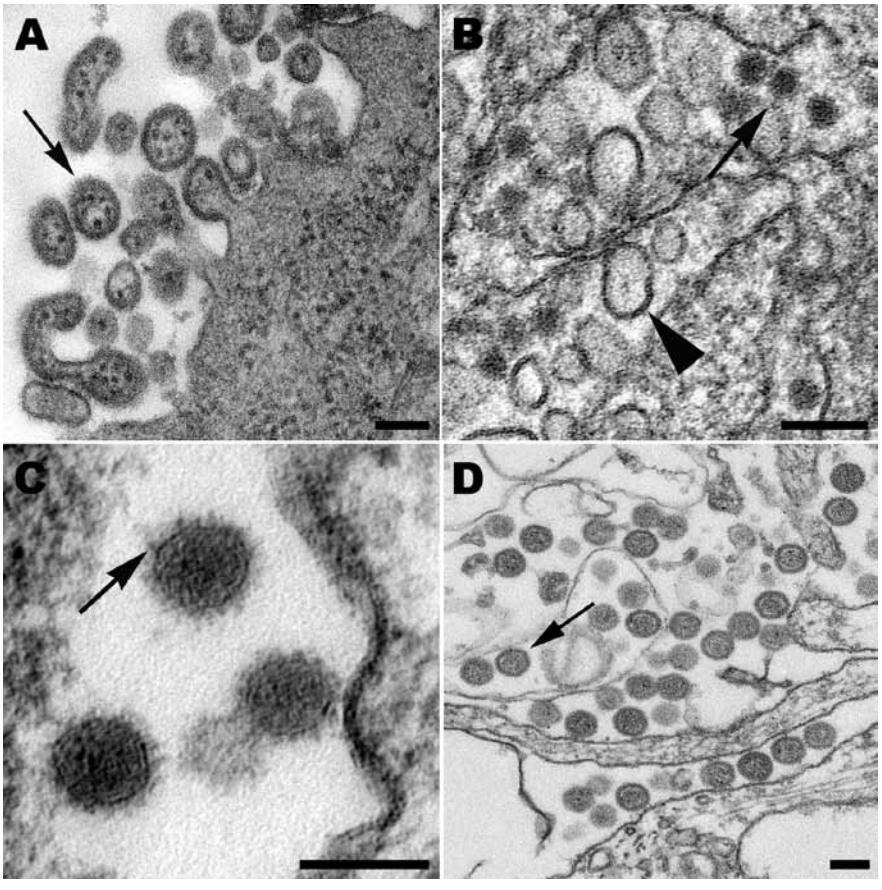

Figure 2. A) Extracellular lymphocytic choriomeningitis virus particles (arrow) containing cellular ribosomes. B) West Nile virus particles (arrow) in the lumen of the rough endoplasmic reticulum of an infected cell. Also in the cisternae are smooth membrane vesicles (arrowhead). C) High magnification of Cache Valley virus particles within a Golgi vesicle, showing small surface projections (arrow). D) Extracellular, spherical Homeland virus particles (arrow) with a slightly granular core. Scale bars = 100 nm.

handled as routine microbiological isolations in hospitals or public health departments equipped to perform routine virus isolation.

Other useful laboratory methods for diagnosis of an unknown virus include serologic testing; IFA, histopathologic, and IHC assays; PCR; and sequencing. Metagenomics with deep sequencing is a recent advancement of a molecular technique that enables genomic analysis of microorganisms without the need to isolate and culture pathogens. High-throughput sequencing uses random amplified DNA products and compares the obtained product sequences with available extensive banks of sequences for final identification of the agent detected. Because random primers are used to nonspecifically amplify templates for sequencing, there is no need for prior knowledge of the suspected target. This technology is advancing rapidly and improvements in the field will undoubtedly solidify its use in the field of pathogen discovery. Previously unknown viruses that have been recognized by using this technique include Schmallenberg virus (*30*), Lloviu virus (*31*), and Bas-Congo virus (*32*).

As molecular diagnostic techniques progress in scope and magnitude, it is critical to retain and use classical techniques, including cell culture and EM, that complement the advances in molecular methods. There is a continuing need to train younger scientists in these traditional methods to maintain an underlying expertise. In particular, with EM, the electron microscopist needs to be able to differentiate between infectious agents and artifacts or look-alike structures. The combination of cell culture and EM is an unbiased approach to identification of a previously unrecognized pathogen. Similarly, unbiased thinking and collaboration among clinicians, epidemiologists, microbiologists, and laboratorians who use different technologies are critical for successful investigations of diseases of unknown origin.

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Ms Goldsmith is an electron microscopist at the Centers for Disease Control and Prevention. Her research interests include ultrastructural identification and characterization of viruses, bacteria, and protozoa.

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Iatrogenic Blood-borne Viral Infections in Refugee Children from War and Transition Zones

Paul N. Goldwater

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Learning Objectives

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

- Describe observations regarding and factors contributing to iatrogenically transmitted blood-borne virus (BBV) infection in refugee children from central Asia, Southeast Asia, and Sub-Saharan Africa, based on a literature review and case reports
- Describe the role of contaminated injections and unsafe blood transfusions in health care settings in contributing to increased prevalence of BBVs in refugee children from central Asia, Southeast Asia, and Sub-Saharan Africa, based on a literature review and case reports
- Describe the role of other factors contributing to increased prevalence of BBVs in refugee children from central Asia, Southeast Asia, and Sub-Saharan Africa, based on a literature review and case reports.

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Authors

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Pediatric infectious disease clinicians in industrialized countries may encounter iatrogenically transmitted HIV, hepatitis B virus, and hepatitis C virus infections in refugee children from Central Asia, Southeast Asia, and sub-Saharan Africa. The consequences of political collapse and/or civil war—work migration, prostitution, intravenous drug use, defective public health resources, and poor access to good medical care—all contribute to the spread of blood-borne viruses. Inadequate infection control practices by medical establishments can lead to iatrogenic infection of children. Summaries of 4 cases in refugee children in Australia are a salient reminder of this problem.

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Blood-borne viruses (BBVs) have benefitted from internal political strife, migration, prostitution, intravenous/injection drug use, and defective public health resources in some Central Asian republics and Southeast Asian and sub-Saharan African countries. Iatrogenic transmission of

HIV in children in Romania (*1*) and the Russian republic of Kalmykia (*2*) are well-known examples. Refugee children are a special risk category for infection with BBVs (*3*). When iatrogenic transmission was encountered in a pediatric infectious diseases clinic in Adelaide, South Australia, Australia, concern was raised about whether it was an isolated or a more widespread phenomenon.

The United Nations High Commissioner for Refugees estimates that there were 43.7 million forcibly displaced persons worldwide at the end of 2010, the highest number in 15 years. Of these, 27.5 million were internally displaced persons, 15.4 million were refugees, and 837,500 were asylum seekers (*4*). Children constituted more than half of the humanitarian refugee population in Australia (*5*). A refugee is legally defined as a person who is outside his or her country of nationality and is unable to return due to a well-founded fear of persecution because of race, religion, nationality, political opinion, or membership in a particular social group. By receiving refugee status, persons are guaranteed protection of their basic human rights and cannot be forced to return to a country where they fear persecution (*4*).

Australia receives refugees from all countries experiencing internal conflict. Some arriving refugees have parasite infestations and bacterial and viral infections, especially undiagnosed BBVs (*6*,7). During 2010–2011, a total of 13,799 persons were admitted under Australia's Humanitarian Program.

The extent of the unusual problem of iatrogenic transmission of BBVs remains unknown because modes of transmission of individual cases are difficult to document. This report summarizes cases in 4 children from South Asia that illustrate the conditions extant in 1 city in Uzbekistan (Andijan), where medical procedures have resulted in transmission of HIV, hepatitis B virus (HBV), and hepatitis C virus (HCV). Among the case-patients are 2 children with BBV co-infection.

Methods and Definitions

Detecting possible iatrogenic BBV infections in children relies on a careful history. However, accurate histories are difficult to obtain because, in many cases, details are acquired through interpreters and facts are lost in translation. Nevertheless, several encounters with a family, during which family members are encouraged to tell their life stories (*8*), usually results in an accurate medical history. For orphans, learning the mode of BBV acquisition usually is impossible, except in cases of maternal HIV-associated deaths and mother-to-child transmission. The Australian Paediatric Surveillance Unit collects data for all HIV-positive children in Australia and reports these data to the National HIV Registry. For HIV-positive children from high-risk countries whose mothers are known to be HIV negative, the information recorded does not indicate mode of transmission (compare surrogate breast-feeding); nevertheless, such cases should be considered suspicious in regard to the manner by which the virus was acquired. The Australian Paediatric Surveillance Unit has recorded a few cases of HIV in children from high-risk countries whose mothers were HIV negative, thus indicating the problem. Since data collection began in May 1993, a total of 77 HIV infections have been reported in children (*9*); 8 cases in children (≤12 years of age) from high-prevalence countries were notified to the Australian National HIV Registry through the end of 2011. The mother of 1 child was reported as HIV negative. No information was available about the HIV status for the mothers of the 7 other children (A. McDonald, pers. comm.).

Case Discovery

Refugee families undergo voluntary health screening after arrival. In South Australia, screening usually is conducted by the Migrant Health Unit of the South Australian Department of Health but sometimes by another health unit. Children in whom BBV infection is diagnosed are referred to the Women's and Children's Hospital (WCH) Infectious Diseases Clinic for assessment and management. Some children are not screened on arrival, and undiagnosed BBV infections may be discovered later.

Iatrogenic BBV Infection

BBV infection acquired through injection of blood or blood products and for which vertical (mother-to-child) infection (including breast milk and surrogate breast-feeding) was ruled out by serologic testing of the mother and by history of sexual abuse and evidence of tattooing, piercing, and scarification. Iatrogenic infection, a form of horizontal transmission, usually involves mediation through injection of the BBV by a third party who might or might not be infected with the virus(es) in question. For this study, I defined an iatrogenic BBV infection according to all of the following criteria: BBV infection in a refugee child <18 years of age who was seen at the WCH Infectious Diseases Clinic during 2008–2010, who had been exposed to medical hollow-bore needles and blood products, who had acquired a BBV infection that was absent in his/her mother, and who had not been breast-fed by a surrogate or sexually abused but for whom other modes of horizontal infection could not be completely ruled out.

Search Methods and Patients

Internet searches of databases, including PubMed and Google Scholar, were performed to obtain peer-reviewed and other journal articles by using the following search terms: iatrogenic blood-borne virus infection, refugee children, blood-borne viral infections in war and transition zones, HIV, HBV, HCV. In addition, case reports of 4

refugee children seen in the WCH pediatric infectious diseases clinic during January 2008–December 2010 (Table) are summarized to illustrate the insidious nature of BBV infection. These case-patients, included here with parental written consent, were victims of their country's chaotic or absent infection control practices consequent to war and political strife.

Case Reports

Case-patient 1

An 8-year-old girl was brought for treatment in early 2010. She was born vaginally in a hospital in Uzbekistan after a full-term normal pregnancy. There were no neonatal concerns. At 18 months of age, severe hematemesis developed. Blood loss required admission to a clinic in Andijan and transfusions of donated plasma and of blood donated by her father. At 7 years of age, she underwent a tonsillectomy in the same clinic. She had no illness typical of HIV seroconversion. Her mother was hepatitis B surface antigen (HBsAg) positive and HIV negative. The girl's 2 younger siblings were BBV negative but anti-HBs positive. As part of migrant health screening on arrival in Australia in 2009, family members underwent serologic tests for HIV, HBV, and HCV. The girl was anti-HIV-1 and HBsAg positive. Her HIV load was 1,010 HIV RNA copies/mL, and HBV DNA was not detectable (<20 IU/mL) (HBeAg and anti-HBe negative) in 2009. Her father was negative for BBV markers. Her CD4 count was 260 cells/mL (19%), indicating severe immunosuppression. Examination was largely unremarkable except for severe dental caries and an ulcer on the lower gums (PCR positive for herpes simplex type 1). She began antiretroviral therapy soon after diagnosis and has responded well.

Case-patient 2

On arrival to Australia in 2010, a 9-year-old boy was discovered to be anti-HIV-1 positive on arrival. Both parents tested negative for anti-HIV-1 and anti-HCV. Through an interpreter, it was established that he had exposure to medical needles in the hospital. In 2004, he had undergone an appendectomy at the same Andijan clinic mentioned above; in summer 2008, he was admitted with "hepatitis" (presumably hepatitis A; see case-patient 3 below) to an Andijan hospital, where he received several injections (possibly vitamin K) by medical and nursing staff, but as far as is known, received no blood products and had no seroconverting-like illness. He had been asymptomatic. His prenatal history was unremarkable; he was born at term by normal vaginal delivery. He was vaccinated in Uzbekistan but to an unknown extent. He looked reasonably well. There was no lymphadenopathy. He had severe dental caries. He began antiretroviral therapy soon after his HIV-1 infection was diagnosed and has responded well.

Case-patient 3

This child, the younger sibling of case-patient 2, arrived in Australia with his family in 2010 when he was 5 years 9 months of age. At arrival screening, he was anti-HIV-1 negative and anti-HCV positive. He had attended the clinic in Andijan in November 2007 for "management" of jaundice, presumably associated with hepatitis A infection acquired from his sibling (case-patient 2), who had contracted hepatitis A and received the described treatment ≈40 days before case-patient 3 received his. Case-patient 3 was in the Andijan clinic for 10 days and received daily injections (the nature of which is unknown)–presumably the source of the HCV infection. When first seen at WCH on referral, he was asymptomatic. There were no abnormal physical findings. He was repeatedly anti-HCV positive and HCV RNA negative, indicating resolved infection.

Case-patient 4

This boy was born in Andijan in 2002. Immigration serology screening was not performed. He was first seen at WCH as a refugee in 2008 at 6 years of age after his general practitioner found him to be anti-HCV positive (anti-HIV-1 and HBsAg testing was not performed at the time). His mother was anti-HCV negative. He was born at 38 weeks' gestation and had some neonatal problems for which he received multiple intravenous injections and

Table. Presumptive iatrogenic BBV infections in refugee children from Uzbekistan, Australia, 2008–2010*

Case-patient	BBV Infection	Age, y/sex	Risk factor	Year of diagnosis	Maternal serostatus	Sibling serostatus
1	HIV/HBV	8/F	Blood transfusion, plasma transfusion	2010	Anti-HIV–, HBsAg+	Seronegative (2 sibs)
2†	HIV	9/M	Possible exposure to nonsterile injections	2010	Anti-HIV–	Anti-HBs+, anti-HCV+
3†	HCV	5/M	Possible exposure tononsterile injections	2010	Anti-HCV–	Anti-HIV+, anti-HCV–
4	HCV/HIV	6/M	Blood transfusion; IV; possible exposure to nonsterile injections	2008; 2012, respectively	Anti-HCV–, anti-HIV–	Anti-HCV–, anti-HIV– (1 sib)

*BBV, blood-borne virus; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; +, positive; –, negative; HCV, hepatitis C virus; IV, intravenous.
†Case-patients 2 and 3 are siblings.

blood transfusion(s) in an Andijan hospital. His 2 younger siblings were anti-HCV negative. Examination findings were normal except for serious dental caries requiring extractions. His liver function showed slightly elevated alanine aminotransferase (50 U/L [reference value 5–45 U/L]). Initially, nonquantitative HCV RNA was detectable in his peripheral blood, and in early 2011, his viral load was 623 IU/mL, at which time his liver function was entirely normal. In 2012, because of rising alanine aminotransferase (57 U/L) and increasing viral load (206,000 IU/mL), he was referred to a pediatric gastroenterologist, who noticed that anti-HIV and HBsAg/antibody tests had never been performed; results were positive for anti-HIV-1 (HBsAg and anti-HBs negative). The patient's viral load was 18,300 RNA copies/mL. His CD4 count was 810 cells/μL (27%). Examination indicated no abnormality. His siblings and parents are anti-HIV negative.

Discussion of Cases

Vertical transmission of the viruses involved in these 4 cases was ruled out by the mothers' negative serostatus, as well as by exclusion based on history of surrogate breastfeeding and sexual abuse. Notwithstanding a small risk for horizontal transmission, the most likely mode of transmission is iatrogenic. Incomplete documentation made other cases speculative. One case-patient had an additional risk factor of rape. Three others with HBV infection in whom the serostatus of the mothers was tested and had revealed anti-HBs positivity could imply vertical transmission with maternal infection resolution and natural immunity. The presumptive cases demonstrate intended or unintended effects of nonfunctional infection control in the chaos left by political collapse and/or civil war on health systems and the blood supply. Such recent events have affected Central Asian countries, including Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and to a lesser extent, Uzbekistan (*10*–*14*). Patients with speculative cases were from several sub-Saharan African countries, including Ethiopia, Kenya, Somalia, Liberia, and Ghana.

The extent of the problem remains unknown. A prospective study of 1,026 child refugees conducted in a tertiary pediatric health unit in Western Australia (March 2006– December 2008) showed an incidence of hepatitis B of 8.1% with no cases of HIV or HCV infection (*3*). The study did not report cases of iatrogenic transmission. The literature examining iatrogenic BBV infection is limited, but conflict and war clearly go hand in hand with increased prevalence of BBVs, especially in women and children. The cases reported here may represent the "tip of the iceberg." Cases showing up in 1 city in Australia might or might not be an isolated event. The absence of conclusive data prevents a conclusion about whether these cases are isolated events or represent a widespread problem, but the cases provide a stimulus for reception countries to be alerted to a possible problem.

The 4 case-patients illustrate the price paid by children caught up in the effects of social disintegration. Such situations provide opportunities for sexually transmitted infections (including HIV) to increase in prevalence (through prostitution and intravenous/injection drug use) and therefore form an environment for possible iatrogenic transmission. Hamers and Downs (*15*) indicated the extent of the problem. Escalating intravenous/injection drug use, which highlights Central Asia's geographic position along major drug-trafficking routes, has led to a corrupted blood supply. These upheavals seem to have formed the nidus of the documented epidemic of HIV in Central Asia (*16*). In Shymkent, 93 children contracted HIV either directly or indirectly through blood transfusion (*17*). Transmission of HIV to babies and children by medical personnel through unscreened blood transfusion has been reported in Kazakhstan and Kyrgyztan in 2007 and 2008, respectively (*18*).

Zahed (*3*) described the situation for children worldwide and pointed to Central Asia as a major problem area, particularly for spread of HIV infection. Thorne et al. (*16*) indicated that Uzbekistan is one of several hot spots for HIV transmission in Central Asia, with a few HIV cases in children having been reported, most of which resulted from nosocomial outbreaks in hospitals (*16*). No data indicate whether refugees are at higher or lower risk for BBV infection than others living in conflict zones. Nevertheless, the following modes of transmission could, arguably, inevitably increase the background prevalence of BBVs and, by inference, increase the risk for children living in these high-risk areas. Some of these children will reach reception countries as refugees or orphans.

Modes of Transmission Contributing to Increased BBV Prevalence

Contaminated Injections in Health Care Settings

According to the US Agency for International Development, "consecutive wars have made it nearly impossible to conduct effective and sustainable HIV prevention activities" (*19*). The World Health Organization estimated that 17%–19% of injections performed in sub-Saharan Africa during 2000 were administered unsafely (*20*). Simonsen et al. reviewed injection practices in the developing world with disturbing findings (*21*).

HIV and AIDS in Africa

Iatrogenic spread of HIV through inadequate screening facilities and improper use of needles and syringes by impoverished and undertrained health care workers puts patients at risk (*22*). Twenty percent of blood transfusions in 3 hospitals in Ashanti, Ghana, were unscreened (*23*).

According to Purdin et al. (*24*), conflict and HIV dramatically intersect in sub-Saharan Africa. The 22.5 million HIV-infected persons in sub-Saharan Africa contain ≈90% of HIV-positive children and 68% of HIV-positive adults infected worldwide. Twenty-one sub-Saharan African countries have ongoing political conflict. Within their borders, as recorded in 2007, are 77% (1.6 million) of the world's AIDS-related deaths (*25–27*).

Jenkins and Robalino (*28*) alluded to the hidden epidemic in Middle Eastern and North African countries affected by war and internal strife where no epidemiologic monitoring occurs and where iatrogenic transmission thus remain undetected. The rise in HIV prevalence for each country affected by poverty or internal political strife and dysfunctional medical services directly correlates with these situations (*28*).

HCV in Africa

In southern Cameroon, where west-central African chimpanzee strains of simian immunodeficiency virus, the source of HIV-1 group M, is prevalent among wild chimpanzees, ≈50% of some human birth cohorts were infected with HCV through unclear mechanisms (*29*) but suggesting high levels of iatrogenic transmission (*30*). Some have speculated that this level of iatrogenic transmission jumpstarted the HIV/AIDS pandemic through injection treatment of trypanosomiasis before 1951 (*31*). Excess deaths among trypanosomiasis patients treated before 1951 support this hypothesis (*32*).

Unsafe Injections and Blood Transfusions

The pandemic of non-A, non-B hepatitis (now attributed to HCV infection) emerged in the second half of the 20th century. Almost certainly it was triggered and fed iatrogenically by the increasing use of injections and blood transfusion. In industrialized countries, the introduction of anti-HCV screening for blood donors sharply decreased the incidence of iatrogenic hepatitis C, but HCV continues to spread in developing countries, where the virus is still transmitted through unscreened blood transfusions and nonsterile injections (*33*). Even in countries without conflict, failure to protect the blood supply still can occur, In western India, 23 children with thalassemia were reported as being positive for HIV infection after receiving blood transfusions during January–August 2011 (*34*).

Surrogate Breast-feeding and Feeding with Expressed Milk

In 2004, Shisana et al. (*35*) assessed blood and breastmilk exposures in children recruited from primary health clinics in the Free State province of South Africa and tested the children and their biological mothers for HIV infection. HIV positivity in children of HIV-negative mothers was associated with dental injections, surrogate breast-feeding, and feeding with expressed milk from a hospital milk room.

Other Possibilities

No data exist on sharing of contaminated equipment during self-injection in children and adolescents in countries of high BBV prevalence (*36*). Also, tattoos, skin piercing, and scarification are common in Africa and are detectable on examination.

Horizontal Infection Not Otherwise Specified

Information about modes of transmission in HIV-infected African children who have HIV-uninfected mothers is generally lacking. Since 1984, horizontally acquired HIV infection in African children who have HIV-negative mothers has been reported from 13 sub-Saharan countries (*37*). Injections related to health care provision or dental surgery (especially by informal providers) were more common in infected children, suggesting that horizontal HIV transmission through blood exposures is common in some sub-Saharan African countries.

In a 2005 serosurvey, Rehle et al. (*38*) estimated that the annual incidence of HIV infection was 0.5% in South African children 2–14 years of age. HIV infection in children who have HIV-negative mothers provided an estimate of horizontal HIV transmission. Medical injections, blood transfusion, and hospitalization almost certainly play a role; however, little is known about the extent and modes of horizontal HIV transmission in African children. Other contributors to the spread of BBVs include prostitution and rape in war; BBV-positive persons provide a link in the chain of infection that may lead to iatrogenesis.

Implications and the Future

Estimates of up to 160,000 HIV, 4.7 million HCV, and 16 million HBV infections each year are attributable to unsafe injections (*39*). Developing world conflict and maldistribution of resources remain major contributors to the prevalence of BBV infection and affect the poor, the young, and the victims of rape in war. Preventing this is a challenge for resource-rich countries. A 2002 report that seems to have preceded the HIV epidemic in Central Asia, including Uzbekistan, indicated that the situation was ripe for an epidemic among children (*40*). This report illustrates the failure of United Nations to effectively address the drug problem and epidemics of BBVs. The "War on Drugs" has clearly failed and seems to enhance the effectiveness of drug barons and warlords in promulgating their trade. The practice of evidence-based medicine would resolve to abandon this wasteful and ill-conceived war. How industrialized countries can help is problematic. The World Health Organization has insufficient resources to monitor and maintain prevention programs. The affected countries

have multitudinous difficulties, including providing food, water, and sanitation. Infection control competes with these and other priorities. The protection of vulnerable children thus remains unaddressed.

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Dr Goldwater is a clinical microbiologist and infectious diseases physician whose responsibilities include the management of HIV in pregnancy and most of the children with HIV in South Australia. His scientific interests include the pathogenesis of sudden infant death syndrome, cerebral palsy, and hemolytic uremic syndrome.

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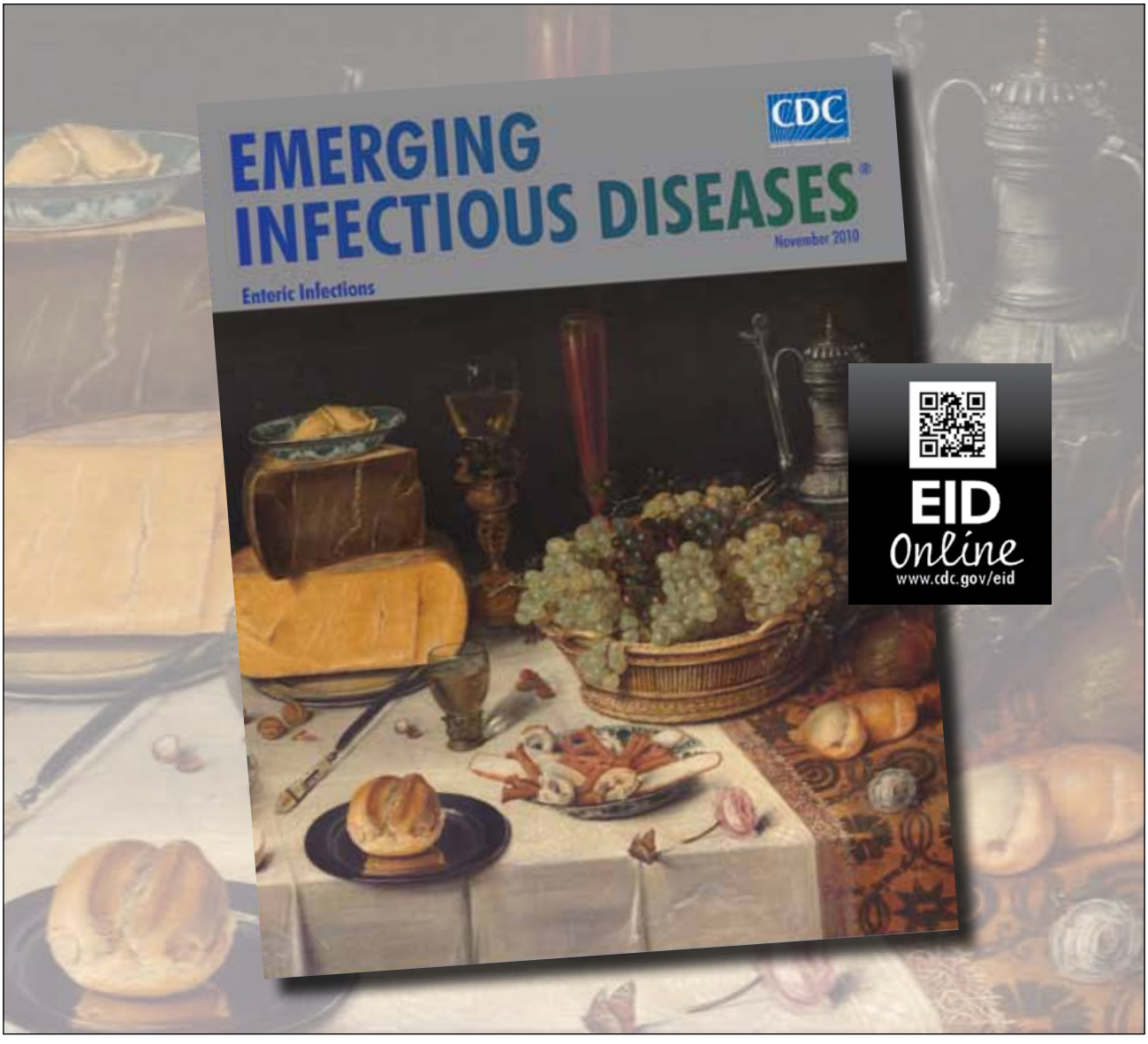

Zoonotic *Mycobacterium bovis*–induced Tuberculosis in Humans

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We aimed to estimate the global occurrence of zoonotic tuberculosis (TB) caused by *Mycobacterium bovis* or *M. caprae* infections in humans by performing a multilingual, systematic review and analysis of relevant scientific literature of the last 2 decades. Although information from many parts of the world was not available, data from 61 countries suggested a low global disease incidence. In regions outside Africa included in this study, overall median proportions of zoonotic TB of ≤1.4% in connection with overall TB incidence rates ≤71/100,000 population/year suggested low incidence rates. For countries of Africa included in the study, we multiplied the observed median proportion of zoonotic TB cases of 2.8% with the continental average overall TB incidence rate of 264/100,000 population/year, which resulted in a crude estimate of 7 zoonotic TB cases/100,000 population/year. These generally low incidence rates notwithstanding, available data indicated substantial consequences of this disease for some population groups and settings.

Tuberculosis (TB) is among the most devastating human infectious diseases worldwide. An estimated 8.8 million new cases, a global average incidence rate of 128/100,000 population/year, and 1.5 million deaths were attributed to TB in 2010 (*1*). Human TB is caused principally by *M. tuberculosis*. The main causative agents of bovine TB are *M. bovis* and, to a lesser extent, *M. caprae*; however, zoonotic transmission of these pathogens is well described and occurs primarily through close contact with infected cattle or consumption of contaminated animal products such as unpasteurized milk (*2*,*3*). TB cases caused by transmission of other mycobacteria from other animal reservoirs (e.g., wildlife) have been anecdotally reported (*4*,*5*). Globally, most cases of zoonotic TB are caused by *M. bovis*, and cattle are the major reservoir (*2*,*3*). Therefore, for the purpose of this study and the remainder of this report, we refer to zoonotic TB as TB in humans caused by *M. bovis* or *M. caprae*.

There is evidence to suggest that zoonotic TB accounted for a significant proportion of the TB cases in the Western world before the introduction of regular milk pasteurization programs (*6,7*). Currently, in high-income countries, bovine TB is well controlled or eliminated in most areas, and cases of zoonotic TB are rarely seen (*6*,*7*). However, reservoirs of TB in wildlife populations have been linked to the persistence or increase of the incidence of bovine TB in some countries, most notably the United Kingdom (UK) (*6*). The absence of zoonotic TB despite an upsurge in the incidence of bovine TB in the United Kingdom sparked a controversy over the large financial expenditures for disease control in cattle (*6*).

The situation may be fundamentally different in other regions. For example, in most countries in Africa, bovine TB is prevalent, but effective disease control, including regular milk pasteurization and slaughterhouse meat inspection, is largely absent (*2*,*3*). This situation is exacerbated by the presence of multiple additional risk factors such as human behavior and the high prevalence of HIV infections (*2*,*3*,*7*). Although HIV/AIDS is thought to facilitate transmission and progression to active disease of any form of TB, some studies showed a significantly increased proportion of *M. bovis* infections among HIV–co-infected TB patients compared with HIV-negative TB patients (*8*–*12*).

No assessment of the global consequences of zoonotic TB has yet been done. This may have been partially caused by the difficulty of differentiating TB caused by *M. tuberculosis* or *M. bovis*, which requires mycobacterial culture and the subsequent use of biochemical or molecular

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Table 1. Number of countries within World Health Organization regions for which the respective data on zoonotic *Mycobacterium bovis*–induced TB in humans was obtained*

Variables	No. (%) of countries by WHO region				
	Africa	Americas	Europe	Eastern Mediterranean	Western Pacific
Total number of countries in WHO region†	46 (100.0)	35 (100.0)	53 (100.0)	22 (100.0)	27 (100.0)
Any data for countries of WHO region indicated‡	13 (28.3)	12 (34.3)	31 (58.5)	2 (9.1)	3 (11.1)
Nationwide data	0	10 (83.3)	30 (96.8)	0	2 (66.7)
Subnational data	13 (100.0)	5 (41.7)	11 (35.5)	2 (100.0)	2 (66.7)
Surveillance data	6 (46.2)	5 (41.7)	31 (100.0)	1 (50.0)	3 (100.0)
Convenience sample/unknown sampling strategy	10 (76.9)	11 (91.7)	6 (19.4)	1 (50.0)	2 (66.7)
Nationwide surveillance data	0	2 (16.7)	30 (96.8)	0	2 (66.7)
Molecular-based detection of *M. bovis*	10 (76.9)	7 (58.3)	9 (29.0)	2 (100.0)	3 (100.0)
Biochemical detection of *M. bovis*	6 (46.2)	3 (25.0)	4 (12.9)	0	1 (33.3)
Other detection method	1 (7.7)	3 (25.0)	3 (9.7)	0	1 (33.3)
Unknown detection method	0	10 (83.3)	30 (96.8)	0	2 (66.7)
Data on average yearly prevalence	9 (69.2)	6 (50.0)	5 (16.1)	0	2 (66.7)
Data on average yearly prevalence per 100,000 population	0	0	0	0	0
Data on average yearly incidence	6 (46.2)	10 (83.3)	31 (100.0)	1 (50.0)	3 (100.0)
Data on average yearly incidence per 100,000 population	2 (15.4)	7 (58.3)	30 (96.8)	1 (50.0)	2 (66.7)
Data on average yearly mortality	0	1 (8.3)	2 (6.5)	0	0
Data on average yearly mortality per 100,000 population	0	1 (8.3)	0	0	0
Data on proportion among all TB cases	13 (100.0)	12 (100.0)	30 (96.8)	2 (100.0)	3 (100.0)
Data on proportion among all TB deaths	0	1 (8.3)	0	0	0
Data on proportion of deaths among all TB cases	0	1 (8.3)	2 (6.5)	0	0

*TB, tuberculosis; WHO, World Health Organization; Freq, Number of countries represented by records reporting on the indicated variable; %, proportions related to the total number of countries in a given WHO region for which data were available (unless otherwise indicated).
†No data were obtained for Southeast Asia.
‡Proportions relate to the total number of countries in the respective WHO region.

(e.g., genotyping) diagnostic methods. Therefore, in low-income countries, facilities to identify the causative agent of TB are largely absent (*2*,*3*,*7*). A previous comprehensive review on zoonotic TB was published 15 years ago with inferences based primarily on the presence of risk factors rather than the occurrence of actual cases (*2*). Since then, several studies of zoonotic TB in different parts of the world have been published, enabling a more detailed evaluation of the current importance of the disease. The current study was mandated by the World Health Organization (WHO) Foodborne Disease Burden Epidemiology Reference Group with the aim to determine, on the basis of previously published literature, the global occurrence of zoonotic TB and its contribution to the overall TB prevalence in affected settings.

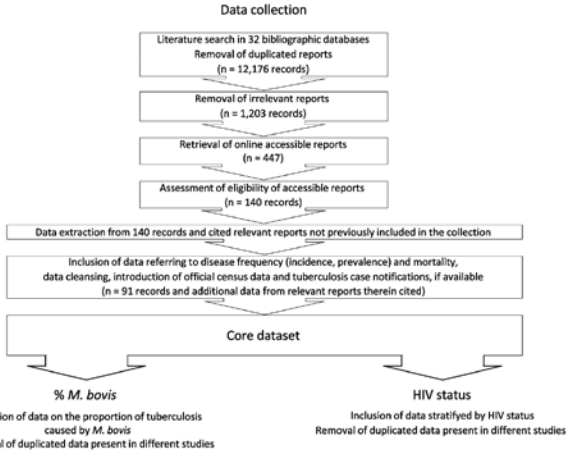

Figure 1. Selection procedure for reports included in this analysis. A list of all identified 1,203 potentially relevant reports and the core dataset is available as supplemental material (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/ 19/6/12-0543-Techapp2.xlsx). TB, tuberculosis; *M. bovis, Mycobacterium bovis*.

Materials and Methods

A systematic multilingual literature search was performed according to international guidelines with certain modifications (www.cochrane-handbook.org/). Potentially relevant reports on putative zoonotic TB caused by *M. bovis* or *M. caprae* were identified by a search of 32 bibliographic databases by using a highly sensitive search syntax. All publications/reports documented in the various databases and published until March 2010 were considered (Table 1, online Technical Appendix 1, wwwnc.cdc.gov/EID/article/19/6/12-0543-Techapp1.pdf, and online Technical Appendix 2, wwwnc.cdc.gov/EID/article/19/6/12-0543-Techapp2.xlsx). Reference Manager v11.0.1 bibliographic software was used to store and remove duplicated reports, leaving 12,176 records (Figure 1). Titles and abstracts of these reports were screened to remove studies unlikely to contain pertinent information. Altogether, 1,203 potentially relevant reports were identified (online Technical Appendix 1, 2) of which 447

(37%) were available online and assessed for eligibility (Figure 1).

Eligible records (written in English, French, German, Spanish, or Portuguese) reported data for at least 50 persons tested on the frequency (prevalence, incidence) or death rate of putative zoonotic TB and contained data from no earlier than 1990. In connection with other ongoing studies, records reporting information on disease sequelae and transmission routes were also included. For 100 randomly selected reports of the 1,203 potentially relevant reports, availability and eligibility was assessed independently by 3 operators. Differently appraised records were reassessed, and the screening procedure was harmonized accordingly. The remaining records were randomly assigned to 1 of the 3 individual operators for further assessment; 140 records were considered eligible and subjected to data extraction. The data were stratified by multiple variables, if possible (e.g., country or province, HIV status). If any of the reports included referred to relevant external data or eligible reports that were not identified during the earlier steps of our literature search, the respective data were also included in this analysis. For 15 randomly selected reports of the 140 eligible records and additional data cited by or referring to these records, data extraction was performed independently by the 3 operators. Differently extracted data was reassessed and the extraction procedure was harmonized accordingly. The remaining records were randomly assigned to one of the 3 operators for data extraction. Of 140 eligible records, 91 reported or referred to data on disease frequency or mortality rates of putative zoonotic TB (online Technical Appendix 1 Figure 2). If accessible, additional population estimates and official TB notifications were included in the database. This core dataset was used to analyze, by geographic region and country, the global occurrence of zoonotic TB in humans and its contribution to the overall TB prevalence in affected settings. A subset of data stratified by HIV status was used to assess a potential association between TB caused by *M. bovis* and HIV co-infection. For each of these analyses, duplicated data, present in >1 report was removed from the core dataset (Figure 1). Statistical analyses were performed in IC Stata 10.0 (StataCorp LP, College Station, TX, USA).

Results

Data Availability

Data obtained from eligible reports covered 5 of 6 WHO regions (Table 1; online Technical Appendix 1 Table 1), namely Africa, the Americas, Europe, the Eastern Mediterranean and the Western Pacific. No data were obtained for any country in Southeast Asia. With the exception of Europe, data were acquired for only a few countries of the regions represented in this analysis (online Technical Appendix 1 Table 1). Notably, for the Eastern Mediterranean and the Western Pacific regions, respectively, data from only 2 and 3 countries were obtained. The considerable lack of data precluded a credible estimation of the global prevalence and incidence of zoonotic TB.

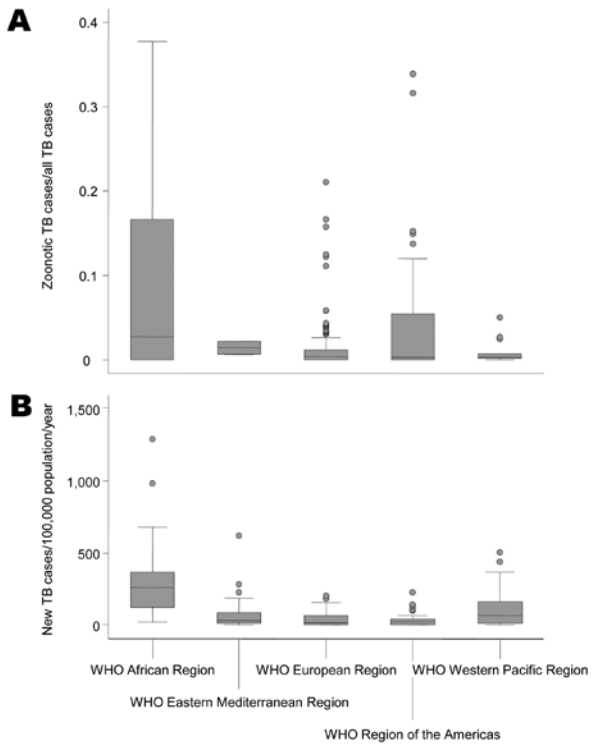

Figure 2. Proportion of zoonotic tuberculosis (TB) among all TB cases (A) and estimated overall TB incidence (B) stratified by World Health Organization (WHO) region. Overall TB incidence rates estimated for 2010 by WHO regions covered all countries of the respective regions. The lower end of each gray box represents the 25th percentile, and the upper end represents the 75th percentile; horizontal lines inside the gray boxes represent the median. Whiskers indicate upper and lower adjacent values; circles indicate outliers. Numbers were obtained from WHO (1).

Zoonotic TB in Africa

Among all studies included from Africa, a median of 2.8% (range 0%–37.7%) of all TB cases in humans were caused by *M. bovis* (Figure 2). In 10 of the 13 countries in Africa included in this study, median proportions of TB caused by *M. bovis* were below 3.5% and in 5 of these countries no cases were detected (Table 2; Figure 3). In contrast, for Ethiopia, Nigeria and Tanzania, respectively, the median proportion of TB cases caused by *M. bovis* was 17.0% (range: 16.7%–31.4%) (*13*–*15*), 15.4% (1 study available) (*16*) and 26.1% (range 10.8%–37.7%) (*17*–*19*). Percentages of ≈30% were reported in 4 regionally based studies in Tanzania and Ethiopia (Figure 3, Table 2) (*13*,*17*). However, many of these studies showed a low sample size, resulting in a high statistical error (Table 2). The lack of large-scale, population-based data did not

Table 2. Overview of selected reports from Africa on zoonotic *Mycobacterium bovis*–induced TB in humans*

ID	Country	Setting	Sampling	Detection	Years	*M. bovis*†	TB‡	%§	95% CI	Location
1131	Tanzania	NA	Conv.	Mol.	NA	20	53	37.7	24.8–52.1	EPTB
1131	Tanzania	Arusha region	Conv.	Mol.	1994	4	11	36.4	10.9–69.2	EPTB
1074	Ethiopia	Butajira health center, southeastern Ethiopia	System.	Mol.	2000/2001	11	35	31.4	16.9–49.3	EPTB
1074	Ethiopia	NA	Conv.	Biochem.	NA	14	48	29.2	17.0–44.1	PTB
15	Ethiopia	Felegehiwot hospital	Conv.	Biochem.	2007/2008	8	47	17.0	7.6–30.8	Both
65	Ethiopia	NA	Conv.	Biochem.	NA	7	42	16.7	7.0–31.4	Both
1074	Ethiopia	Fitche Hospital TB clinic	Conv.	Biochem.	2004/2005	7	42	16.7	7.0–31.4	Both
294	Tanzania	Arusha region and Southern Highlands	Surv.	Biochem.	1993–1996	7	44	15.9	6.6–30.1	Both
73	Nigeria	Jos	Conv.	Biochem.	NA	10	65	15.4	7.6–26.5	PTB
68	Uganda	Karamojo region	Conv.	Mol.	NA	3	24	12.5	2.7–32.4	EPTB
159	Tanzania	Three districts in Arusha region	Surv.	Biochem.	1999–2001	7	65	10.8	4.4–20.9	EPTB
459	Malawi	Blantyre, Queen Elizabeth Central Hospital	Conv.	Biochem.	NA	1	30	3.3	0.1–17.2	PTB
62	Ghana	Korle-Bu Teaching Hospital	Conv.	Biochem.	2003	2	64	3.1	0.4–10.8	PTB
464	Madagascar	Institut d'Hygiène Sociale, Antananarivo	Conv.	Mol.	1994	3	126	2.4	0.5–6.8	PTB
340	Madagascar	Antananarivo	Surv.	Other	1994/1995	2	156	1.3	0.2–4.6	EPTB
292	Madagascar	Antananarivo, Antsirabe, Fianarantsoa, and Mahajanga	Rand.	Mol.	1994/1995	4	316	1.3	0.3–3.2	PTB
243	Uganda	Kampala	Conv.	Biochem.	1995–1997	1	234	0.4	0.0–2.4	PTB
52	Uganda	Kampala, Rubaga division	Surv.	Mol.	2006	1	386	0.3	0.0–1.4	PTB
222	Cameroon	Ouest Province	Surv.	Mol.	1997/1998	1	455	0.2	0.0–1.2	PTB
80	Burkina Faso	Health centers in Ouagadougou and Bobo Dioulasso	Conv.	Mol.	2001	0	120	0.0	0.0–3.0¶	PTB
12	Burundi	Bujumbara, Bubanza Hospital	Conv.	Mol.	1987–1994	0	117	0.0	0.0–3.1¶	Both
165	Chad	Chari-Baguirmi	Conv.	Mol.	2002	0	10	0.0	0.0–30.8¶	Both
12	Côte d'Ivoire	TB and rural health centers in Côte d'Ivoire	Cluster	Mol.	1994–1996	0	320	0.0	0.0–1.1¶	PTB
464	Madagascar	Antananarivo prison	Conv.	Mol.	1994	0	36	0.0	0.0–9.7¶	PTB
12	Sierra Leone	Western Area and Kenema districts	Surv.	Mol.	2003/2004	0	97	0.0	0.0–3.7¶	PTB
32	Uganda	Mbarara district	Rand.	Mol.	2004/2005	0	70	0.0	0.0–5.1¶	Both

*TB, tuberculosis; ID, record identification number (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/19/6/12-0543-Techapp2.xlsx); NA, not available; Conv., convenience sample/not specified; Mol., molecular-based detection method; EPTB, extrapulmonary TB; System, systematic sampling; Biochem, biochemical detection method; PTB, pulmonary TB; Surv.,surveillance data; Rand., random sampling; Cluster, cluster sampling.
†Number of putative zoonotic cases of *Mycobacterium bovis*.
‡Total number of bacteriologically confirmed and characterized TB cases.
§%, proportion of zoonotic TB among all TB cases.
¶One-sided 97.5% CIs are indicated.

allow for an identification of specific risk groups associated with *M. bovis* infections.

Zoonotic TB in the Americas

A median of 0.3% (range 0%–33.9%) of *M. bovis* infections among human TB cases was found for all reports included. For most countries, *M. bovis* accounted for a negligible percentage of the TB cases (Figure 2). Conversely, high proportions were reported for specific areas of Mexico and the United States (Figure 4). For Mexico, the median percentage of *M. bovis* cases was 7.6% (range 0%–31.6%); proportions >10% were detected in 3 independent studies (*20*–*22*). However, overall TB incidence in Mexico is relatively low, with a rate of 16/100,000 population/year (*1*). In the United States, TB caused by *M. bovis* is strongly linked to persons in Hispanic communities, mostly with origins in Mexico (Table 3). A study including data from 41 states of the United States suggested that ≈90% of all TB cases caused by *M. bovis* affect persons of Hispanic ethnicity (*8*). This association is attributed to the consumption of unpasteurized, contaminated cheese produced in Mexico. Moreover, when multivariate logistic regression analyses was used, several studies in the United States showed an independent association of TB caused by *M. bovis* with TB cases in children, HIV coinfection and extrapulmonary disease (*8*–*12*). Surveys in San Diego County, California indicated a steady increase in the incidence of TB caused by *M. bovis* and a decrease in TB incidence caused by *M. tuberculosis* infection (*9*,*12*). In this setting, the odds for TB patients

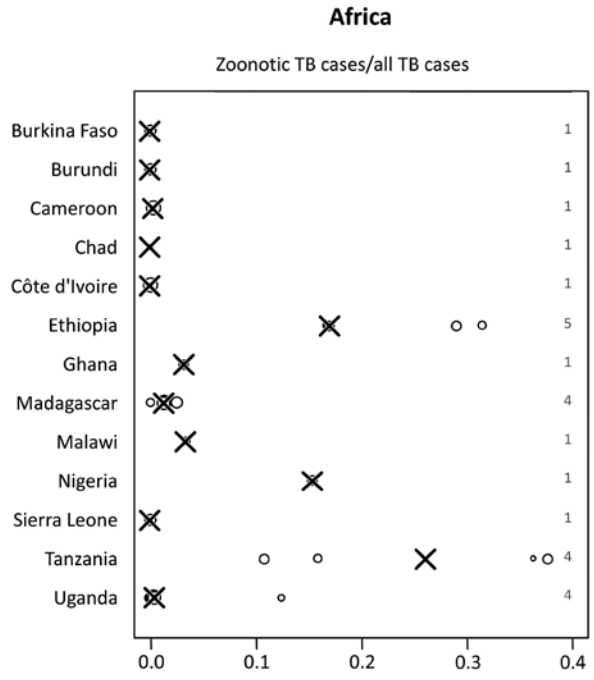

Figure 3. Proportion of zoonotic tuberculosis (TB) among all TB cases stratified by country: Africa. X-axis values are proportion of zoonotic TB cases among all TB cases. Each circle represents a study with the circle diameter being proportional to the $\log_{10}$ of the number of isolates tested. A gray rhombus indicates that the number of samples tested was not reported or could not be inferred from the data available. The median proportion of all studies for a given country is indicated by X. Numbers on the right side of the figures indicate the number of studies included for any given country.

infected with *M. bovis* to die during treatment was >2 times as high as for patients infected with *M. tuberculosis* (*9*,*10*,*12*). In San Diego County, during 1994–2003 and 2001–2005, respectively, *M. bovis* accounted for 25 and 19 deaths, corresponding to 27% and 17% of all TB deaths and a mortality rate of ≈0.1/100,000 population/year (*9*,*10*). The reasons for increased deaths among patients infected with zoonotic TB compared with those infected with *M. tuberculosis* remained unidentified, although health care inequality or treatment differences were stated as possible explanations (*9*). However, overall incidence rates of zoonotic TB in the United States are low, at a median of 0.7/100,000 population/year. Although zoonotic TB causes minor consequences of disease in the Americas, available data corroborate the finding that *M. bovis* infections can be a substantial cause of deaths from TB among humans in certain population groups and settings.

Zoonotic TB in Europe

Studies from Austria, Germany, Greece, and Spain included in this analysis identified *M. caprae* as a causative agent of zoonotic TB in addition to *M. bovis* (*23*–*26*). Our analysis revealed a median proportion of 0.4% (range 0%–21.1%) of *M. bovis* or *M. caprae* infections among all bacteriologically confirmed TB cases reported. Median proportions for individual countries never reached rates >2.3%, although higher percentages were found for specific populations and settings (Figure 5). Three of 5 studies reporting proportions >10% were conducted in settings with very low incidences of human TB (<20 cases/year on a countrywide level) (*27*). In another hospital-based study in Germany, 4 *M. bovis* cases were identified among 19 TB cases with molecular speciation results, 2 of which probably represented disease caused by the treatment of urothelial carcinoma with *M. bovis* BCG (*28*). A study in Spain characterized the transmission of a multidrug-resistant strain of *M. bovis* as the cause of 2 nosocomial TB outbreaks that accounted for 12.2% of multidrug-resistant TB isolates (*29*). However, these cases did not represent cases of zoonotic TB, because transmission occurred from humans to humans. Reported incidence rates for TB caused by *M. bovis* or *M. caprae* for all studies included from European countries were <1/100,000 population/year if TB cases caused by multidrug-resistant strains of *M. bovis* in Spain were excluded (online Technical Appendix 2). Moreover, available data suggested decreasing trends in the number of zoonotic TB cases over time (online Technical Appendix 2).

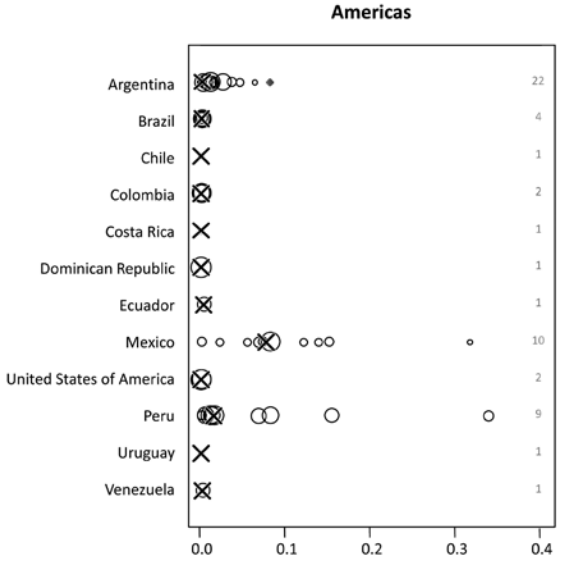

Figure 4. Proportion of zoonotic tuberculosis (TB) among all TB cases stratified by country: Americas. X-axis values are are proportion of zoonotic TB cases among all TB cases. Each circle represents a study with the circle diameter being proportional to the $\log_{10}$ of the number of isolates tested. A gray rhombus indicates that the number of samples tested was not reported or could not be inferred from the data available. The median proportion of all studies for a given country is indicated by X. Numbers on the right side of the figures indicate the number of studies included for any given country.

Table 3. Selected reports from the United States on zoonotic *Mycobacterium bovis*-induced TB in humans*

ID	Setting	Study period	Ethnicity or place of birth	No. cases†	No. tested‡	%§
46	41 states in the United States	1995–2005	Native American, non-Hispanic	0	243	0.0
			Asian, non-Hispanic	5	2,938	0.2
			Black, non-Hispanic	6	3,447	0.2
			Hispanic	147	2,724	5.4
			White, non-Hispanic	7	2,449	0.3
			Mexico-born	102	1,399	7.3
			Non-US/Mexico-born	15	4,907	0.3
			US-born	47	5,531	0.8
48	San Diego County, California	2001–2005	Hispanic	128	657	19.5
			White	3	154	1.9
			Asian	1	421	0.2
			Black	0	86	0.0
			Other	0	6	0.0
			Mexico-born	79	461	17.1
			The Philippines	0	248	0.0
			Non-US/Mexico/The Philippines-born	0	260	0.0
			US-born	53	355	14.9
164	San Diego County, California	1994–2003	Hispanic	156	531	29.4
			Non-Hispanic	11	564	2.0
			Mexico-born	93	374	24.9
			Non-US/Mexico-born	3	416	0.7
			US-born	71	305	23.3
155	New York, New York, USA	2001–2004	Mexico-born	20	155	12.9
			Non–Mexico-born	15	2925	0.5
233	San Diego County, California	1994–2000	Mexico-born	70	553	12.7
			The Philippines	1	423	0.2
			Non-US/Mexico/The Philippines-–born	2	403	0.5
			US-born	56	552	10.1

*TB. tuberculosis; ID, record identification number (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/19/6/12-0543-Techapp2.xlsx).
†N number of zoonotic TB cases identified.
‡Number of TB case-patients tested for the presence of *M. bovis* infection.
§ Proportion of zoonotic TB among all TB cases.

Zoonotic TB in the Eastern Mediterranean

One study from the Suez Canal region of Egypt reported a rate of 2.2% (95% CI 0.1%–11.8%) for pulmonary TB cases caused by *M. bovis* (*30*). A second nationwide study in Djibouti detected 0.6% (95% CI 0.0%–3.5%) of the TB lymphadenitis cases for which samples were tested to be caused by *M. bovis* (*31*). No other studies were obtained for the WHO region of the Eastern Mediterranean. The proportion of zoonotic TB among all TB cases in the eastern Mediterranean is shown in Figure 6.

Zoonotic TB in the Western Pacific

Data obtained from the Western Pacific region was from Australia, New Zealand, and parts of China only. For these settings, respectively, median proportions of *M. bovis* infections of 0.2% (range 0.1%–0.7%), 2.7% (range 2.4%–5%) and 0.2% (range 0%–0.5%) among all TB cases analyzed were observed, indicating that zoonotic TB had minor importance Median incidence rates were 0.03 (range 0.00–0.60) and 0.16 (range 0.11–0.27)/100,000 population/year for Australia and New Zealand, respectively. The infrequent occurrence of zoonotic TB in these settings notwithstanding, New Zealand showed a generally higher proportion and incidence rate for TB caused by *M. bovis* than did Australia (Figure 6). While a steadily increasing proportion of TB caused by *M. bovis* was observed in New Zealand, trends were decreasing in Australia (online Technical Appendix 2).

Influence of HIV Co-infection

Seven surveys covering Ethiopia, Tanzania, Argentina, Mexico, and different parts of the United States provided data for analysis of a potential association between HIV co-infection and zoonotic TB (Table 4) (*8–10*,*13*,*18*,*20*,*32*). Among these studies, only studies from the United States showed a significantly higher proportion of TB caused by *M. bovis* for HIV co-infected TB patients (Table 4). For studies from the USA, the relative risk for an infection with *M. bovis* among TB patients was 2.6–8.3 times higher in HIV–co-infected patients than in HIV-negative patients. No significant association between *M. bovis* infection and HIV status was identified in surveys in Africa or other countries of the Americas (Table 4).

Discussion

Naturally, the occurrence of zoonotic TB is greatly dependent on the presence of TB in cattle. Information on the global distribution and prevalence of bovine TB

is scarce, but available data suggest that TB in cattle is prevalent in virtually all major livestock-producing countries of the developing world and Africa, specifically (*2*,*3*,*7*). Disease control in cattle is largely absent in these regions. Consequently, the majority of the human population is at risk for exposure to bovine TB; and globally, the occurrence of zoonotic TB likely mirrors TB prevalence in cattle.

Figure 5. Proportion of zoonotic tuberculosis (TB) among all TB cases stratified by country: Europe. X-axis values are are proportion of zoonotic TB cases among all TB cases. Each circle represents a study with the circle diameter being proportional to the $\log_{10}$ of the number of isolates tested. A gray rhombus indicates that the number of samples tested was not reported or could not be inferred from the data available. The median proportion of all studies for a given country is indicated by X. Numbers on the right side of the figures indicate the number of studies included for any given country.

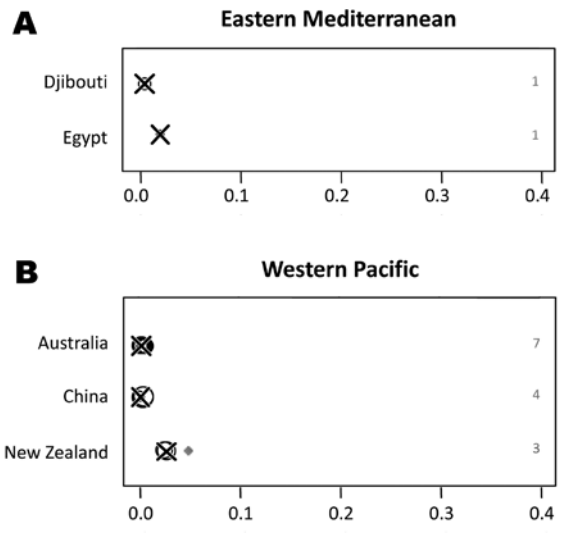

Figure 6. Proportion of zoonotic tuberculosis (TB) among all TB cases stratified by country: A) Eastern Mediterranean; B) Western Pacific. X-axis values are are proportion of zoonotic TB cases among all TB cases. Each circle represents a study with the circle diameter being proportional to the $\log_{10}$ of the number of isolates tested. A gray rhombus indicates that the number of samples tested was not reported or could not be inferred from the data available. The median proportion of all studies for a given country is indicated by X. Numbers on the right side of the figures indicate the number of studies included for any given country.

Our systematic literature search on the occurrence of zoonotic TB provided no data for the WHO region of Southeast Asia, including major cattle producing middle- and low-income countries (e.g., India, Bangladesh, Pakistan, Myanmar, Indonesia) (online Technical Appendix 1) (*33*). Moreover, except for Europe, data were acquired for few countries of the regions represented in this analysis (Table 1) with some, in terms of livestock production, particularly relevant countries missing (e.g., Canada, Kenya, Russia, South Africa, Sudan, and Turkey) (*33*). Nationwide surveillance data were almost exclusively available for high-income countries that have programs in place for bovine TB control and regular milk pasteurization (Table 1). Although ample data were obtained for many low-risk, high-income countries, the lack of nationwide surveys in potential high-risk settings precluded a credible estimation of the global occurrence of zoonotic TB.

Recorded incidence rates for zoonotic TB in Europe, the United States, Australia, and New Zealand were consistently below 1/100,000 population/year (online Technical Appendix 2). Incidence rates were unavailable for other countries. However, a crude estimate could be obtained by

Table 4. Available data on zoonotic *Mycobacterium bovis*–induced TB in humans, stratified by HIV-positive and -negative persons*

ID	Region	Country	HIV status	No. cases	No. tested	%	95% CI	RR
1074	Africa	Ethiopia	+	4	10	40.0	12.2–73.8	1.43
1074	Africa	Ethiopia	–	7	25	28.0	12.1–49.4	NA
1147	Africa	Tanzania	+	2	29	6.9	0.8–22.8	0.50
1147	Africa	Tanzania	–	5	36	13.9	4.7–29.5	NA
NA	Africa	Total	+	6	39	15.4	5.9–30.5	0.78
NA	Africa	Total	–	12	61	19.7	10.6–31.8	NA
57	Americas	Argentina	+	2	240	0.8	0.1–3.0	0.88
57	Americas	Argentina	–	95	10,000	1.0	0.8–1.2	NA
57	Americas	Argentina	+	8	1,391	0.6	0.2–1.1	2.66
57	Americas	Argentina	–	12	5,551	0.2	0.1–0.4	NA
39	Americas	Mexico	+	11	80	13.8	7.1–23.3	1.90
39	Americas	Mexico	–	6	83	7.2	2.7–15.1	NA
46	Americas	USA	+	21	891	2.4	1.5–3.6*	2.59
46	Americas	USA	–	59	6,472	0.9	0.7–1.2*	NA
48	Americas	USA	+	33	140	23.6	16.8–31.5*	3.24
48	Americas	USA	–	48	659	7.3	5.4–9.5*	NA
164	Americas	USA	+	39	64	60.9	47.9–72.9*	8.32
164	Americas	USA	–	35	478	7.3	5.2–10.0*	NA
NA	Americas	Total	+	114	2806	4.1	3.4–4.9*	3.70
NA	Americas	Total	–	255	23,243	1.1	1.0–1.2*	N/A

*TB, tuberculosis; ID, Record identification number (online Technical Appendix 2, wwwnc.cdc.gov/EID/article/19/6/12-0543-Techapp2.xlsx); Cases, number of zoonotic tuberculosis (TB) cases identified; Tested, number of TB cases tested for the presence of *M. bovis* infection; %, proportion of zoonotic TB among all TB cases; RR, relative risk ratio; NA, not available.
Significantly higher proportion of *M. bovis* infection among TB patients with HIV coinfection.

multiplying the observed country-specific median proportion of zoonotic TB by the respective overall TB incidence rates. This suggested incidence rates of zoonotic TB of ≈1/100,000 population/year or lower for all countries outside Africa included in this survey, except for the Republic of Djibouti (WHO Eastern Mediterranean Region) which reported ≈4 zoonotic TB cases/100,000 population/year.

Africa is assumed to bear the highest consequences of zoonotic TB worldwide because of the frequent and concurrent presence of multiple risk factors (*2*,*3*,*7*). This is supported by the highest reported median proportions of TB caused by *M. bovis* in connection with the worldwide highest overall TB incidence rates (Figure 2). Given an observed median proportion of zoonotic TB of 2.8% and the continental average overall incidence of TB of 264/100,000 population/year (Figure 2), an incidence rate of 7 zoonotic TB cases/100,000 population/year could be estimated (≈20 times lower than the global overall TB incidence rate) (*1*). Presumably, this is an overestimate as high-risk livestock producing countries of Africa (e.g., Ethiopia, Madagascar, Nigeria, and Tanzania) were overrepresented in this analysis (*33*) and because studies reporting the presence of zoonotic TB can be expected to be overrepresented among the surveys included (see limitations of the study below). Together, this suggests a low incidence of zoonotic TB in Africa.

Individual studies from various regions reported high proportions of zoonotic TB for specific population groups and settings (Figures 3–7). For example, in the Hispanic community in the United States, zoonotic TB appeared to be a considerable proportion of all TB cases (Table 3) and was associated with the consumption of unpasteurized cheese from Mexico (*8*–*12*). The highest median proportions for TB caused by *M. bovis* were observed in countries in Africa: Ethiopia, Nigeria, and Tanzania (Figure 3). However, the specific populations affected and risk factors of zoonotic TB in these settings remain largely elusive. The highest proportions of zoonotic TB in Africa were reported in studies investigating cases of extra-pulmonary TB (Table 2). For example, of a total of 26 studies, 11 studies reported proportions of zoonotic TB >10%; 9 of those included cases of extrapulmonary TB; of the 15 studies reporting a proportion of zoonotic TB <3.3%, only 4 included extrapulmonary TB cases (Table 2). This may mirror a widely stated association of zoonotic TB with extra-pulmonary disease, perhaps reflecting the consumption of contaminated animal products as one of the main drivers of zoontic TB (2,3,7). It has been postulated that pastoralist and rural communities would be at greatest risk for zoonotic TB (*2*,*3*,*7*), but the lack of data for these population groups prevents confirmation of this assumption. Collected individual studies reporting high proportions of TB caused by *M. bovis* suggest pockets of zoonotic transmission of TB for specific population groups and settings.

Outside Africa, large proportions of *M. bovis* infections among TB case-patients have been found mostly in low-TB incidence settings such as Mexico. In Cyprus, Iceland, and Malta, proportions of TB caused by *M. bovis* of >10% were observed (Figure 5); however, these countries, nationwide, reported <20 human TB cases in the respective years. Similarly, a study in San Diego County, California,

USA, showed an overall decreasing incidence of human TB while the incidence of zoonotic TB and therefore also the relative proportion of zoonotic TB has steadily increased (*9*,*12*). This suggests that commonly applied control efforts targeting *M. tuberculosis* transmission have little effect on the occurrence of zoonotic TB and probably reflects the distinct drivers of *M. tuberculosis* and zoonotic TB infection (e.g., aerosol transmission vs. foodborne infection) (*2*,*3*). Similarly, differences in the epidemiology of zoonotic TB are likely to exist between different regions. This could be mirrored by the association of zoonotic TB with HIV in the United States, but not in other areas included in this analysis (Table 4). Ascertaining the factors contributing to an association between HIV and zoonotic TB in some regions will require more in-depth research, thus eliminating potential confounders such as socioeconomic status, education level, national origin, and other factors.

The current study is affected by several biases. The sensitivity of this systematic literature review was affected by the selection of eligible reports and data extraction by a single operator. Also, only reports available online and written in English, French, German, Spanish, or Portuguese were included. Selected reports are biased toward surveys which identified or aimed to identify TB cases caused by *M. bovis*, possibly resulting in an overestimation of the proportion of zoonotic TB cases. Data from low-income countries included in this study were rarely comparable and not representative of the respective nationwide populations. Nonetheless, it seems unlikely that our conclusions were fundamentally affected by these biases. Lastly, our results are influenced by the technical constraints of the studies included. Specifically, biochemical methods may be relatively unreliable for the identification of *M. bovis* or *M. caprae* strains and routine culture methods for *M. tuberculosis* are suboptimal to detect strains of *M. bovis* (*2*,*3*). Thus, TB cases caused by *M. bovis* may be systematically underreported.

Reports published after the completion of this systematic review revealed information from countries not covered by this study. A study from Bangladesh analyzed isolates from 350 TB patients but did not identify any infections by *M. bovis* (*34*). In a study from Bamako, Mali, 0.8% of TB cases analyzed were caused by *M. bovis* (*35*). In Turkey and the West Bank, Palestine, respectively, 5.3% and 6.5% of clinical TB cases analyzed were caused by *M. bovis* (*36*,*37*); however, zoonotic TB can be considered rare in these areas, given the low overall incidence rates of TB of 28 and 0.7/100,000 population/year (*1*,*38*). Together, available data suggest a minor global importance of zoonotic TB. However, pockets of more frequent zoonotic transmission of TB seem to be present in certain population groups. More research is needed to identify the main transmission drivers in these areas.

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Group A *Streptococcus* Strains Circulating during Scarlet Fever Epidemic, Beijing, China, 2011

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Scarlet fever is one of a variety of diseases caused by group A *Streptococcus* (GAS). During 2011, a scarlet fever epidemic characterized by peak monthly incidence rates 2.9–6.7 times higher than those in 2006–2010 occurred in Beijing, China. During the epidemic, hospital-based enhanced surveillance for scarlet fever and pharyngitis was conducted to determine characteristics of circulating GAS strains. The surveillance identified 3,359 clinical cases of scarlet fever or pharyngitis. GAS was isolated from 647 of the patients; 76.4% of the strains were type *emm*12, and 17.1% were *emm*1. Almost all isolates harbored superantigens *speC* and *ssa*. All isolates were susceptible to penicillin, and resistance rates were 96.1% to erythromycin, 93.7% to tetracycline, and 79.4% to clindamycin. Because *emm*12 type GAS is not the predominant type in other countries, wider surveillance for the possible spread of *emm*12 type GAS from China to other countries is warranted.

Streptococcus pyogenes, also known as group A *Streptococcus* (GAS), is a common human pathogen that can induce a wide spectrum of diseases, ranging from noninvasive diseases, such as pharyngitis, scarlet fever, and impetigo, to invasive diseases, such as erysipelas, cellulitis, pneumonia, bacteremia, necrotizing fasciitis, and toxic shock syndrome. Moreover, GAS can cause rheumatic fever and acute poststreptococcal glomerulonephritis (*1*,*2*). In the late1980s, a change in the epidemiology of invasive GAS diseases and the emergence of streptococcal toxic shock syndrome were documented (*3*,*4*), and the current number of invasive GAS disease cases worldwide is high (*2*).

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Many virulence factors contribute to the pathogenesis of GAS diseases (*1*,*5*). However, the matrix (M) protein, encoded by the *emm* gene, has the most critical role, mainly by antiphagocytic mechanisms (*6*,*7*), and the amino-terminal region of M protein is the most promising target for designing a vaccine (*8*,*9*). *emm* gene sequencing is a standard method for typing the M protein (*10*), but the distribution of *emm* types varies greatly by geographic location, time, and collection site of clinical specimens (*9*,*11*–*14*).

Streptococcal pyrogenic exotoxins also play a major role in the pathogenesis of GAS infections by acting as superantigens. When these exotoxins cross-link major histocompatibility complex class II molecules and T cell receptors, they trigger intense activation of a subset of T cells within a specific b-chain variable region. This process induces a tremendous release of a series of cytokines and may lead to cell, tissue, and organ damage (*15*,*16*).

Several antimicrobial drugs effectively treat GAS infections (*1*). In recent years, however, considerable attention has been given worldwide to the issue of antimicrobial drug–resistant GAS. Macrolide-resistant GAS strains have been isolated from various regions of the world (*17*–*19*). Macrolides are used as an alternative treatment for GAS in patients allergic to penicillin, and clindamycin, in combination with b-lactam antimicrobial drugs, is a recommended treatment for invasive GAS disease (*20*). Thus, it is critical that surveillance for macrolide- and clindamycin-resistant GAS be continued.

In China, scarlet fever is the only GAS disease reported by the National Notifiable Infectious Disease Surveillance System (NNIDSS) (*21*). According to NNIDSS, the incidence of scarlet fever in Beijing, China, before 2011 had persistently remained within normal threshold

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limits. However, in late spring 2011, an epidemic of scarlet fever occurred in Beijing and many other regions of China. In response to the epidemic, enhanced surveillance for GAS diseases was conducted in Beijing during May–July 2011 to determine characteristics of the circulating GAS strains.

Methods

National Notifiable Infectious Disease Surveillance System

China established NNIDSS in 2004, after the 2003 outbreak of severe acute respiratory syndrome. At that time, NNIDSS covered 37 infectious diseases, which were classified into 3 categories (A–C), in a descending order according to disease severity; scarlet fever belonged to category B.

A clinical case of scarlet fever is defined as acute illness onset with fever, pharyngitis, and sandpaper-like red rash with or without strawberry tongue, Pastia lines, or circumoral pallor. In China, clinicians and hospitals are to report clinical cases of scarlet fever to NNIDSS within 6 hours of diagnosis. To respond to the 2011 epidemic of scarlet fever and to monitor its severity, each clinical case of scarlet fever reported in Beijing was followed for 3 weeks after the onset of disease.

Enhanced Surveillance for GAS Diseases

Enhanced surveillance for GAS diseases was conducted in the pediatric clinics of 36 hospitals within Beijing's 18 districts during May–July 2011. The surveillance system was designed and managed by the Beijing Center for Disease Prevention and Control (Beijing CDC). The Beijing CDC laboratory and 18 collaborating district laboratories were involved. The study was approved by the Institutional Review Board and the Human Research Ethics Committee of Beijing CDC.

The surveillance included children with scarlet fever or pharyngitis diagnosed by clinicians in the surveillance hospitals. All children with scarlet fever were invited to participate in the study after their parent(s)/guardian(s) gave informed consent. Each week, 10 children with pharyngitis were randomly selected from each hospital to participate in the study. Trained clinic staff used a standardized questionnaire to collect information (e.g., sex, age, date of illness onset, date medical care was sought, clinical symptoms and signs, and antimicrobial drug treatment) for each study participant. In addition, at each clinic, trained personnel obtained pharyngeal swab samples from study participants, and, the same day, a designated hospital staff member collected and sent all specimens to the corresponding district laboratory. The collaborating district laboratories isolated and identified GAS strains and then sent the isolates to Beijing CDC for *emm* typing, superantigen determination, and antimicrobial drug susceptibility testing. Patients with scarlet fever or pharyngitis from whom GAS was isolated were identified as confirmed GAS patients.

Isolation and Identification of GAS Strains

After pharyngeal swab samples arrived at a collaborating district laboratory, they were immediately spread onto 5% sheep blood agar plates and incubated overnight at 37°C in 5% CO_2. We tested b-hemolytic isolates for susceptibility to bacitracin and used the Streptococcal Grouping Kit (Oxoid Ltd., Basingstoke, UK) to determine the Lancefield group for each isolate.

emm Typing

All GAS isolates were subjected to *emm* typing, as described (*22*). We extracted DNA and amplified and sequenced the 5′ region of the *emm* gene by using recommended primers and cycling conditions (*22*). We aligned the sequence of the 5′ region of the *emm* gene with sequences in the Blast-*emm* database (www.cdc.gov/ncidod/biotech/strep/strepblast.htm; Centers for Disease Control and Prevention, Atlanta, GA, USA). The *emm* type and subtype of GAS isolates were identified on the basis of the 90 bases encoding the N terminal 30 residues of the processed M protein and the exact 150 base sequences encoding the N terminal 50 residues of the mature M protein, respectively. The fairly conserved 30 bases encoding the last 10 residues of the M protein signal sequence were referred to for identifying the start of the sequence encoding the mature M protein.

Superantigen Detection

Thirteen superantigens (*speA–speC*, *speF–speM*, *smeZ*, and *ssa*) were detected by subjecting each GAS isolate to real-time PCR. Specific PCR primers were used to amplify the gene of each superantigen in a 40-mL real-time PCR reaction system under the following cycling condition: 2 min at 94°C, followed by 40 cycles of 15 s at 93°C and 60 s at 55°C. Superantigen profiles were investigated for various *emm* types of GAS isolates.

Antimicrobial Drug Susceptibility Testing

We used the VITEK2 Compact (bioMérieux, Marcy l'Etoile, France) to test all GAS isolates for susceptibility to penicillin, ampicillin, quinupristin-dalfopristin, linezolid, vancomycin, tigecycline, levofloxacin, erythromycin, clindamycin, and tetracycline. We estimated the minimal inhibitory concentration of each antimicrobial drug for individual GAS isolates and determined the corresponding susceptibility according to the 2011 criteria of the Clinical and Laboratory Standards Institute (*23*).

Statistical Analysis

We entered data by using Microsoft Excel 2003 software (Microsoft Corp., Redmond, WA, USA) and analyzed data by using the SPSS 16.0 statistical package (SPSS Inc., Chicago, IL, USA). The mean and SD were calculated for continuous variables, and percentages were calculated for categorical variables. Differences in distributions of *emm* types and superantigens of GAS isolates were compared between subgroups of participants by using the χ^2 test or Fisher exact test. In addition, we compared antimicrobial drug susceptibilities by strain *emm* type by using the χ^2 test. We used multivariate unconditional logistic regression analyses to determine factors associated with various clinical signs of scarlet fever in patients with confirmed GAS infection. All statistical tests were 2-sided, and statistical significance was defined as $p<0.05$.

Results

Comparison of the 2011 Epidemic and 2006–2010 Cluster Outbreaks

According to NNIDSS, the annual number of scarlet fever cases in Beijing during 2006–2010 ranged from 1,193 to 2,264, and annual incidence rates ranged from 7.0 cases to 14.3 cases/100,000 population. In 2011, however, the number of scarlet fever cases in Beijing rose to 6,152, and the incidence rate rose to 31.4 cases/100,000 population. Peak monthly incidence rates in 2011 were 2.9–6.7 times those in 2006–2010. During the epidemic and during 2006–2010, scarlet fever cases peaked twice yearly: 1 peak occurred in early summer, and a second, less pronounced peak occurred in winter, except in 2010, when the winter peak was more pronounced (Figure, panel A).

Scarlet fever primarily affected children 3–8 years of age during the epidemic and nonepidemic years (Figure, panel B). During these years, the ratio of males to females in Beijing was 1.5:1.8, respectively, and the annual incidence of scarlet fever was persistently higher among males than females (Figure, panel C).

In Beijing, a cluster of scarlet fever was defined as onset of ≥2 clinical cases within a 7-day period in a school or kindergarten. According to this definition, 37–131 clusters (85–316 cases) occurred during 2006–2010 in Beijing, and 401 clusters (1,116 cases) occurred during 2011. In addition, case-patient follow-up showed that all cases reported during May–December, 2011, were fully resolved 3 weeks after illness onset without complications.

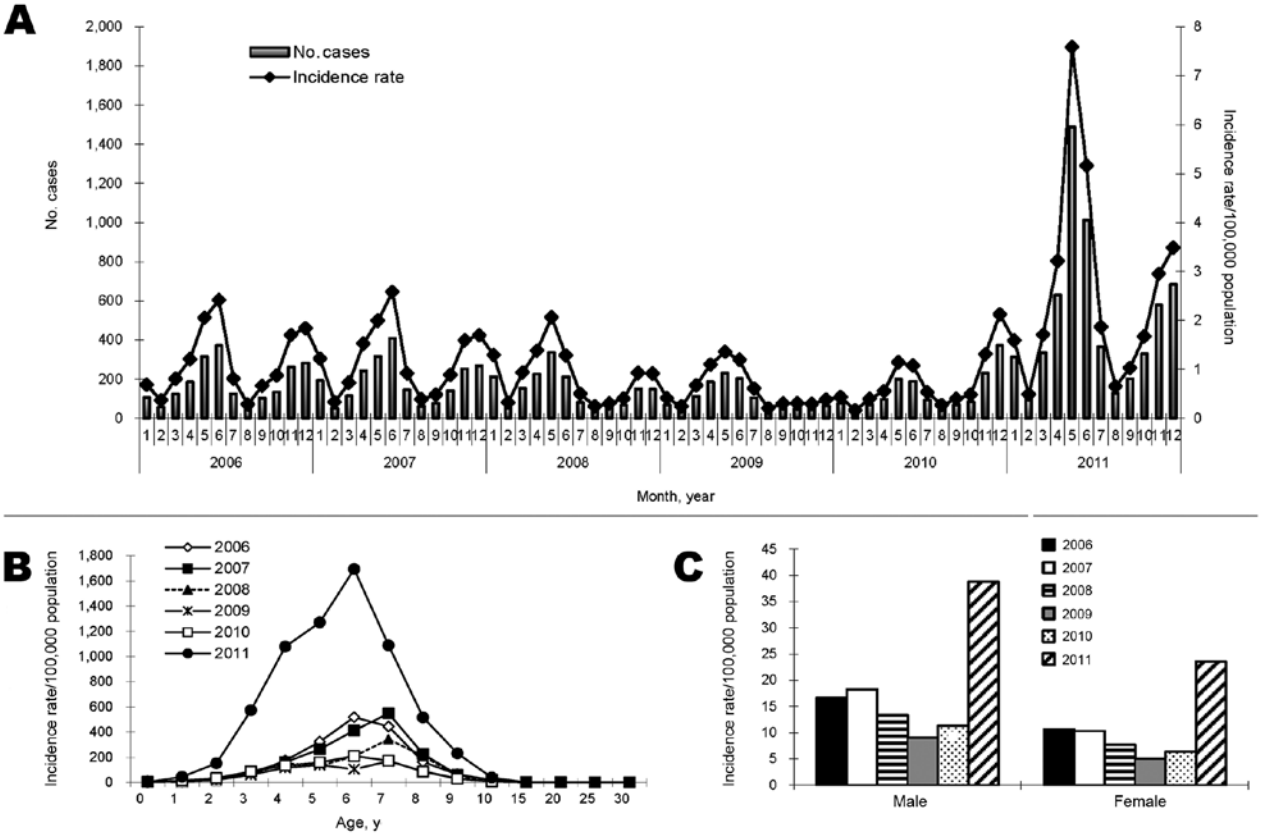

Figure. Scarlet fever incidence, Beijing, China, 2006–2011, as reported in the National Notifiable Infectious Disease Surveillance System. A) Number of cases and incidence rate by month. B) Incidence rate by age. C) Incidence rate by sex.

Characteristics of Children Enrolled in Enhanced Surveillance

A total of 3,359 children, representing 972 clinical cases of scarlet fever and 2,387 cases of pharyngitis, were enrolled in enhanced surveillance for GAS disease. Of the 3,359 enrollees, ≈41.0% were female. The mean age was 5.8 years (SD ± 3.3 years). The mean interval from illness onset to first medical visit was 1.5 days (SD ± 1.5 days). Approximately 41.2% of the children received antimicrobial drugs at home before seeking medical care.

Of the 3,359 enrollees, 647 (19.2%) were confirmed to have GAS infection. GAS was isolated from clinical samples for 44.2% (430/972) of the children with scarlet fever and from 9.1% (217/2,387) of the children with pharyngitis.

emm Subtypes and Superantigens

*emm*12 type accounted for 76.4% (494/647) of all GAS isolates. The leading *emm* subtypes were *emm*12.0 (65.7%), *emm*1.0 (16.8%), *emm*12.19 (5.7%), and *emm*12.1 (2.5%). There was a statistically significant difference in the proportion of *emm*12 type GAS in children ≤5 years of age and those >5 years of age (p = 0.004) and in the proportion of *emm*1 scarlet fever cases and pharyngitis cases (p = 0.042) (Table 1).

All of the GAS isolates harbored *speB*, and almost all possessed *speC*, *speF*, *speG*, *smeZ*, and *ssa*. Approximately 20% of the isolates harbored *speA* or *speJ*, and ≈75% possessed *speH* or *speI*; however, the percentage of isolates that harbored *speK*, *speL*, or *speM* was extremely low (Table 1). A total of 25 profiles of superantigens were found in the GAS isolates. Of the 494 *emm*12 isolates, 411 (83.2%) had the following superantigen profile: *speA* (-), *speB* (+), *speC* (+), *speF* (+), *speG* (+), *speH* (+), *speI* (+), *speJ* (-), *speK* (-), *speL* (-), *speM* (-), *smeZ* (+), *ssa* (+). Of the 111 *emm*1 isolates, 93 (83.8%) had the following superantigen profile: *speA* (+), *speB* (+), *speC* (+), *speF* (+), *speG* (+), *speH* (-), *speI* (-), *speJ* (+), *speK* (-), *speL* (-), *speM* (-), *smeZ* (+), *ssa* (+).

Antimicrobial Drug Susceptibility

All GAS strains isolated during the scarlet fever epidemic were susceptible to penicillin, ampicillin, quinupristin-dalfopristin, linezolid, vancomycin, and tigecycline, and 96.6% of them were susceptible to levofloxacin. Resistance to erythromycin, tetracycline, and clindamycin was found in 96.1%, 93.7%, and 79.4% of the isolates, respectively. A statistically significant difference was found between the percentage of *emm*1 and *emm*12 strains resistant to clindamycin (87.4% vs. 77.9%, respectively; p = 0.025) but not between the percentage of those resistant to erythromycin (99.1% vs. 96.4%, respectively; p = 0.134) or tetracycline (97.3% vs. 93.1%, respectively; p = 0.097).

Factors Associated with Clinical Signs of Scarlet Fever

The odds of having strawberry tongue was higher for GAS-infected study participants ≤5 years of age (odds ratio [OR] 2.04, 95% CI 1.46–2.83; p < 0.001). The odds of having a red rash was higher for participants infected with an *emm*1 versus *emm*12 type strain (OR 1.63, 95% CI 1.01–2.62; p = 0.046) and for participants ≤5 years of age (OR 2.52, 95% CI 1.74–3.65; p<0.001). Compared with patients with *emm*12 type strains, those with *emm*1

Table 1. SAgs and *emm* types of group A *Streptococcus* circulating during a scarlet fever epidemic, Beijing, China, 2011*

	No. (%) patients, by age			No. (%) patients, by sex			No. (%) patients, by clinical diagnosis			
emm type or SAg	≤5 y, n = 246	>5 y, n = 401	p value	Male, n = 402	Female, n = 245	p value	Scarlet fever, n = 430	Pharyngitis, n = 217	p value	Total, n = 647
emm type										
12	203 (82.5)	291 (72.6)	**0.004**	303 (75.4)	191 (78.0)	0.453	331 (77.0)	163 (75.1)	0.599	494 (76.4)
1	34 (13.8)	77 (19.2)	0.078	73 (18.2)	38 (15.5)	0.386	83 (19.3)	28 (12.9)	**0.042**	111 (17.1)
Other†	9 (3.7)	33 (8.2)	**0.022**	26 (6.5)	16 (6.5)	0.975	16 (3.7)	26 (12.0)	**<0.001**	42 (6.5)
SAg										
SpeA	47 (19.1)	96 (23.9)	0.150	91 (22.6)	52 (21.2)	0.675	99 (23.0)	44 (20.3)	0.427	143 (22.1)
SpeB	246 (100.0)	401 (100.0)	NA	402 (100.0)	245 (100.0)	NA	430 (100.0)	217 (100.0)	NA	647 (100.0)
SpeC‡	243 (98.8)	400 (99.8)	0.156	398 (99.0)	245 (100.0)	0.303	427 (99.3)	216 (99.5)	1.000	643 (99.4)
SpeF‡	245 (99.6)	400 (99.8)	1.000	401 (99.8)	244 (99.6)	1.000	429 (99.8)	216 (99.5)	1.000	645 (99.7)
SpeG‡	246 (100.0)	399 (99.5)	0.528	400 (99.5)	245 (100.0)	0.529	428 (99.5)	217 (100.0)	0.554	645 (99.7)
SpeH	192 (78.0)	301 (75.1)	0.387	299 (74.4)	194 (79.2)	0.164	326 (75.8)	167 (77.0)	0.747	493 (76.2)
SpeI	190 (77.2)	302 (75.3)	0.578	301 (74.9)	191 (78.0)	0.373	327 (76.0)	165 (76.0)	1.000	492 (76.0)
SpeJ	47 (19.1)	92 (22.9)	0.249	87 (21.6)	52 (21.2)	0.900	99 (23.0)	40 (18.4)	0.180	139 (21.5)
SpeK ‡	1 (0.4)	3 (0.7)	1.000	1 (0.2)	3 (1.2)	0.155	0	4 (1.8)	**0.012**	4 (0.6)
SpeL ‡	2 (0.8)	5 (1.2)	0.715	4 (1.0)	3 (1.2)	1.000	2 (0.5)	5 (2.3)	**0.046**	7 (1.1)
SpeM	5 (2.0)	9 (2.2)	0.857	8 (2.0)	6 (2.4)	0.697	9 (2.1)	5 (2.3)	0.862	14 (2.2)
SmeZ‡	245 (99.6)	400 (99.8)	1.000	401 (99.8)	244 (99.6)	1.000	428 (99.5)	217 (100.0)	0.554	645 (99.7)
Ssa	241 (98.0)	391 (97.5)	0.705	394 (98.0)	238 (97.1)	0.477	427 (99.3)	205 (94.5)	**<0.001**	632 (97.7)

*__Boldface__ indicates statistical significance. SAg, superantigen.
†Other *emm* types included types 4, 11, 22, 75, and 89.
‡Fisher exact test was used.

type strains had a higher probability of having Pastia lines (OR 1.80, 95% CI 1.02–3.16; p = 0.043) or circumoral pallor (OR 2.15, 95% CI 1.17–3.93; p = 0.013) (see Table 2, Appendix, wwwnc.cdc.gov/EID/article/19/6/12-1020-T1.htm, for details).

Discussion

We found that an epidemic of scarlet fever occurred in Beijing in 2011. *emm*12 was the predominant type among the circulating GAS strains, and resistance to erythromycin, tetracycline, and clindamycin was high among the isolates.

Although the incidence of scarlet fever in Beijing in 2011 was much higher than that in preceding years, the basic characteristics, including the seasonality of epidemic peaks, the most vulnerable age group, and the difference in susceptibility to infection by sex, did not change. In Beijing, the incidence of scarlet fever usually peaks twice a year in 2 distinct seasons–summer and winter–with the highest peak in early summer; however, it is well-recognized that scarlet fever most commonly occurs in winter/spring in other locations (*24*,*25*). This finding indicates that in China, GAS is transmitted more easily in early summer than in winter, possibly because in early summer, compared with winter, each year an *emm* type that is relatively new to young children sweeps through a population of children that does not have an effective level of population immunity. In addition, the winter peak in 2010 was higher than the summer peak, which implies that this epidemic of scarlet fever started at the end of 2010.

Among the GAS strains we identified circulating in Beijing during 2011, types *emm*12 and *emm*1 were most predominant (≈76% and ≈17%, respectively); these percentages were higher (≈40%) and lower (>40%), respectively, than those reported in China during previous years (*13*,*26*,*27*). Scarlet fever outbreaks also occurred in Hong Kong and Shanghai, China, during 2011, and *emm*12 was the predominant circulating type (*28*–*30*). These findings suggest that some intrinsic factors might have facilitated the spread of *emm*12 strains and led to the epidemic. Two previously unreported genomic insertions (64.9 kb and 46.4 kb) were identified in *emm*12 GAS strains isolated during the 2011 scarlet fever outbreak in Hong Kong. However, analysis of *emm*12 strains isolated during 2005–2010 showed that the insertions were also present in those strains (*28*), and the study concluded that mobile genetic elements, environmental factors, and host immune status might have contributed to the 2011 scarlet fever outbreak in Hong Kong.

*emm*12 is not known to be the exclusively predominant GAS type in other countries (*9*,*31*,*32*), but it is possible that the *emm*12 type strains circulating in China in 2011 could spread to other regions. Therefore, surveillance for the increased presence of *emm*12 strains outside of China is warranted. A 30-valent GAS vaccine that contains the most prevalent *emm* types found in our study (*emm*12 and *emm*1) is under development (*8*); such a vaccine would help prevent and control the spread of GAS diseases in China.

Superantigens in the GAS isolates in our study were similar to those found in isolates from other studies, except for *speC* and *ssa*. In contrast to findings in other studies, we found that almost all *emm*1 GAS strains from the 2011 Beijing epidemic harbored *speC*, and *ssa* became the primary superantigen of *emm*1 and *emm*12 isolates (*11*,*32*,*33*). Consistent with findings in earlier studies in other locations, we found that an extremely low number of GAS isolates harbored *speK*, *speL,* or *speM* (*11*,*33*,*34*).

All GAS isolates from the 2011 Beijing epidemic were susceptible to penicillin, a standard antimicrobial drug for the treatment of scarlet fever. However, >96% and ≈94% of the isolates were resistant to erythromycin and tetracycline, respectively, and ≈80% were resistant to clindamycin. These findings compare with earlier findings of 99.5%, 97.1%, and 99.5% resistance to erythromycin, tetracycline, and clindamycin, respectively (*27*). The extent of the resistance of GAS strains to erythromycin and tetracycline in many other countries has been very low (*11*,*17*,*18*). The high resistance rate in China might be attributed to the overuse of antimicrobial drugs in humans or animals.

In our study, the odds of having strawberry tongue or red rash with GAS infection was higher for younger patients. This could indicate that older patients had acquired partial immunity to GAS from a previous exposure or infection, resulting in milder clinical manifestation of the disease during subsequent infection.

This study had limitations. First, to track case outcomes, we followed up on the scarlet fever case-patients for 3 weeks after illness onset, which may not have allowed sufficient time to capture later-occurring complications; thus, outcome profiles may have been incomplete. Second, the enhanced surveillance in Beijing did not include invasive GAS diseases, so we could not report on all GAS strains circulating during the 2011 scarlet fever epidemic.

The 2011 scarlet fever epidemic in Beijing was characteristic of other scarlet fever epidemics and occurred after an abnormal peak winter incidence of the disease in 2010. *emm*12 type GAS became predominant. The level of GAS resistance to clindamycin was lower than that to erythromycin and tetracycline.

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Transmission Potential of Rift Valley Fever Virus over the Course of the 2010 Epidemic in South Africa

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A Rift Valley fever (RVF) epidemic affecting animals on domestic livestock farms was reported in South Africa during January–August 2010. The first cases occurred after heavy rainfall, and the virus subsequently spread countrywide. To determine the possible effect of environmental conditions and vaccination on RVF virus transmissibility, we estimated the effective reproduction number (R_e) for the virus over the course of the epidemic by extending the Wallinga and Teunis algorithm with spatial information. Re reached its highest value in mid-February and fell below unity around mid-March, when vaccination coverage was 7.5%–45.7% and vector-suitable environmental conditions were maintained. The epidemic fade-out likely resulted first from the immunization of animals following natural infection or vaccination. The decline in vector-suitable environmental conditions from April onwards and further vaccination helped maintain R_e below unity. Increased availability of vaccine use data would enable evaluation of the effect of RVF vaccination campaigns.

Rift Valley fever (RVF) is a zoonotic arbovirosis caused by infection with a phlebovirus (family *Bunyaviridae*, genus *Phlebovirus*). The main vectors are specific *Aedes* and *Culex* spp. mosquitoes, and primary hosts are sheep, goats, and cattle (*1*,*2*). RVF epidemics usually occur after heavy rainfalls, which inundate ephemeral wetlands and enable large numbers of *Aedes* spp. mosquito eggs to hatch; it has been hypothesized that these mosquitoes harbor RFV virus (*3*–*5*). Virus transmission is sustained in locations with more persistent surface water, which provides suitable breeding conditions for other vectors, such as *Culex* sp. mosquitoes (*6*). RVF epidemics among animal herds cause abortion storms, affecting all stages of pregnancy, and high death rates among neonates. Epidemics among humans often cause influenza-like illness, although severe conditions (e.g., hemorrhagic fever and death) have been reported (*1*,*2*).

RVF epidemics occurred in South Africa in 1950–1951 (*7*), 1973–1975 (*8*), and 2010–2011. The 2010 wave started in January and February in Free State Province and subsequently spread to almost all provinces in South Africa (Figure 1, panel A). Animals from a variety of species were affected (e.g., cattle, sheep and goats, buffaloes, camels, and other wild animals), and 95% (n = 470) of the affected farms raised cattle, small ruminants (sheep/goats), or both (*9*). The incidence peaked in March, and the last case of that wave was reported in August 2010. The epidemic resumed in January 2011, affecting 124 farms, mainly in Eastern Cape Province (Figure 1, panel B) (*10*). The start of the 2010 epidemic was attributed to heavy rainfall in January and February (*11*,*12*). The fade-out of the 2010 wave could be attributed to several factors: a depletion of susceptible hosts after natural infection or vaccination (*13*); a change of environmental conditions affecting the sustainability of vector breeding, such as a decrease in temperature (*14*); the drying of wetlands; or a combination of these factors.

The effective reproduction number (R_e) is a key epidemiologic parameter that measures the transmission potential of the causative agent of a disease during an epidemic. R_e is defined by the number of secondary infections resulting from 1 infectious case in a population in which some

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members are already immune (*15*). When R_e is above 1, the infection spreads; maintenance of R_e below 1 is required to stop an outbreak (*16*).

The objective of this study was to estimate R_e at the farm level over the course of the 2010 RVF epidemic wave in South Africa by applying the Wallinga and Teunis transmission tree–reconstruction method (*17*), extended to use geographic information. By tracking the transmission potential of the virus and comparing our findings with data on vaccination and climate (rainfall and temperature), we determined plausible reasons for fade-out of the epidemic wave.

Methods

RVF Dataset and Study Period

A total of 470 RVF cases were reported over the study period (January–August 2010). A case was defined as an outbreak reported from a farm raising cattle, small ruminants, or both (*9*). Available information comprised the global positioning system coordinates and outbreak starting dates for the affected farms.

Estimation of Effective Reproduction Number

The Wallinga and Teunis method (*17*), extended with spatial information, enables estimation of R_e at the farm level by calculating the relative likelihood, or probability (p_{ij}), that a specific farm (i) gets infected from another specific farm (j). This probability, p_{ij}, is equal to the probability that farm j infects farm i, divided by the probability that farm i had been infected from any other farm (k) in the dataset (Figure 2). These probabilities depend on the number of days separating the onset of symptoms on the 2 farms (i and j) and the distance (in kilometers) separating i and j, and the probabilities were extracted from a probability density function of the generation

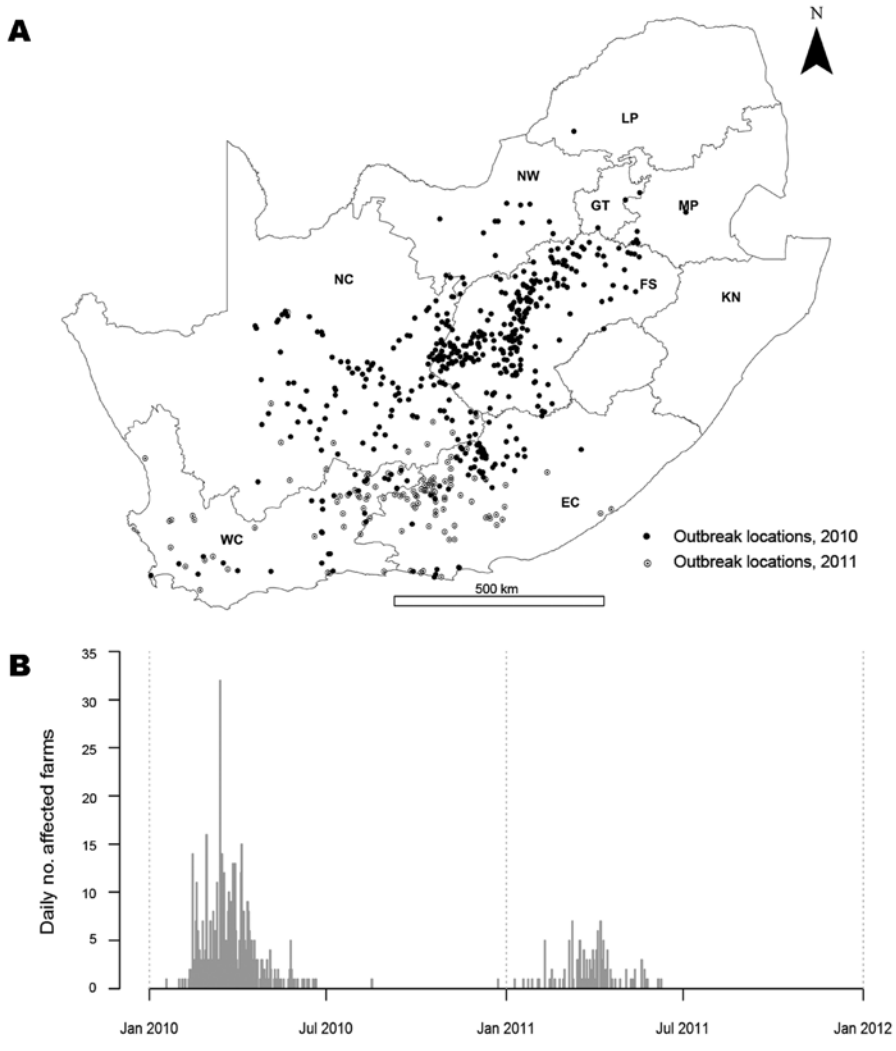

Figure 1. Rift Valley fever epidemic, South Africa, 2010–2011. A) Location of cases. Unmarked area in center right is Lesotho (no data). B) Epidemic curve for the 2 years. NC, Northern Cape; NW, North West; LP, Limpopo; GT, Gauteng; MP, Mpumalanga; FS, Free State; KN, KwaZulu-Natal; EC, Eastern Cape; WC, Western Cape.

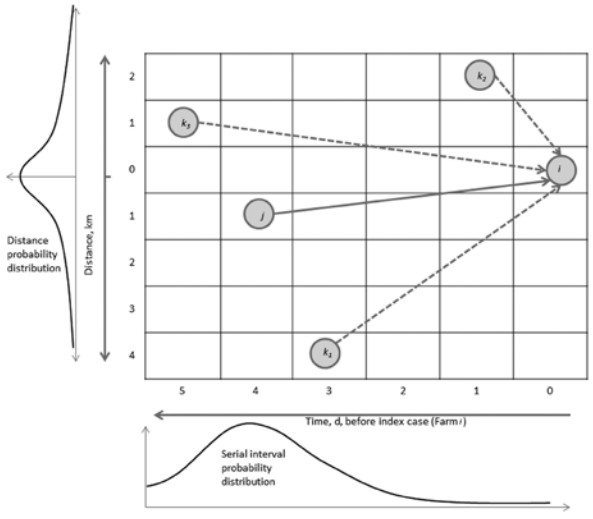

Figure 2. Schematic representation of the Wallinga-Teunis algorithm extended with spatial information. Farm *i* could get infection from Farm *j*, but it also could get infection from Farms k_1, k_2, and k_3. In this example, the most likely time difference between onset of symptoms is 4 days (based on the serial interval distribution, given below the *x*-axis), and the most likely distance between farms is short (<1 km). Therefore, Farm *j* is the most likely farm to have infected Farm *i* (this scenario maximizes the probability in both dimensions). See the online Technical Appendix (wwwnc.cdc.gov/EID/article/19/6/12-1641-Techapp1.pdf) for details.

interval (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/6/12-1641-Techapp1.pdf). The generation (or serial) interval was defined as the time between onset of symptoms for a primary case and the onset of symptoms for its secondary case (*18*). In the stylized example in Figure 2, the most likely time difference was 4 days (determined on the basis of the serial interval distribution, given below the *x* axis), and the most likely distance is short (<1 km). Therefore, farm *j* is the most likely farm to have infected farm *i* (this maximized the probability in both dimensions).

Because no independent dataset (i.e., from another epidemic in another country) was available to estimate a generation interval for RVF at the farm level and in 2 dimensions (i.e., distance and time), we used the dataset for the 2011 RVF outbreak in South Africa. In a previous analysis, Métras et al. (*19*) estimated the spatiotemporal interaction (or proximity) from the 2011 dataset [denoted $D_0(s,t)$] by using the space–time *K*-function (*20*). These $D_0(s,t)$ values were used as a generation interval distribution to calculate p_{ij} (online Technical Appendix).

Sensitivity Analysis

The shape of the $D_0(s,t)$ plot, peaking for short space–time windows (Figure 3), suggested that most of the transmission was attributed to short-distance mechanisms (e.g., local vector dispersal) rather than long-distance mechanisms (e.g., movement of infectious animals or wind carriage of vectors) (*19*). By using this generation interval for the duration of the epidemic, a constant and high importance of short-distance transmission mechanisms was assumed. However, as the epidemic grew, these short-distance transmission mechanisms were likely to be less important; or in, other words, as farms around a case became infected and immune, short-distance transmission was likely to be less involved in disease spread. Thus, we investigated the variations of R_e by giving less weight to short-distance transmission and more weight to long-distance transmission. To obtain such serial interval distributions, the $D_0(s,t)$ distribution was flattened by using a 2-dimensional double exponential kernel function with bandwidth values equal to 1, 3, and 5, resulting in 3 smoothed surfaces (Figure 3). It was assumed that the bandwidth equal to 1 would better correspond to the serial interval distribution at the early stage of the epidemic and that bandwidth values 3 and 5 would better describe the intensity of the transmission when the population started to be immune (i.e., at the later stages of the epidemic).

Vaccination Coverage and Climate Data

We collected information on animal vaccination and climate to determine the potential effect of these factors on the fade-out of the 2010 RVF epidemic. RVF vaccination in South Africa is not compulsory and is not implemented by the government. Although the government can strongly advise farmers to vaccinate their animals, implementation of vaccination on a farm depends on the individual farmer's decision. Therefore, data on vaccination are especially limited.

Onderstepoort Biologic Products Ltd. (Onderstepoort, South Africa), the sole provider of RVF vaccine in South Africa, calculates its yearly sales from April of one year to March of the next year (*21*). During April 1, 2009–March 31, 2010, ≈3.4 million RVF vaccine doses (live attenuated Smithburn and inactivated) were sold, and during April 1–May 31, 2010, ≈5.8 million doses were sold (Table 1) (*22*). In our study, Period 1 corresponded with the time before the 2010 epidemic (April 1, 2009–January 18, 2010); Period 2 corresponded with the start of the 2010 epidemic and the end of the 2009 vaccine sales year (January 19, 2010–March 31, 2010); and Period 3 corresponded with April 1, 2010–May 31, 2010, beyond which no vaccine sales data were available (Table 1). Vaccination coverage was estimated up to March 31, 2010 (end of Period 2) and up to May 31, 2010 (end of Period 3). Since no spatial (i.e., location-specific) information on vaccine sales was available, vaccination coverage was estimated under 3 scenarios (A, B, and C): Scenario A assumed that vaccination coverage

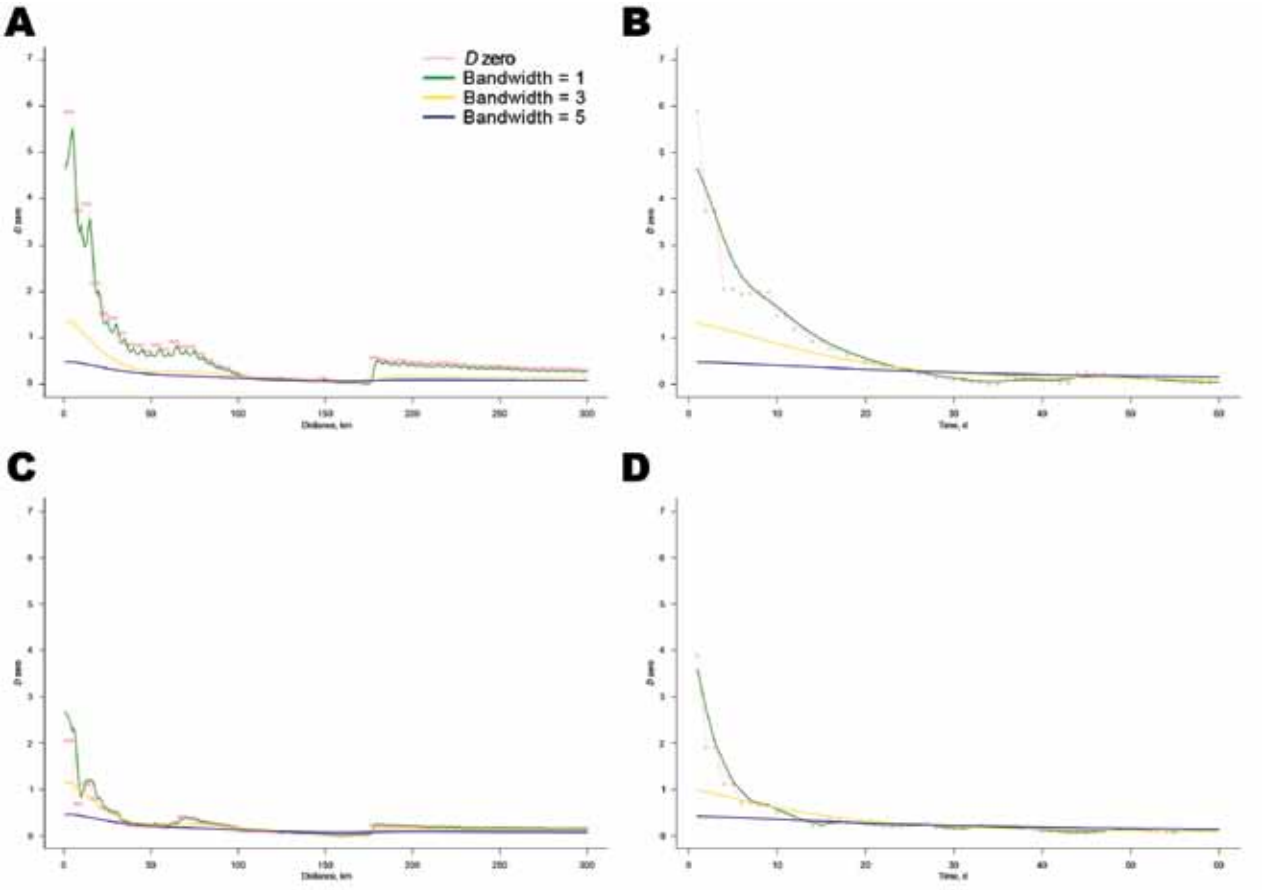

Figure 3. Distribution of D_0 by time and distance [$D_0(s,t)$]. $D_0(s,t)$ is a measure of spatiotemporal interaction between cases that was estimated by using the space–time *K*-function (*19*,*20*); the distribution is indicated by the pink dashed line. The green, yellow, and blue lines are the smoothed distributions, which were obtained with bandwidth values of 1, 3, and 5, respectively. A) Plot of $D_0(s,t)$ values by distance on day 1. B) $D_0(s,t)$ values by time at distance of 5 km. C) Plot of $D_0(s,t)$ values by distance on day 5. D) $D_0(s,t)$ values by time at distance of 15 km.

was applied throughout South Africa proportional to the livestock population; Scenario B assumed that the number of vaccines used in a province over a specific period was proportional to the number of cases reported in that province over that same period; Scenario C assumed that all vaccines were used in Free State Province during Periods 2 and 3 and that no vaccine had been used before the epidemic (Period 1). Therefore, using Scenario C, we could estimate the maximum coverage for Free State Province, which was the first and most affected province and also the one in which the government strongly supported vaccination (*13*). Formulas used to calculate vaccination coverage are available in the online Technical Appendix.

Most RVF cases were reported in Free State Province, although Northern Cape Province had the most cases in Period 3 (Table 1). Therefore, we averaged the daily minimum and maximum temperatures and total monthly rainfall from 5 weather stations in Free State (Bloemfontein, Kroonstad, Welkom, Fauresmith, and Gariep Dam) and 4 weather stations in Northern Cape (Kimberley, Prieska, De Aar, and Noupoort) (South African Weather Service, pers. comm.).

Herd Immunity Threshold

Herd immunity threshold (HIT) is defined as the proportion of animals that needs to be immune to a pathogen to control transmission (*15*):

$$\mathrm{HIT} = 1 - 1/R_0$$

In the equation, R_0, the basic reproduction number, is the expected number of secondary cases generated by a primary case in a totally susceptible population and measures the potential for an infectious agent to start an outbreak. To compare the estimated vaccination coverage at the end of March with the proportion of farms on which animals should have been immune (either by natural infection or vaccination) to control transmission, we approximated HIT by replacing R_0 in the equation by the highest value of R_e (and its 95% CI values) at the start of the epidemic.

Table 1. Number of farms affected by Rift Valley fever before and during first 4.5 months of the 2010 epidemic, South Africa

	No. (%) farms affected			
	Before the epidemic	First 4.5 months of the epidemic		
Province	Period 1, April 1, 2009–January 18, 2010*	Period 2, January 19–March 31, 2010*	Period 3, April 1–May 31, 2010†	Periods 2 and 3, January 19–May 31, 2010
Free State	0 (0)	208 (66.9)	41 (27.2)	249 (53.9)
Northern Cape	19 (67.9)	61 (19.6)	54 (35.8)	115 (24.9)
Eastern Cape	0	24 (7.7)	26 (17.2)	50 (10.9)
Kwazulu-Natal	8 (28.6)	0	0 (0)	0
North West	0	7 (2.3)	8 (5.3)	15 (3.2)
Mpumalanga	1 (3.6)	5 (1.6)	0	5 (1.1)
Western Cape	0	4 (1.3)	20 (13.2)	24 (5.2)
Gauteng	0	2 (0.6)	1 (0.7)	3 (0.6)
Limpopo	0	0	1 (0.7)	1 (0.2)
All provinces	28 (100.0)	311 (100.0)	151 (100.0)	462 (100.0)

*A total of 3.4 million Rift Valley fever vaccine doses were sold during Periods 1 and 2.
†5.8 million Rift Valley fever vaccine doses were sold during Period 3.

Software

The analysis and plots were done by using R version 2.14.0 (*23*). Kernel smoothing was performed by using the image.smooth function in the fields package (*24*).

Results

Estimation of Effective Reproduction Number

The estimated transmission potential of RVF virus from farms with infected animals peaked in mid-February (R_e = 4.3, 95% CI 2.0–6.5), dropped sharply within a few days (R_e = 1.8, 95% CI 1.21–2.43), and then remained at ≈1.5 until mid-March, at which time it dropped below unity, where it remained until the end of the epidemic (Figure 4). In addition, the lower bound of the 95% CI dropped and remained below 1.0 from mid-February onwards. In January and February, the most highly infectious farms ($R_e \geq 2$) were located in Free State Province (Figure 5, panels A–C), and although the data suggest the epidemic was still contained in Free State Province in February, a rapid fall in the R_e value was observed (Figure 4). In March, the epidemic had spread to other provinces, mainly Northern Cape, and transmission was ongoing. In April, the spatial extent of the virus was similar to that in March, but most of the affected farms were not sources of ongoing transmission ($R_e < 1$). By May, only 7 spatially isolated farms had R_e above unity.

Figure 6 shows the variability of R_e for the different serial interval distributions used in our analyses. In the early stages of the epidemic, R_e was smaller when using input distributions that gave more weight to short-distance transmission [$D_0(s,t)$ and bandwidth 1] because it used only those cases closer in time and space, whereas when R_e was estimated with flatter distributions (bandwidths 3 and 5), it also encompassed longer-distance transmission. However, for all distributions, the important variations of R_e followed the same trend over time: a marked peak in January and February and stable transmission between late February and early March.

Vaccine Coverage and Climate Data

At the end of March, we estimated vaccination coverage in Free State and Northern Cape Provinces to be 7.5% by applying vaccine coverage throughout the country in proportion to the livestock population (Scenario A, Table 2). When the number of vaccines used in each province was proportional to the number of RVF cases in each province, vaccination coverage was 28.2% in Free State and 11.0% in Northern Cape (Scenario B, Table 2). When all vaccines were used at the early stages of the epidemic in Free State Province only, vaccination coverage reached its highest value (45.7%) (Scenario C, Table 2). At the end of May, vaccination coverage in Free State was 20.4%, 49.4%, and 100.0% for Scenarios A, B, and C, respectively; vaccination coverage in Northern Cape Province was 39.6% for Scenario B (Table 2). In Scenario C, the total number of vaccines sold at the end of March was greater than the number of livestock in Free State Province. Thus, assuming that the spillover vaccine was used in Northern Cape, the estimated vaccination coverage in that province was 24.3%.

In Free State Province, monthly rainfall peaked in January (152 mm total). Substantial rainfall, although declining, persisted until April (58 mm total) and dropped in May (9 mm total), eventually approaching zero in September (Figure 4). The average daily temperature dropped from 24°C to 18°C during the study period; a decrease of 6°C (from 21°C to 15°C) occurred from mid-March to mid-May. Minimum daily temperatures fell below 13°C from early April onwards, but most of the time, the maximum daily temperature remained above 15°C. Rainfall and temperature estimates followed a similar trend in Free State and Northern Cape Provinces (Figure 7).

Herd Immunity Threshold

In early February, the highest R_e value was 4.3 (95% CI 2.0–6.5). The HIT at that time was therefore estimated at ≈78.9%, varying between 50.0% and 84.6%.

Discussion

R_e reached its highest value in early February (R_e = 4.3, 95% CI 2.0–6.5). Although R_e fell below unity in mid-March, the lower bound of its 95% CI dropped below 1.0 in mid-February. Until the end of March, most RVF cases were recorded in Free State Province, and vaccination coverage was estimated between 7.5% and 45.7%. During this time, rainfall was substantial (73 mm total in March), so water was maintained in water bodies, and average temperature ranges (17°–24°C) were recorded (*14*). In addition, the minimum HIT was estimated at 50%. In April and May, R_e was maintained below 1.0, more RVF cases were reported in Northern Cape, and vaccination coverage in Free State and Northern Cape varied between 20.4% and 100.0%. The level of rainfall was maintained until the end of April (58 mm total) and dropped to 9 mm in May; temperatures averaged below 20°C most days.

The R_e peak observed in February followed the heavy rain observed in January, which, together with warm temperatures, created suitable environmental conditions for a massive hatching of *Aedes* spp. mosquito eggs (specifically, *Aedes juppi*, *Ae. caballus*, and *Ae. linneatopennis* in South Africa [*25*]) and initiation of the RVF outbreak. The virus originated from infected *Aedes* mosquito eggs (*5*,*26*) or possibly from other sources (e.g., long-distance vectors or movement of infected animals). Despite the decline in rainfall during January–March (from 152 mm to 73 mm/month) in Free State Province, transmission of RVF virus continued, although at a lower intensity. The continued transmission suggested that the lower amount of rainfall was sufficient to keep water bodies with good retention capacity filled and, thus, enable secondary vectors (e.g., *Cx. theileri* and *Anopheles cinereus*) (*25*) to sustain virus transmission in Free State and Northern Cape Provinces. From April onwards, the drop in rainfall may have contributed to a decreased abundance of *Culex* spp. mosquitoes; lower temperatures may have also slowed virus replication and shedding in *Culex* spp. vectors, as has been observed for *Cx. pipiens* (*27*,*28*), and thereby reduced virus transmission.

Given the environmental conditions, the RVF epidemic could have continued at least until the end of March in Free State Province. However, a depletion of susceptible animals after natural infection or vaccination probably caused the R_e to fall below unity 2 weeks earlier (mid-March) and the lower bound of its 95% CI to fall in mid-February. In addition, the minimum HIT was estimated at 50.0%, but at the end of March, the estimated

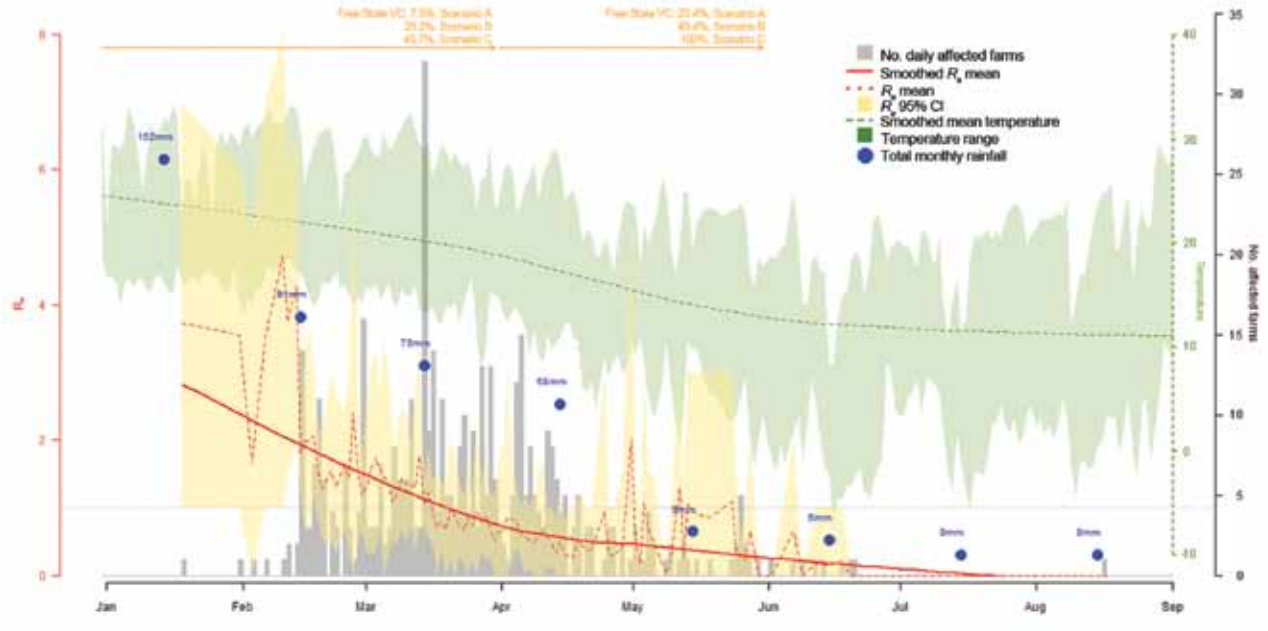

Figure 4. Rift Valley fever incidence (bars), daily effective reproduction number (R_e; red dashed line), and smoothed mean of R_e (solid red line) over the course of 2010 epidemic in Free State Province, South Africa. Blue dots, estimates of concurrent total monthly rainfall; dashed green line, average daily temperature. Vaccination coverage (VC) by March 31, 2010, and May 31, 2010, for Scenarios A–C (descriptions follow) are indicated at the top of the graph. Scenarios: Scenario A assumed that vaccination coverage was applied throughout South Africa in proportion to the livestock population; Scenario B assumed that the number of vaccines used in a province over a specific period was proportional to the number of cases reported in that province over that same period; Scenario C assumed that all vaccines were used in Free State Province during Period 2 (January 19–March 31, 2010) and Period 3 (April 1–May 31, 2010) and that no vaccine had been used before the epidemic (Period 1, April 1, 2009–January 18, 2010). The horizontal dashed line represents the threshold value R_e = 1.

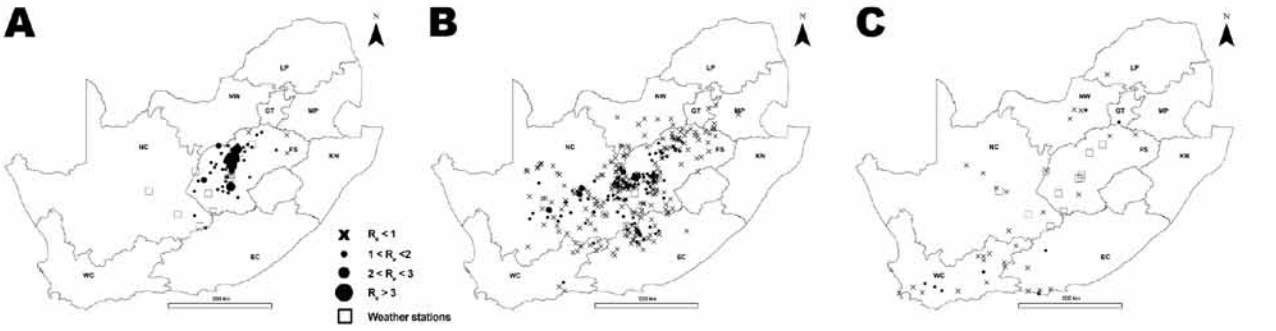

Figure 5. Effective reproduction number (R_e) per affected farm, by province, over the 2010 Rift Valley fever epidemic, South Africa. A) January and February. B) March and April. C) May and June. July and August are not displayed because no cases were reported in July, and R_e was 0 for the only farm reported in August. NC, Northern Cape; NW, North West; LP, Limpopo; GT, Gauteng; MP, Mpumalanga; FS, Free State; KN, KwaZulu-Natal; EC, Eastern Cape; WC, Western Cape. The unmarked area to the right of center is Lesotho (no data).

maximum vaccination coverage in Free State was 45.7% (Scenario C). By the end of May, vaccination coverage was higher, rainfall was very low, and temperatures continued to decrease, all of which probably contributed to preventing further virus transmission.

Several limitations with regard to the methods and data used might have altered the results of this study and their interpretation. First, the validity of the Wallinga and Teunis method assumes that all cases are reported and reported in a timely manner. The RVF cases used were those reported to the World Organisation for Animal Health. RVF is a notifiable disease that causes obvious signs in affected herds, so it is unlikely that underreporting was a major limitation. However, underreporting cannot be excluded, and we acknowledge that an assessment of its extent would increase the quality of the data. Another assumption of the Wallinga and Teunis estimation method is that the generation interval remains constant over the course of the epidemic. The sensitivity analysis showed that the shape of the generation interval chosen was important only in the early stages of the outbreak, when a high number of initial cases in the epidemic would equally involve short- and long-distance transmission mechanisms. Another limitation is that in the absence of identified distinct cases resulting from initial virus emergence or introduction, we considered that all cases for the entire epidemic as resulting from transmission from a single index case. In that setting, the initial values of R_e could have been overestimated. If multiple index cases could be identified, the model could be improved by studying transmission within clusters. The algorithm could also be extended to include other information, such as contact between farms caused by movement of animals or environmental data at a higher resolution.

The second limitation is that the 2011 South African RVF dataset was used to build the serial interval distribution because no data from another country or from another epidemic period were available. Although the use of an external dataset would have been more appropriate, the fact that the 2010 and 2011 waves occurred 1 year apart and had a different spatial extent (Figure 1) suggested that both datasets were reasonably independent. However, as a consequence of the 2010 wave, it is possible that

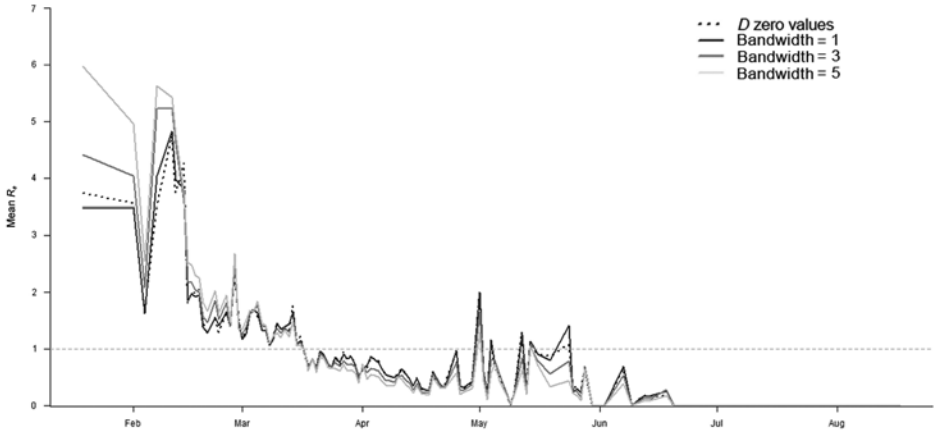

Figure 6. Mean effective daily reproduction number (R_e) during Rift Valley fever epidemic, South Africa, 2010. R_e was estimated by using $D_0(s,t)$ values (dashed black line) and $D_0(s,t)$ smoothed surfaces obtained with bandwidth values of 1 (dark gray), 3 (medium gray), and 5 (light gray). $D_0(s,t)$ values were estimated by using the space–time *K*-function (*19*,*20*) and are a measure of the spatiotemporal proximity between cases. The horizontal dashed line represents the threshold value R_e = 1.

vaccination was implemented in the 2011 affected area before the second wave actually started. If applied, vaccination would not modify the shape of the space–time interaction in 2010 and 2011, but it might explain the difference in the intensity of the interactions (*19*). In all cases, the values measuring the intensity of the space–time interaction [$D_0(s,t)$] in 2010 would lie between the 2011 values smoothed with a bandwidth between 1 and 3, and because the sensitivity analysis suggested that the key variations of R_e over time were not affected by these various surfaces, results remain robust.

Another limitation is that vaccine, rainfall, and temperature data used to discuss the plausibility of different reasons for fade-out of the 2010 epidemic were centered on Free State Province. This is where the epidemic started, where vaccination by the government was first applied (*13*), and where 53.9% of the cases were reported by the end of May. Rainfall and temperature data were recorded for Free State Province, which is centrally situated with respect to the outbreak. There is great spatial variation in temperature and rainfall across Free State Province and the country. However, rainfall countrywide was higher than usual that year (*11*,*12*); observations from the field confirmed a decreased winter temperature in Free State Province, starting in April–May (*14*); and trends in environmental variables in Northern Cape Province were similar to those in Free State (Figure 5).

Furthermore, limited vaccination data were available, so vaccination coverage was estimated under 3 scenarios. It is likely that a large proportion of the 3.4 million vaccine doses sold during April 2009–March 2010 were used in Free State Province at the early stages of the 2010 epidemic (*13*). However, some of those doses would have been used by farmers earlier in 2009 in KwaZulu–Natal and Northern Cape Provinces, where RVF cases were reported and vaccination was applied (*29*,*30*), and in early 2010 because of the perceived risk of further outbreaks. However, detailed figures on this were not available. Therefore, the most likely scenario might have been between Scenarios B and C, corresponding to vaccination coverage of 28.2%–45.7% in Free State Province.

Table 2. Estimated vaccination coverage, under 3 different scenarios, during an epidemic of Rift Valley fever in 2 provinces in South Africa, 2010*

	% Vaccine coverage			
	Free State Province		Northern Cape Province	
Scenario	March 31	May 31	March 31	May 31
A	7.5	20.4	7.5	20.4
B	28.2	49.4	11.0	39.6
C	45.7	>100.0	0	0† (24.3‡)

*Scenario A assumed that vaccination coverage was applied throughout South Africa in proportion to the livestock population; Scenario B assumed that the number of vaccines used in a province over a specific period was proportional to the number of cases reported in that province over that same period; Scenario C assumed that all vaccines were used in Free State Province during Periods 2 (January 19–March 31, 2010) and 3 (April 1–May 31, 2010) and that no vaccine had been used before the epidemic (Period 1, April 1, 2009–January 18, 2010).
†Assumes that all vaccines are used in Free State.
‡Assumes that spillover vaccines from Free State were used in Northern Cape.

In conclusion, the results of this study suggest that a depletion of RVF-susceptible animals by natural infection or vaccination first contributed to reduce RVF virus transmission in Free State Province and that the effect of further vaccination and the decrease in temperature from April onwards probably helped maintain R_e below unity. Disentangling and quantifying the relative importance of these factors would have benefited from detailed data on monthly vaccine sales and geographic use information. Increasing the public availability of vaccine use data would enable further evaluation of the effect of RVF vaccination campaigns.

Acknowledgments

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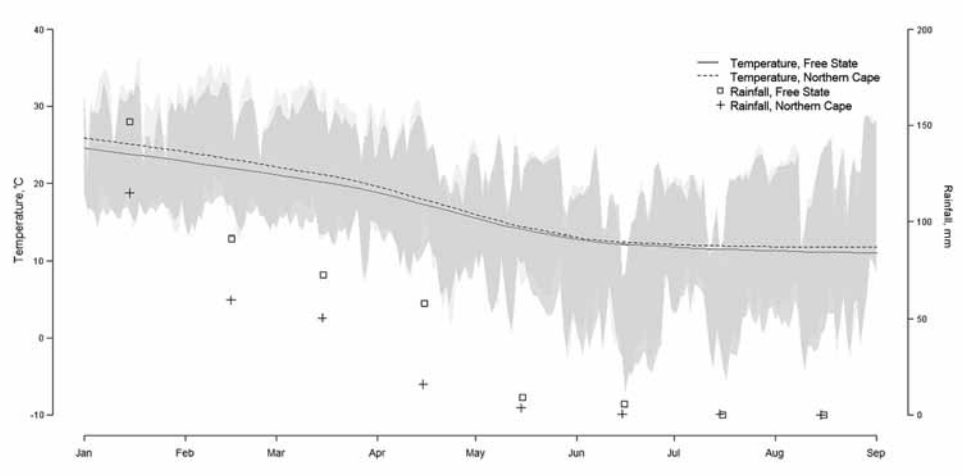

Figure 7. Daily temperature and total monthly rainfall during January–August 2010, Free State and Northern Cape Provinces, South Africa.

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Effect of Travel on Influenza Epidemiology

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To assess the attack and incidence rates for influenza virus infections, during October 2006–October 2007 we prospectively studied 1,190 adult short-term travelers from the Netherlands to tropical and subtropical countries. Participants donated blood samples before and after travel and kept a travel diary. The samples were serologically tested for the epidemic strains during the study period. The attack rate for all infections was 7% (86 travelers) and for influenza-like illness (ILI), 0.8%. The incidence rate for all infections was 8.9 per 100 person-months and for ILI, 0.9%. Risk factors for infection were birth in a non-Western country, age 55–64 years, and ILI. In 15 travelers with fever or ILI, influenza virus infection was serologically confirmed; 7 of these travelers were considered contagious or incubating the infection while traveling home. Given the large number of travelers to (sub)tropical countries, travel-related infection most likely contributes to importation and further influenza spread worldwide.

International tourism has increased tremendously, with ≈908 million tourist arrivals at airports worldwide in 2007 (*1*). The annual number of travelers from the Netherlands to tropical and subtropical countries, in a population of ≈16 million persons, doubled from ≈1 million in 1999 to ≈2 million in 2007 (*2*). Increased health risks, particularly infectious diseases, are associated with travel. Prospective studies estimate that up to 64% of short-term travelers experience an illness related to travel to (sub)tropical countries (*3*–*5*). In these studies, respiratory tract infections were the second most frequent infectious disease contracted during travel, with attack rates (ARs) up to 26%; fever affected 11%–19.9% of travelers while they were abroad (*3*–*6*). Influenza is one of the most frequently acquired infectious diseases among travelers (*7*). Respiratory tract infections, including influenza in 6% of cases, commonly caused illness among patients admitted to a tertiary-care hospital after they returned from travel (*8*). Among febrile travelers examined at hospitals after return, influenza was diagnosed in up to 15% (*9*–*11*).

The World Health Organization (WHO) estimates that ≈5%–15% of the worldwide population is affected by seasonal influenza viruses annually (*3*). Outbreaks of influenza associated with travel by air, ship, or train indicate that international travelers are at risk for this infection (*12*–*14*) and may introduce novel strains into domestic populations (*15*,*16*). Indeed, in Europe in 2009, >29% of all confirmed cases of influenza A(H1N1)pdm09 virus were related to travel (*17*). Also, of patients admitted to Tan Tock Seng Hospital in Singapore with confirmed influenza A(H1N1) infection, 25% had traveled by plane after onset of illness, and 15% became ill while traveling (*18*).

The incubation period for influenza is 1–5 days, with adults most infectious from 1 day before symptom onset to ≈5–7 days after symptom onset. In healthy adults, a wide range of symptoms occur, varying from classic influenza and mild illness to asymptomatic infection (*19*). Because influenza is highly contagious and has a short incubation period, travel probably contributes considerably to the rapid spread of the virus (*20*).

In temperate climates, influenza is seasonal: most influenza activity occurs in winter, in the Northern Hemisphere during November–March and in the Southern Hemisphere during April–October. In the tropics, however, the destination of many short-term travelers, influenza virus circulates at low levels year-round (*21*,*22*).

Prospective research on influenza during travel is sparse. To our knowledge, the only prospective study that estimated the AR and incidence rate (IR) of influenza among travelers was a cohort study conducted during 1998–2000 (*6*). This study reported that 1.2% of all travelers had a confirmed influenza virus infection, defined as a ≥4-fold increase in antibody titers, and an influenza incidence of 1.0 per 100 person-months abroad. We prospectively estimated

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the AR and IR for influenza, risk factors for and proportion of symptomatic and asymptomatic travelers, and geographic areas with particular risk.

Methods

Study Population

Persons attending the travel clinic of the Public Health Service Amsterdam during October 2006–October 2007 were recruited for this prospective study. All immunocompetent persons ≥18 years of age were eligible if they were planning to travel for 1–13 weeks to ≥1 (sub)tropical countries. We used the definition of the United Nations' Department of Economics and Social Affairs (*23*) and categorized these countries in 6 regions: South America; Central America and Caribbean; Middle, Western, and Northern Africa; Southern and Eastern Africa; Southeastern and Eastern Asia; and South-central and Western Asia.

All participants were seen by a doctor or nurse who specialized in travel medicine. They received vaccinations, a prescription for antimalarial chemoprophylaxis if required, and oral and written information about how to avoid acquiring travel-related diseases in accordance with national guidelines of the Netherlands for travelers' health (*24*). No additional information was provided about how to avoid respiratory infections. Influenza vaccination is not routinely advised for healthy travelers (*25*,*26*).

Survey Methods

Before departure and 2–6 weeks after return, participants donated venous blood samples for serologic testing. A standard questionnaire was used before departure to collect data on sociodemographic characteristics, travel history, and purpose of the travel (tourism, work or education, or visiting friends and/or relatives [VFR]). History of influenza vaccination was not recorded. Participants were given a thermometer and asked to take their temperature if they felt feverish. They were also asked to keep a structured travel diary by recording itinerary, symptoms of disease (such as fever, sore throat, or coughing), and self-treatment or involvement of a doctor. Participants made daily diary entries from the day they arrived at their destination to 1 week after their return, to encompass incubation periods of acute travel-related infections. After travel, the diary was checked for entry gaps and interpretations by a nurse in the participant's presence. The study protocol was approved by the Medical Ethics Committee of the Academic Medical Center Amsterdam (MEC 06/016).

Laboratory Methods and Case Definition

All blood samples were immediately stored at 6°C. Blood samples for serologic testing were centrifuged (Hettich Rotixa 50S, APP/407: program 1 [Hettich Rotixa, Beverly, MA, USA] 10 min. 3,000 rpm [210 × *g*]) and frozen at −80°C within 24 h until use. Paired serum samples collected from each study participant before travel and after return were tested simultaneously. The serum samples were tested for antibodies against influenza viruses by using the hemagglutination-inhibition (HI) assay, which was performed in duplicate according to standard methods (*27*,*28*) with turkey erythrocytes and 4 hemagglutinating units of virus propagated in 11-day-old embryonated chicken eggs. For this purpose, vaccine strains IVR-142 (A/Wisconsin/67/05-like [H3N2]), IVR-116 (A/New Caledonia/20/99-like [H1N1]), B/Malaysia/2506/04, and B/Florida/4/06 were used to represent the epidemic strains that circulated worldwide during the study period (*29*). Ferret antiserum raised against the respective vaccine strains were used as positive controls (mean titers for subtype H3N2: 2,560; subtype H1N1: 1,280; strain B/Malaysia: 480; and strain B/Florida: 1,280). For statistical analysis, a titer of 5 was arbitrarily assigned to serum with a titer <10. Titers were transformed to a logarithmic scale, and geometric means were used for further calculations. Because blood samples were collected 2–6 weeks after return of travel, acute influenza virus infections were not expected, and therefore virus isolation and PCR were not used to detect virus in respiratory tract specimens. Pretravel titers of ≥40 for ≥1 influenza viruses were defined as protective antibody titers. If the posttravel titer for ≥1 influenza viruses was ≥40 and showed a ≥4-fold increase above pretravel titer, we defined it as a (serologically) confirmed influenza virus infection. Antibodies to influenza B viruses can cross-react and overlap to a certain extent with the other influenza B virus.

Fever was registered when participants reported a body temperature of ≥38.0°C or when they recorded fever without temperature. In addition to fever, other clinical symptoms were scored as influenza-like illness (ILI) when participants recorded fever with a temperature of ≥38.0°C or recorded fever without temperature and a sore throat and/or cough according to the WHO definition (*30*). Participants who, in addition to fever, had self-reported "flu" or self-reported "cold" also were included in the ILI definition.

Data Analysis

Data analysis was performed with SPSS version 19.0.0.1 (2010; IBM, Somers, NY, USA) and Stata version 11 (StataCorp LP, College Station, TX, USA). ARs were calculated by dividing the number of study participants displaying seroconversion for ≥1 influenza viruses (indicating infection) by the total number of participants at risk. IRs per 100 person-months were calculated by dividing the number of travelers with confirmed influenza virus infections by the total number of travel months in which participants were at risk for infection. For travelers with

confirmed influenza virus infection, we used half of their travel duration; for travelers without confirmed influenza virus infection we used their total travel duration. Pearson χ^2 tests of association were used to compare categorical variables between any 2 groups. Relative risks of confirmed influenza virus infection were expressed as ratios of the IR (IRR). IRRs and 95% CIs were obtained by fitting Poisson regression models that predicted the incidence of confirmed influenza virus infection according to the variables sex, age, country of birth, previous travel, purpose of travel, travel destination, and fever or ILI as a variable for symptoms. The multivariable model included all variables in a backward stepwise regression. Participants with any missing values were omitted from the regression models. We chose the backward stepwise approach to ensure inclusion of variables involved in suppressor effects and thus to reduce the risk of making a type II error. A p value <0.05 was considered statistically significant.

Results

Study Population

Originally, we recruited 1,273 immunocompetent persons who intended to travel to (sub)tropical countries. Of these, 83 (7%) were excluded: 23 had their travel arrangements cancelled; 42 were lost to follow-up; and 18 did not have enough enough serum collected to perform influenza serology. Of the remaining 1,190 persons, 510 (43%) were male (Table 1, Appendix, wwwnc.cdc.gov/EID/article/19/6/11-1864-T1.htm). Median age was 37 years (interquartile range [IQR] 28–51 years). Most (966 [81%]) previously had visited (sub)tropical countries. Most (1,103 [93%]) participants were born in a Western country; 1,017 (86%) traveled for holiday; 100 (8%) traveled for work or education, and 73 (6%) were VFR. Median travel duration was 21 days (IQR 15–28 days). The most frequently visited continent was Asia (46%); 27% traveled to Latin America and 24% to Africa.

Median time between first blood sample and travel departure was 24 days ([IQR 12–37 days); median time between return and second blood sample was 23 days (IQR 19–27 days). Of all participants, 592 (50%) donated the first blood sample, and 493 (41%) donated the second blood sample during the influenza season in the Netherlands.

Protective Antibody Titers

Of the 1,190 travelers, 839 (71%) had protective antibody titers; 633 (75%) were positive for A(H3N2), 328 (39%) for A(H1N1), 307 (37%) for B/Malaysia, and 370 (44%) for B/Florida (Table 2). Of the 839 travelers with protective antibody titers, 243 (29%) were immune to 2 influenza viruses, 140 (17%) for 3 influenza viruses, and 92 (11%) for 4 influenza viruses.

Confirmed Influenza Virus Infections

Eighty-six travelers had a confirmed influenza infection caused by ≥1 viruses (Table 1). The AR was 7% (95% CI 6%–9%). Of these 86 travelers, 72 (84%) were born in a Western country; 31 (36%) were male, and 75 (87%) had traveled previously. Median age was 43 years (IQR 29–55 years). The travel destination with the highest AR and IR was South-central and Western Asia. The IR for serologically confirmed influenza virus infection per 100 person-months was 8.9 (95% CI 7.1–10.9).

Of the 86 participants, 66 (77%) displayed a rise in antibody titer against 1 influenza virus; 11 (13%) against 2 viruses; 4 (5%) against 3 viruses, and 5 (6%) against all 4 viruses, making a total of 120 recent infections. Of all 120 confirmed influenza virus infections, 44 (37%) were caused by A(H3N2); 29 (24%) by A(H1N1); 23 (19%) by B/Malaysia, and 24 (20%) by B/Florida (Table 2). Twenty-two (18%) infections occurred in travelers who had protective antibodies against the same strain before travel.

Symptoms

Of the 1,190 travelers, 117 (10%) had fever with a median temperature of 38.6°C (range 38.0°C–41.3°C); illness of 40 (3%) met the definition for ILI. Influenza virus infection was confirmed in 15 (13%) of the 117 travelers with fever and in 9 (23%) of the 40 travelers with ILI; 6 travelers had only fever, but their illness did not meet the ILI definition.

The AR of symptomatic (ILI) confirmed influenza virus infection for all travelers was 0.8% (95% CI 0.4%–1.4%) with an IR of 0.9 per 100 person-months (95% CI 1.2–3.2). In the analysis of travelers with symptomatic confirmed influenza virus infection, no determinants were found. For none of the 4 viruses did we find an association between level of posttravel titer and ILI. Only 1 traveler had protective antibody titers against all 4 influenza viruses, displayed a confirmed A(H3N2) infection, and also displayed ILI. This 28-year-old woman was known to have asthma and to have been hospitalized and treated for symptoms of asthma with acetylcysteïne, salbutamol, and amoxicillin.

Of the 9 travelers with symptomatic confirmed influenza virus infection, 7 (78%) sought medical attention abroad, but aside from the asthmatic woman, no hospitalizations were recorded. Of the 15 travelers with confirmed influenza virus infection with fever or ILI, in 7 (47%) symptoms started within 1 week before returning home or shortly after return.

Independent Risk Factors for Influenza Virus Infection

Age, country of birth, and ILI were independently associated with confirmed influenza virus infection (Table 1). IRR was significantly higher for persons 55–64 years of age (IRR 2.6 (95% CI 1.1–6.1) than for persons <25 years;

Table 2. Increase in titer and symptoms of influenza-like illness in 1,190 travelers from the Netherlands to (sub)tropical countries, October 2006–October 2007

Strain, subtype, pretravel titer	No. travelers, n = 1,190	Titer ≥4-fold, no. (%), n = 120*	Influenza-like illness, no. (%), n = 11
A			
H3N2			
<40	557	32 (6)	6 (19)
≥40†	633	12 (2)	1 (8)‡
H1N1			
<40	862	24 (3)	1 (4)
≥40†	328	5 (2)	0
B/Malaysia			
<40	883	20 (2)	2 (10)
≥40†	307	3 (1)	0
B/Florida			
<40	820	22 (3)	1 (5)
≥40†	370	2 (1)	0

*≥4-fold rise between pretravel and posttravel titer and posttravel titer ≥40.
†≥40: protective antibodies.
‡Symptoms probably caused by asthma.

IRRs were significantly higher for travelers born in an African (IRR 3.7 [95% CI 1.4–5.5]) or Latin American (IRR 3.8 [95% CI 1.9–7.8]) country than for persons born in a Western country (IRR 3.8 [95% CI 1.9–7.8]); and IRR was significantly higher for travelers with ILI (IRR 2.8 [95% CI 1.4–5.5]) than for travelers without ILI. Travel duration was not associated with influenza virus infection (Pearson χ^2, p = 0.808). For none of the 4 viruses did we find significant associations between confirmed influenza virus infection and travel destination.

Discussion

This prospective study with short-term travelers to (sub)tropical countries supports earlier studies showing that influenza is 1 of the most frequently acquired infectious travel-related diseases (*7*). The AR for confirmed influenza virus infections was 8%; the IR 8.9 per 100 person-months. The AR and IR of symptomatic (ILI) confirmed influenza virus infection for all travelers was 0.8% and 0.9 per 100 person-months, respectively.

The prospective study by Mutsch et al. (*6*) found an AR for infection with a ≥4-fold increase in antibody titers of 1.2% and an incidence of 1.0 per 100 person-months of travel, lower than the overall AR and IR in our study. Mutsch et al. found an AR for symptomatic infection (defined as fever alone) of 0.9%; in our study, AR for confirmed influenza virus infection with fever was 2% (95% CI 0.7%–2%), which is broadly similar to results of Mutsch et al.

However, comparing these 2 studies is difficult because of different study methods and circumstances. First, the characteristics of the populations differed. Mutsch et al. included travelers ≥12 years of age who had travel duration ≤6 months for almost 3 total influenza seasons, and this could have affected the AR and IR. Further, we serologically tested the entire study population, whereas they tested a subgroup of travelers with febrile illness and a matched group of travelers without febrile illness, possibly leading to an underestimation of influenza infections. They also defined a group of probable cases by a 2.0- to 3.9-fold increase in antibody titer. In our study, a ≥4-fold titer rise in antibody was used as serologic evidence for influenza infection, which is an accepted threshold in the field (*31*). Both studies showed that participants born in African and Latin American countries are more likely to have contracted influenza virus infections during travel (p = 0.029 and p<0.001, respectively) than those born in Western countries. Also, in other studies an association was found between country of birth and risk for certain other infections (*8*,*32*,*33*). Possibly VFR travelers have a higher risk for influenza because they tend to have closer contact with the local population (*8*,*34*,*35*). The GeoSentinel surveillance network (*8*) showed that VFRs and a trip duration of >30 days were associated with influenza. In our study and the study by Mutsch et al. (*6*), travel duration was not significantly associated with confirmed influenza virus infection (p = 0.808). Mutsch et al. found the Indian subcontinent to be a higher risk area (*6*). We also found South-central and Western Asia to have the highest AR and IR of all regions (11%; 14.4/100 person-months), although these rates did not differ significantly from other destinations. In our study, the IRR was significantly higher for persons 55–64 years of age than for persons <25 years, for which we do not have an explanation.

In 3 of the travelers who had confirmed influenza virus infection with fever or ILI, symptoms started within 1 week before they returned home; they were thus considered to be contagious during the flight. In 4 travelers who had confirmed influenza virus infection with fever or ILI, the symptoms started within 1 week after return. These 7 travelers probably imported an influenza virus that could spread in the Netherlands. In the tropics, influenza viruses circulate throughout the year (*21*,*22*). That travel occurs year-round suggests that influenza viruses

are imported continuously and spread to other regions of the world. Because 7 of 1,190 travelers in our study could have imported influenza virus into the Netherlands, the ≈2 million travelers from the Netherlands who visit (sub) tropical countries could theoretically represent ≈12,000 persons importing influenza viruses annually. Because asymptomatic travelers also could be infectious, the number of travelers who import influenza virus is probably underestimated. Indeed, evidence suggests that influenza A(H3N2) viruses originate in Southeastern and Eastern Asia and are spread continuously, causing epidemics worldwide (*16*).

Only 1 traveler with protective antibody titers to all 4 influenza viruses had a confirmed subtype H3N2 infection and ILI. Although we did not register influenza vaccination status, protective antibodies in this traveler probably resulted from vaccination. This traveler had asthma, and all asthma patients are offered free influenza vaccinations by their general practitioner annually. The influenza vaccine contained the same 4 strains for which we tested. The traveler was hospitalized during travel with asthma symptoms. Symptoms could have been part of the asthma spectrum and might not have been caused by the influenza virus infection. In that case, none of the participants in our study with protective antibody titers had a symptomatic influenza virus infection (i.e., all symptomatic infections occurred in susceptible travelers) (Table 2). Many travelers had pretravel protective antibody titers to ≥1 influenza viruses, which may be explained by a history of infection with influenza viruses and not by influenza vaccination because, in the Netherlands, influenza vaccination is not routinely advised for healthy travelers.

Our study has several strengths. The prospective nature of the study enabled an estimation of the AR and IR of confirmed influenza virus infection with ILI or fever and of asymptomatic confirmed influenza virus infections. The daily diary entries, which minimize recall bias, provide a good record of symptoms during travel. A high percentage (90%) of study participants with fever used the thermometer that was offered before travel to measure their body temperature. The HI assay is the method of choice for seroepidemiologic surveys because it is relatively easy to perform and can be used to detect recent infections using preconvalescent-phase and convalescent-phase serum samples (*6*,*36*–*38*).

Our study also has some limitations. First, because blood samples were taken some time before and after travel, participants could have been infected with influenza virus in the Netherlands. About half of the follow-up time was spent during the influenza season in the Netherlands and not abroad, which might have resulted in an overestimation of travel-related influenza. Mutsch et al. had the same limitation. In Europe, the 2006–07 influenza season began in November and peaked in January and was reported to be generally mild. In the Netherlands, the 2006–07 influenza epidemic, with a maximum clinical influenza activity of 8.2/10,000 inhabitants/week, was among the 3 smallest registered since 1969 (*39*), suggesting that the number of influenza virus infections contracted in the Netherlands might have been low. Because the Southern Hemisphere also had mild influenza activity during our study period (*40*), the higher incidence of confirmed influenza virus infections in our study compared with that of Mutsch et al. is difficult to explain, possibly because of differences in the sensitivity of the HI assays used. Furthermore, the contribution of other infectious diseases to disease symptoms cannot be excluded. Although influenza B/Malaysia/2506/04 and B/Florida/4/06 viruses belong to different lineages, antibodies against these viruses may cross-react to a certain extent. Therefore, seropositivity to these viruses should not be considered independent events. Because we found 120 infections in 86 travelers, including 23 B/Malaysia and 24 B/Florida virus infections, possible cross-reactions could have led to overestimation of the number of influenza B virus infections.

Vaccination of all travelers against influenza has been discussed (*22*). In Canada the Committee to Advise on Tropical Medicine and Travel recommends influenza vaccination to all healthy travelers. WHO recommends annual influenza vaccination only for travelers who have conditions that place them at high risk for complications of influenza. In the Netherlands, as in many other countries, influenza vaccination is already recommended for these risk groups, irrespective of travel. Because travel is not a risk factor for severe disease, we believe that there is no need to advise influenza vaccinations to all healthy travelers. In case of an influenza pandemic with a new strain, vaccination could play a role in the control of outbreak, but not in the beginning, because a new vaccine will not readily be available (*22*).

In conclusion, short-term travelers to (sub)tropical regions contract influenza regularly, which is probably a major factor in the epidemiology of influenza. Fifty percent of travelers with symptomatic influenza could have imported the virus into the Netherlands. Because travelers often visit (sub)tropical regions, where influenza viruses continuously circulate, after contracting the disease they become vectors that further spread the virus worldwide.

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Haemophilus influenzae Serotype a Invasive Disease, Alaska, USA, 1983–2011

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Before introduction of *Haemophilus influenzae* type b (Hib) vaccines, rates of Hib disease in Alaska's indigenous people were among the highest in the world. Vaccination reduced rates dramatically; however, invasive *H. influenzae* type a (Hia) disease has emerged. Cases of invasive disease were identified through Alaska statewide surveillance during1983–2011. Of 866 isolates analyzed for serotype, 32 (4%) were Hia. No Hia disease was identified before 2002; 32 cases occurred during 2002–2011 (p<0.001). Median age of case-patients was 0.7 years; 3 infants died. Incidence of Hia infection (2002–2011) among children <5 years was 5.4/100,000; 27 cases occurred in Alaska Native children (18/100,000) versus 2 cases in non-Native children (0.5/100,000) (risk ratio = 36, p<0.001). From 12/2009 to 12/2011, 15 cases of Hia disease occurred in southwestern Alaska (in children <5 years, rate = 204/100,000). Since introduction of the Hib conjugate vaccine, Hia infection has become a major invasive bacterial disease in Alaska Native children.

Haemophilus influenzae is a bacterial pathogen that can cause serious invasive disease. The organism is classified by the presence of a capsular polysaccharide (6 STs, a-f) or its absence (nonencapsulated or nontypeable strains). In Alaska, before introduction of *H. influenzae* serotype b (Hib) vaccine, rates of invasive Hib disease among Alaska Native people were among the highest in the world (*1*,*2*). Disease caused by Hib was reduced significantly after the 1991 introduction of the Hib conjugate vaccine, polyribosylribitol phosphate outer membrane protein (PRP-OMP) (*3*); however, this vaccine does not provide protection against the other capsular or nontypeable strains (*4*). *H. influenzae* serotype a (Hia), in particular, has been reported as the cause of serious disease in young children (*5*–*7*). We previously described invasive Hia disease in the North American Arctic (Northern Canada and Alaska) from 2000 through 2005 (*8*) and reported an outbreak of 5 episodes of invasive Hia disease in 3 children during 2003 in southwestern Alaska (*9*). Sporadic cases were reported from 2005 through 2009. In this report, we describe an outbreak of 15 cases of invasive Hia disease that occurred from December 2009 through December 2011 in neighboring areas and review the microbiology and epidemiology of Hia disease in Alaska from 1983 to 2011.

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Methods

Statewide surveillance for all invasive *H. influenzae* disease in Alaska has been conducted since 1980 by the Centers for Disease Control's Arctic Investigations Program (AIP) based in Anchorage, Alaska. Clinical laboratories are requested to send *H. influenzae* isolates recovered from a normally sterile site (e.g., blood, cerebrospinal fluid, pleural fluid, etc.) in a resident of Alaska to AIP for confirmation of identity and serotyping. *H. influenzae* is confirmed by using Gram stain and factor X and V requirements (Differentiation Disks; Difco Laboratories, Detroit, MI, USA). Serotyping is conducted by using slide agglutination (*H. influenzae* types a–f typing antisera; Difco). Since 2005, a small number of *H. influenzae* cases have also been identified by PCR on specimens from patients whose conditions had been treated with antimicrobial drugs before samples were collected and whose samples from sterile sites were culture negative. PCR amplification of serotype-specific genes was done by using primers

and probes as reported by Maaroufi, et al. (*10*). Multilocus sequence typing (MLST) of 7 housekeeping gene loci was done on all invasive Hia isolates according to a previously described method (*11*). The sequence type (ST) assignments were made by using the *H. influenzae* MLST website (http://haemophilus.mlst.net/). Clonal complexes were assigned by using the eBURST algorithm with software available at the MLST website (www.mlst.net). The IS*1016*-bexA deletion was amplified from genomic DNA by PCR by using sense IS*1016* (5′-ATTAGCAAGTATGCTAGTCTAT-3′) and antisense bexA (5′-CAATGATTCGCGTAAATAATGT-3′) primers (*12*). AIP uses a standardized form to collect demographic and clinical data from medical records for each reported case. Each year, a list of submitted samples is reconciled with culture results from each participating laboratory, and case lists are compared with state-reportable disease data. Serotyping of invasive *H. influenzae* isolates was introduced gradually; the practice grew from serotyping isolates from 8% of collected samples in 1980 to serotyping 80% by 1983. Because serotyping was intermittent from 1980 to 1982, we reviewed data from 1983 to 2011.

A case of invasive *H. influenzae* disease was defined by isolation of *H. influenzae* from a normally sterile site, including blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, or joint fluid from a resident of Alaska. Cases in patients with clinical epiglottitis (who had *H. influenzae* isolated from an epiglottis swab sample) were also reportable. The clinical description of *H. influenzae* infection was determined by a review of the discharge diagnoses in each case-patient's medical record. When case-patients had multiple discharge diagnoses, the diagnoses related to invasive Hia infection were ranked according to severity, from highest to lowest, in the following order: meningitis, epiglottitis, pneumonia, pericarditis, osteomyelitis, septic arthritis, and septicemia with unknown focus.

We defined an outbreak of invasive Hia disease as the occurrence of confirmed cases of invasive Hia infection by isolates within the same clonal complex among persons residing in the same region with incidence rates above baseline. Population denominator data for Alaska were obtained from the Alaska Department of Labor and Workforce Development website (www.labor.state.ak.us). Estimates for calculating rates of Hia disease in Alaska reflect population figures derived from the 2000 and 2010 census counts; rates were calculated for the years 2002–2011. During 2002–2011, Alaska's population ranged from 640,841 to 722,190; Alaska Native peoples comprised 19% of the population. Seventy-five percent of the population resided in urban centers; the remaining population was dispersed widely across 586,412 square miles. We defined Region A as the southwestern region of Alaska, which has a population of ≈25,000 persons.

Results

Descriptive Epidemiology

We identified 958 cases of invasive *H. influenzae* disease during 1983–2011 (data not shown); of these, 858 isolates were available for serotyping by slide agglutination. Eight additional culture-negative sterile site samples obtained from patients with clinically compatible illnesses who had been treated with antimicrobial drugs were identified, and isolates were serotyped by using PCR. Among samples serotyped, 617 were serotype b and 158 were nontypeable isolates. Of the remaining 91 typeable, non–serotype b isolates, 44 (48%) were serotype f, 32 (35%) were serotype a, 13 (14%) were serotype e, and 2 (2%) were serotype d (Table 1). Hif and Hie isolates were identified from 1985 onward; the first Hia isolate was identified in 2002. During 1983–2001, none of the 30 encapsulated, non–serotype b isolates were Hia; however, from 2002 through 2011, 32 (52%) of 62 non–serotype b isolates were Hia ($p<0.001$ vs. 1983–2001) (Figure 1). Five (16%) of 32 Hia cases were identified by PCR.

Invasive Hia Cases, 2002–2011

Among the 32 Hia cases, 28 (88%) occurred in children <2 years of age, 1 (3%) occurred in a child 2–4 years, and 3 (9%) occurred in persons ≥5 years (range 8–48 years). The median age of case-patients was 0.7 years (range 0.3–48 years). Ethnicity data were available for all cases; 28 (88%) occurred in Alaska Native persons. Cases occurred in 5 areas of Alaska; 21 (66%) cases occurred in Region A (Figure 2). Among all invasive Hia cases, the most common clinical syndromes were meningitis (12 [38%]), pneumonia with bacteremia (11 [34%]), and septic arthritis (6 [19%]). All cases of meningitis occurred in children <2 years of age; there were no cases of epiglottitis. Twenty-seven (84%) case-patients were hospitalized. Three case-patients <1 year of age died (case-fatality ratio 9%). Twenty-five (78%) case-patients <5 years of age had been vaccinated

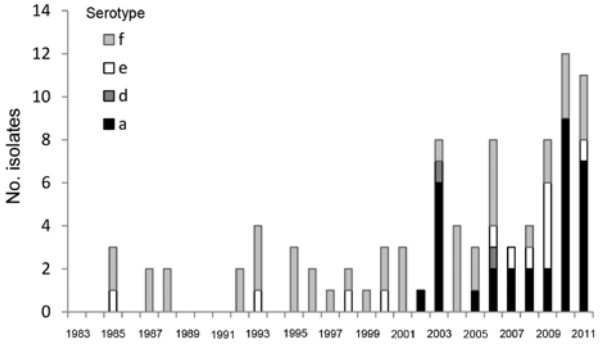

Figure 1. Reported cases of non-b encapsulated *Haemophilus influenza* disease, Alaska, 1983–2011.

Table 1. Typeable and nontypeable *Haemophilus influenzae* isolates, Alaska 1983–2011

Year	Isolate type							
	a	b	c	d	e	f	NT*	Total
1983	0	71	0	0	0	0	4	75
1984	0	73	0	0	0	0	5	78
1985	0	88	0	0	1	2	2	93
1986	0	82	0	0	0	0	3	85
1987	0	52	0	0	0	2	4	58
1988	0	60	0	0	0	2	0	62
1989	0	44	0	0	0	0	1	45
1990	0	44	0	0	0	0	2	46
1991†	0	22	0	0	0	0	3	25
1992	0	3	0	0	0	2	0	5
1993	0	8	0	0	1	3	2	14
1994	0	2	0	0	1	0	11	14
1995	0	4	0	0	0	3	10	17
1996	0	8	0	0	0	2	4	14
1997	0	9	0	0	0	1	6	16
1998	0	5	0	0	1	1	8	15
1999	0	8	0	0	0	1	2	11
2000	0	9	0	0	1	2	3	15
2001	0	1	0	0	0	3	6	10
2002	1	1	0	0	0	0	8	10
2003	6	2	0	1	0	1	8	18
2004	0	2	0	0	0	4	5	11
2005	1	4	0	0	0	2	2	9
2006	2	3	0	1	1	4	7	18
2007	2	0	0	0	1	0	10	13
2008	2	3	0	0	1	1	13	20
2009	2	4	0	0	4	2	8	20
2010	9	3	0	0	0	3	11	26
2011	7	2	0	0	1	3	10	23
Total	32	617	0	2	13	44	158	866

*NT, not typeable.
†*Haemophilus influenza type* b conjugate vaccine introduction in Alaska.

for Hib at appropriate ages. No seasonal pattern among Hia cases was observed. Two distinct outbreaks, 1 in 2003 (5 cases) (*9*) and the other in 2009–2011 (15 cases), comprised 63% of the 32 invasive Hia cases that occurred from 2002–2011. Although there were 20 Hif cases during the same period, the epidemiology differs from Hia cases; Hif cases tend to occur in older persons (median age of case-patients: 55 years), in non–Alaska Native persons (70%), and a higher proportion of case-patients (45%) have a clinical syndrome of pneumonia with bacteremia.

Description of the 2009–2011 Hia Outbreak

From December 1, 2009, through December 31, 2011, 15 cases of Hia occurred in Region A, 10 (67%) of which were identified either in the largest community (4 cases) or in nearby villages (6 cases). Thirteen of the 15 cases occurred in Alaska Native children <2 years of age; 1 was in an Alaska Native child 8 years of age, and 1 was in a non-Native adult, who was 48 years of age. The median age of case-patients was 0.8 years (range 0.3–48 years). Of the 13 cases in Alaska Native children <5 years of age, 8 (62%) were in girls. There was 1 death (case-fatality ratio 8%). Primary clinical conditions included meningitis (n = 6), pneumonia with bacteremia (n = 3), septic arthritis (n = 2), cellulitis (n = 1), and bacteremia (n = 1). Four case-patients had underlying conditions, including a history of otitis media, chronic pulmonary disease, premature birth, esophageal reflux, abnormal heart murmur, or pulmonary valve stenosis, and 1 child experienced a stroke while hospitalized. Of these children, 1 was not vaccinated with PRP-OMP at the appropriate age.

Incidence Rates

The overall rate of invasive Hia disease in Alaska during 2002–2011 was 5.4/100,000 population (95% CI 3.6–7.7) among children <5 years of age; however, the rate in Alaska Native children <5 years was 36 times higher than in non-Native children (18/100,000 versus 0.5/100,000, p< 0.0001). Overall rates of invasive Hib and Hif disease in children in Alaska <5 years of age during 2002–2011 were 2.8/100,000 (95% CI: 1.6–4.6) and 0.9/100,000 (95% CI: 0.3–2.2), respectively. Invasive Hia disease occurred primarily in Region A, where the rate among children <5 years of age was 72.3/100,000 compared with 1.2/100,000 in children <5 years of age in the rest of the state (p<0.0001). During 2010, the year in which the most cases occurred during the most recent outbreak, the rate of invasive Hia disease in children <5 years of age in Region A was 250/100,000 compared to the rate for previous years of 45/100,000 (p<0.001 Fisher exact test).

Subtyping Data

MLST of the 27 Hia isolates yielded 3 sequence types (STs), which are shown by month and year (Table 2; Figure 2). eBURST analysis showed that these 3 STs fell into a single clonal complex: CC23. ST576 was identified 4 times during the 26-year surveillance period and is a double locus variant of ST23. Of 14 ST23 isolates, 9 (64%) were found during 2003–2007. All of the ST56 isolates, which are single locus variants of ST23, were found during 2008–2011. Each of these STs first appeared in a port or major city in Alaska and was subsequently isolated from samples from patients in the western rural regions of the state (Figure 2). None of the Hia isolates had evidence of the IS*1016*-bexA partial deletion.

Discussion

The 2009–2011 outbreak investigation of invasive Hia disease in Region A, highlighted the emergence of a pathogen that was first identified in Alaska in 2002. Three MLST STs were identified during the 10-year period of 2002–2011; the same 3 STs were recently identified in British Columbia by Shuel, et al. (*13*). Although the first appearance of each ST occurred in a port or major city outside of Region A, outbreaks of disease occurred only in Region A, the region that reported the highest rate of childhood respiratory hospitalizations in the state and the

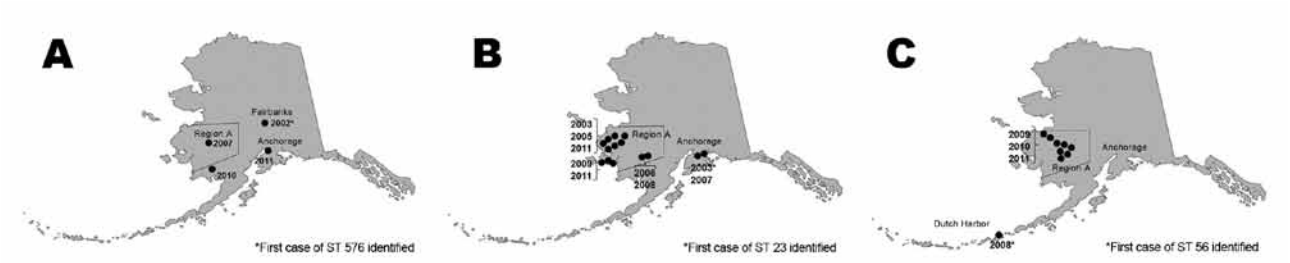

Figure 2. Geographic distribution of invasive *Haemophilus influenzae* type a disease in Alaska, by sequence type (ST). A) ST 576; B) ST 23; C) ST 56.

highest rate of Hib disease in the prevaccine era (*3*). Patterns of spread of these Hia STs showed a tendency of slow expansion within a limited geographic region over a period of years. These data suggest several directions for future research and prevention activities.

Extensive evaluation of isolates of invasive Hi disease (1983–2011), and a large study of *H. influenzae* throat carriage in 1982 (*14*) revealed no confirmed isolates of Hia in Alaska before 2002. Reports of Hia carriage in children in Alaska during the early 1980s (*14*) and invasive Hia disease before 2002 (*3*) were incorrect; subsequent MLST evaluation of these isolates and confirmatory re-evaluation by serotyping determined that they were either nontypeable *H. influenzae* or encapsulated *H. influenzae* of other serotypes. Suboptimal accuracy of serotyping by slide agglutination alone for identification of capsular types in general (*15*) and Hia in particular (*16*) has recently been well characterized and likely explains the original false-positive Hia results.

Hia was not identified in Alaska until 2002 and has since increased and caused outbreaks. Although it is possible that Hia existed in Alaska before 2002, it is unlikely that it was commonly circulating during the 1980s and 1990s. The primary focus of the surveillance and carriage studies in those decades was Hib, and thus, surveillance may have missed an occasional Hia case. Serotyping for non–type b encapsulated strains was routinely performed on available *H. influenzae* isolates in Alaska from 1985 onward, and multiple isolates of nontypeable *H. influenzae*, Hid, Hie, and Hif were identified over the 32 years covered in this report. The subsequent rapid expansion throughout the rest of the study period also suggests recent introduction, as opposed to the uncovering of a previously prevalent strain, as apparently occurred for Hia disease in the American Indian population in the American Southwest after introduction of the Hib conjugate vaccine (*17*).

The epidemiologic pattern of invasive Hia disease in Alaska differs strikingly by geographic region. Single episodes of invasive disease caused by each of the 3 Hia STs were initially identified outside of Region A; later appeared within Region A, where they proliferated and spread. Although disease outside of Region A remained rare, within Region A, invasive Hia disease caused by 2 of the 3 ST groups expanded slowly, but persistently, to contiguous geographic areas. In the central part of Region A, rates of Hia disease during the 2009–2011 outbreak among children <5 years of age approached those of prevaccine invasive Hib disease in Alaska (250/100,000) (*3*).

The disparity in rates of disease by geographic region may be partly explained by several factors that are known to contribute to the elevated risk for respiratory infection in general in Region A. Previous studies have shown associations between the rate of respiratory infections and household crowding, decreased availability of in-home piped water service, indoor smoke, and poverty (*18*,*19*). Each of these factors could play a role in facilitating transmission or invasiveness of Hia disease in human populations. These same factors likely play a role in other populations for whom high rates of Hia disease are routinely described, including residents of the American Southwest (*17*) and northwestern Ontario, Canada (*5*, *6)*, although the factors contributing to increased rates of disease in Utah are less clear (*7*). Recently, invasive Hia cases have also been identified in British Columbia and Manitoba, Canada (*13, 20*). In contrast, Hia is not a major cause of invasive *H. influenzae* disease in other areas of Canada, the United States, and Europe, where these risk factors are not as prevalent and Hif plays a more prominent role (*21–23*). It should also be noted that in the other areas of Alaska, where the above-mentioned contributory factors are less prevalent, the rate of invasive Hia disease between 2002 and 2011 (0.08/100,000) was similar to previously noted rates in the rest of the United States before vaccine introduction (0.04/100,000) (*24*).

The clinical syndrome associated with Hia infection in Alaska from 2002–2011 was similar to that described in Canada, Utah, and the American Southwest (*5,7,17*,*20*): most cases occurred in children <2 years of age, 84% of case-patients were hospitalized, and the case-fatality ratio was 9%. Of the 13 Alaska Native children infected during

Table 2. Sequence type results for *Haemophilus influenzae* type a strains, by date of onset, Alaska, 2002–2011

Year	Month	Sequence type
2002	Nov	576
2003	Jun	23
	Jul	23
	Aug	23
	Oct	23
	Nov	23
	Dec	23
2005	Jul	23
2006	Feb	23
2007	Jul	23
	Oct	576
2008	Apr	23
	Oct	56
2009	Jun	56
	Dec	23
2010	Feb	56
	Jun	56
	Jul	576
	Aug	56
	Oct	56
	Dec	56
2011	Jan	23
	Jan	56
	May	23
	Aug	23
	Sep	576

the 2009–2011 outbreak, 6 (46%) had meningitis, 1 (8%) child died, and 1 child had serious neurologic complications following a stroke the child experienced in the hospital. Although clinical disease associated with Hia was often severe, the Hia isolates from this outbreak in Alaska do not exhibit the IS*1016*-bexA partial deletion, a characteristic classically associated with more severe disease (*12,25*).

Hia infection in infants causes a serious disease with severe sequelae. The severity of disease and increasing rates in recent years have increased interest in identifying prevention and control options. A promising Hia conjugate vaccine, modeled after the Hib conjugate vaccines, has been suggested for control of invasive Hia disease (*4*). However, the population at risk for high rates of Hia disease is so small that further development of this potentially useful tool has not progressed. The presumptive ecologic and transmission characteristics of Hia disease, which rely heavily on analogies with invasive Hib disease, raise the possibility that interrupting person-to-person transmission through chemoprophylaxis may be effective. Current clinical practice in Region A often includes chemoprophylaxis of close and household contacts of Hia case-patients (*26*). However, to this point, there are no reports of secondary invasive disease cases (i.e., a case of invasive Hia disease occurring in a close or household contact of a case-patient with Hia disease), and thus, a key component of the justification for a chemoprophylaxis control strategy is missing: identification of those at risk for secondary disease once a sentinel case has occurred. Finally, several identified risk factors for increased respiratory disease are modifiable, and continuing efforts are underway to address issues of lack of running water, housing ventilation, indoor wood smoke, and other factors, in the high risk area, which may lead to reduced rates of Hia disease. To better define carriage and transmission patterns and further clarify risk factors for invasive Hia disease, AIP is currently evaluating risk factors for disease and Hia carriage among contacts and noncontacts of case-patients in Alaska. These data may contribute to future recommendations for prevention and control of Hia disease in this environment.

The primary limitations of this study are a result of the changing laboratory and surveillance methodologies used over the past 3 decades. Although statewide surveillance of invasive *H. influenzae* disease began in 1980, it was primarily focused on Hib disease, and compliance with the program expanded gradually. We cannot, therefore, confirm that all invasive *H. influenzae* isolates from the early years of surveillance were sent to AIP, or that there was not preferential selection of Hib strains for surveillance. However, by 1983, both nontypeable and non-b encapsulated strains were being received and identified routinely in the AIP laboratory. These isolates were retained, and all Hia strains were subsequently evaluated by MLST by AIP laboratory personnel to confirm strain identity. Although earlier serotyping results suggested the presence of invasive Hia disease in the mid-1990s (and Hia in carriage studies from the early 1980s), subsequent molecular testing and re-serotyping resulted in reclassification of all of these isolates, mostly to nontypeable *H. influenzae*. This experience illustrates the importance of molecular methods in evaluation of epidemiologic patterns, inter-laboratory quality control (*27*), and the importance of close collaboration between laboratory and epidemiology groups.

We confirmed the presence of Hia in Alaska in 2002, and its subsequent emergence as a cause of invasive bacterial disease in an area in rural Alaska associated with high risk for the disease. Its pattern of spread is consistent with slow, person-to-person transmission, with persistence in the high-risk area. Continued surveillance is needed to monitor for outbreaks and galvanize meaningful control efforts. Studies currently in progress in Alaska and other populations should help shape those efforts by providing additional information on risk factors and disease transmission.

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Effect of Winter School Breaks on Influenza-like Illness, Argentina, 2005–2008

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School closures are used to reduce seasonal and pandemic influenza transmission, yet evidence of their effectiveness is sparse. In Argentina, annual winter school breaks occur during the influenza season, providing an opportunity to study this intervention. We used 2005–2008 national weekly surveillance data of visits to a health care provider for influenza-like illness (ILI) from all provinces. Using Serfling-specified Poisson regressions and population-based census denominators, we developed incidence rate ratios (IRRs) for the 3 weeks before, 2 weeks during, and 3 weeks after the break. For persons 5–64 years of age, IRRs were <1 for at least 1 week after the break. Observed rates returned to expected by the third week after the break; overall decrease among persons of all ages was 14%. The largest decrease was among children 5–14 years of age during the week after the break (37% lower IRR). Among adults, effects were weaker and delayed. Two-week winter school breaks significantly decreased visits to a health care provider for ILI among school-aged children and nonelderly adults.

Children play a major role in the transmission of influenza within schools and households (*1–3*). These findings have garnered interest in use of school closures as critical nonpharmaceutical interventions during severe influenza epidemics to mitigate the spread of disease in the community (*4*). These closures might be especially useful in lower resource countries, where access to antiviral drugs and vaccines is relatively limited.

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Recent studies have suggested that school closures might be effective for controlling the spread of influenza during a pandemic and reducing the spread of seasonal influenza (*5–11*). Results from modeling studies vary considerably; estimated case reductions because of school closures range from 40% to 90% (*8*,*11*). Studies that have relied on empirical analysis of disease data have reported narrower ranges, from 0 to 42% (*6*,*7*,*12–14*). For better understanding of the effectiveness of this mitigation strategy, additional studies that rely on multiple years of disease data from other influenza-related school closure experiences are needed.

Argentina, a middle-income country in the Southern Hemisphere, has annual winter school breaks in all provinces. We examined the weekly syndromic surveillance data for influenza-like illness (ILI) from Argentina and estimated the effectiveness of these breaks on incidence of ILI in the community.

Methods

Overview

For all provinces in Argentina, we used province-specific, age-stratified surveillance data on weekly reported hospitalizations and outpatient visits attributable to ILI during 2005–2008 to construct Poisson regression models. We then correlated these data with province-specific school calendars for the same periods. We compared the observed and expected rates of ILI cases during 3 periods: before, during, and after the 2-week winter school break.

Data

Each week, each of the country's 23 provinces and the city of Buenos Aires report all visits to a health care provider for ILI (hospitalizations and outpatient visits, hereafter

referred to as ILI cases) from all Argentina government health care providers and facilities, including hospitals and clinics, to the Argentina Ministry of Health through its National System for Health Surveillance (Sistema Nacional de Vigilancia de la Salud; SNVS). According to the Argentina Census Bureau, 48.1% of the population has no form of health insurance. The SNVS captures health care visits made by these persons as well as by those who seek health care at government facilities (*15*).

Implementation of the SNVS began in 2000 and became fully functional nationwide in 2005. Weekly surveillance data are stratified in the following 10 age groups: <1 y, 1 y, 2–4 y, 5–9 y, 10–14 y, 15–24 y, 25–34 y, 35–44 y, 45–64 y, and ≥65 y. The case definition of ILI for SNVS reporting is temperature >100.4°F with cough or sore throat, possibly accompanied by weakness, muscle pain, nausea or vomiting, runny nose, conjunctivitis, inflammation of the lymph nodes, or diarrhea.

School calendars for each province, including the dates for winter school breaks for public primary and secondary schools, were obtained directly from the Argentina Ministry of Education. Each province independently determines its school calendar at the beginning of the school year; thus, the dates of winter school breaks vary across provinces and years and might not coincide with seasonal influenza peaks. This variation provides a natural experiment for our evaluation. We used data from Argentina's 2001 population census (*16*) for province-level population estimates and assumed that populations remained constant over the study period.

Analysis

To estimate the effect of winter school breaks on ILI cases, we fitted a statistical regression model to ILI surveillance data for each age group and then measured the difference between observed and expected incidence of ILI cases. For our statistical model, we used Poisson regressions with a Serfling specification, a sinusoidal equation that accounts for annual seasonal patterns in ILI outcomes (*17*). By incorporating annual seasonality of influenza activity into the regression model, we could estimate the effect of timing of winter breaks on ILI incidence while controlling for decreases and increases in ILI incidence associated with the annual seasonal patterns characteristic of influenza. The dependent variable was the number of ILI visits for each province, by age group and week. The independent variables were time trends (linear and quadratic effects), sinusoidal terms to account for seasonal patterns of ILI or influenza activity; fixed effects for geographic region, year, weeks with winter school breaks; and variables for each of the 3 weeks that immediately preceded or followed winter school breaks (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/6/12-0916-Techapp1.pdf). We also included interaction terms between year and region, year and sinusoidal terms, and region and seasonal terms (online Technical Appendix). Our model allowed for differences in the seasonality of ILI visits across regions and years, which might result from climate variety in Argentina, province response to ILI, and the timing and peak of influenza circulation in the local community. Model fit was evaluated by using pseudo R^2 values and comparing plots of predicted versus reported ILI cases.

Effects of winter school breaks on ILI cases were estimated separately for each of the following age groups: <5 y, 5–14 y, 15–24 y, 25–44 y, 45–64 y, and ≥65 y. These age groups represent aggregate data from the SNVS that better match groups of persons at different school grades or different stages of life. We report the results of these estimations as ILI incidence rate ratios (IRRs) for the 3 weeks immediately before, 2 weeks during, and 3 weeks immediately after the winter school breaks. IRRs, as used in our analysis, estimate whether incidence of ILI-associated visits to a physician in a particular week were lower, higher, or did not deviate from the expected seasonal ILI patterns. That is, statistically significant IRRs <1 or >1 indicate that ILI cases during a particular week for a specific age group were below or above the estimated seasonal trend, respectively. Conversely, an IRR that is not statistically significant suggests that the number of ILI cases in a particular week did not deviate from expected cases of ILI. We repeated this analysis for each of Argentina's 6 regions: Argentine Northwest, Gran Chaco, Mesopotamia, Cuyo, Pampas, and Patagonia.

We also estimated the number of ILI episodes prevented by winter school breaks, defining them as the difference between observed and expected ILI in a scenario without winter school breaks. That is, we assumed that there were no winter school breaks and used the results of the regression model to predict ILI cases without the reductions in ILI visits with the weeks during and immediately after school breaks.

The investigation protocol was reviewed by the Argentina Ministry of Health and the Centers for Disease Control and Prevention and was given a nonresearch determination. All analyses were performed by using Stata statistical software version 10.1 (StataCorp LP, College Station, TX, USA).

Results

During 2005–2008, a total of 4,376,181 cases of ILI were reported to the SNVS; an average of ≈20,900 cases occurred per week, or an average of 63 cases per 100,000 population nationwide (based on population estimates from the 2001 population census) (*16*). Rates of reported ILI varied significantly across provinces over the 4-year

Table 1. Incidence of visits to a health care provider for influenza-like illness and timing of first week of winter school break in Argentina, by province, 2005–2008

City or province	Region	2001 Argentina population, no. (%)	Average weekly incidence (95% CI)*	Epidemiologic weeks of winter break†			
				2005	2006	2007	2008
Buenos Aires City	Pampas	2,776,138 (7.7)	16 (0.63–75.64)	28–29	28–29	30–31	31–32
Province							
Buenos Aires	Pampas	13,827,203 (38.1)	43 (3.63–149.38)	28–29	29–30	30–31	31–32
Catamarca	Northwest	334,568 (0.9)	113 (14.47–299.56)	28–29	29–30	29–30	29–30
Cordoba	Pampas	3,066,801 (2.7)	56 (5.83–184.77)	28–29	29–30	28–29	28–29
Corrientes	Mesopotamia	930,991 (1.1)	59 (7.81–199.56)	28–29	29–30	28–29	29–30
Chaco	Gran Chaco	984,446 (8.5)	143 (36.75–401.55)	29–30	29–30	29–30	30–31
Chubut	Patagonia	413,237 (2.6)	100 (22.93–244.67)	28–29	29–30	28–29	28–29
Entre Rios	Mesopotamia	1,158,147 (3.2)	113 (19.09–341.92)	28–29	29–30	28–29	29–30
Formosa	Gran Chaco	486,559 (1.3)	122 (24.31–411.54)	28–29	29–30	28–29	29–30
Jujuy	Northwest	611,888 (1.7)	127 (33.03–325.24)	28–29	28–29	29–30	29–30
La Pampa	Pampas	299,294 (0.8)	84 (0–263.86)	28–29	28–29	28–29	29–30
La Rioja	Northwest	289,983 (0.8)	11 (0–38.81)	28–29	29–30	28–29	29–30
Mendoza	Cuyo	1,579,651 (4.4)	57 (0.35–171.09)	28–29	29–30	28–29	29–30
Misiones	Mesopotamia	965,522 (2.7)	169 (28.49–604.50)	29–30	29–30	28–29	29–30
Neuquen	Patagonia	474,155 (1.3)	78 (9.55–233.61)	29–30	29–30	29–30	29–30
Rio Negro	Patagonia	552,822 (1.5)	57 (10.40–149.10)	30–31	29–30	29–30	29–30
Salta	Northwest	1,079,051 (3.0)	158 (58.76–333.69)	28–29	29–30	28–29	29–30
San Juan	Cuyo	620,023 (1.7)	36 (2.66–107.17)	28–29	28–29	28–29	29–30
San Luis	Cuyo	367,933 (1.0)	75 (9.37–217.36)	28–29	28–29	28–29	29–30
Santa Cruz	Patagonia	196,958 (0.5)	58 (9.48–138.03)	29–31	28–29	28–29	29–30
Santa Fe	Pampas	3,000,701 (8.3)	38 (2.59–141.07)	28–29	29–30	28–29	29–30
Santiago del Estero	Gran Chaco	804,457 (2.2)	90 (15.83–278.47)	29–30	29–30	29–30	29–30
Tucuman	Northwest	1,338,523 (3.7)	102 (11.39–296.37)	28–29	29–30	28–29	29–30
Tierra del Fuego	Patagonia	101,079 (0.3)	83 (0–223.01)	29–30	29–30	29–30	29–30
All Argentina	Not applicable	36,260,130	63 (11.68–200.37)	Not applicable			

*Visits/100,000 population.
†For reference, epidemiologic week 28 is typically in mid-July.

study period, from 11 cases per 100,000 population in La Rioja Province to 169 cases per 100,000 population in Misiones Province (Table 1). Reports of ILI cases followed a seasonal pattern; yearly peak activity occurred during the winter months of May–August (Figure 1, panel A). Most provinces did not consistently conduct their school breaks during the same week every year, and provinces that began the school breaks earlier in a particular year did not necessarily do so every year (Table 1).

The regression models for each age group provided a good statistical fit to the data; pseudo R^2 values ranged from 0.66 to 0.72. Model goodness-of-fit can also be observed when comparing predicted values against observed number of ILI cases (Figure 1, panel A).

Except for the age groups <5 and ≥65 years, lower incidence rates for ILI visits (i.e., IRR<1) were estimated for all groups for at least 1 of the weeks during or after winter school break, but this effect varied by age group in strength and timing relative to the start of the break (Table 2). Statistically significant IRRs for the age group 15–24 years occurred during the 2 weeks after the winter break. Among adults, 25–44 years of age, significant deviations from seasonal trends in ILI visits were observed for the second week (IRR = 0.83, p = 0.009) after the winter break.

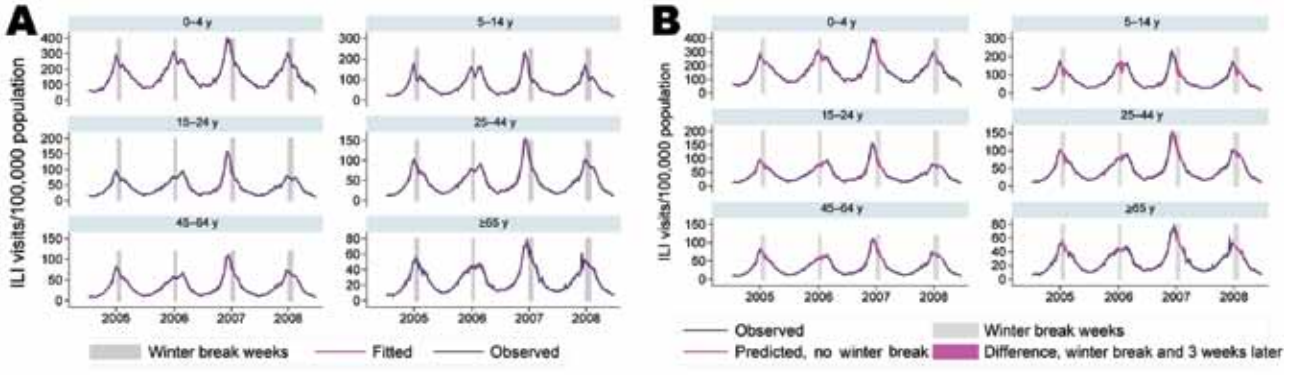

Figure 1. Observed and predicted cases of influenza-like illness (ILI), by age group, Argentina, 2005–2008. A) Observed and model-fitted predictions of incidence. B) Differences between observed cases and model predictions removing the estimated effect of winter school breaks.

Table 2. Estimated IRRs of visits to a health care provider for ILI surrounding winter school breaks, by patient age, Argentina, 2005–2008*

Patient age, y	Time in relation to winter school break, IRR (95% CI)								Pseudo R^2
	3 wk before	2 wk before	1 w before	Wk 1 of break	Wk 2 of break	1 wk after	2 wk after	3 wk after	
0–4	1.09 (0.96–1.24)	1.09 (0.96–1.23)	1.10 (0.97–1.25)	1.01 (0.89–1.15)	0.95 (0.84–1.07)	0.89 (0.79–1.01)	0.93 (0.83–1.05)	1.03 (0.93–1.15)	0.70
5–14	1.09 (0.95–1.26)	1.10 (0.94–1.29)	1.03 (0.90–1.19)	**0.83 (0.72–0.95)**	**0.69 (0.61–0.79)**	**0.67 (0.59–0.76)**	**0.81 (0.71–0.92)**	0.96 (0.85–1.10)	0.70
15–24	1.15 (0.97–1.35)	1.10 (0.95–1.29)	1.04 (0.90–1.19)	0.91 (0.80–1.04)	0.88 (0.77– 1.01)	**0.87 (0.77–0.99)**	**0.86 (0.76–0.97)**	0.95 (0.85–1.08)	0.72
25–44	1.11 (0.94–1.32)	1.07 (0.92–1.26)	1.02 (0.87–1.19)	0.93 (0.80–1.08)	0.90 (0.77–1.05)	0.87 (0.75–1.00)	**0.83 (0.72–0.95)**	0.93 (0.81–1.07)	0.72
45- 64	1.10 (0.94–1.30)	1.07 (0.91–1.26)	1.03 (0.87–1.21)	0.95 (0.82–1.11)	0.92 (0.78–1.08)	0.91 (0.78–1.06)	**0.86 (0.74–0.99)**	0.91 (0.79–1.05)	0.72
≥65	1.05 (0.85–1.31)	1.14 (0.93–1.41)	1.05 (0.87–1.27)	1.04 (0.84–1.28)	1.09 (0.90–1.31)	1.05 (0.87–1.27)	0.99 (0.84–1.17)	1.06 (0.88–1.27)	0.66

*IRRs were used to estimate whether incidence of ILI-associated visits in a particular week were lower, higher, or did not deviate from the expected seasonal ILI patterns. Each row represents a separate regression model. **Boldface** indicates statistically significant changes at the 0.05 confidence level. IRR, incidence rate ratio; ILI, influenza-like illness.

Among all age groups, incidence of ILI visits returned to regular seasonal patterns 3 weeks after the end of the winter break (Figure 2; Table 2).

The largest decrease in observed ILI cases was among school-aged children (5–14 years of age). For this age group, ILI-associated health care visits were 33% ($p<0.05$) lower than expected during the 2 weeks of winter break and the 2 weeks after winter break; this decrease included a 17% decrease (i.e., 1–IRR, where IRR = 0.83, p = 0.008) in the first week of winter school break. The largest deviation from seasonal trends, 33% (IRR = 0.67, $p<0.001$), was observed during the first week after the school break (Table 2; Figure 2). This significant decrease in ILI among school-aged children 5–14 years of age was also found within each of the 6 regions in Argentina (Table 3).

Assuming no winter school breaks, we estimated that during 2005–2008, without school breaks there would have been 77,290 more ILI cases, a 14% increase over observed cases during that period (Table 3; Figure 1, panel B). More than half (38,859 [50.3%]) of the difference in ILI cases occurred among children 5–14 years of age; the group that experienced the second largest difference were young adults, 25–44 years of age (15,989 [20.7%]).

Discussion

Our analysis of weekly rates of ILI cases reported by health care providers throughout Argentina for 2005–2008 found that winter school breaks were associated with significant decreases in the number of cases in school-aged children and in the community at large. The effect on ILI followed a stepwise trend; the 5–14 year age group experienced the initial decrease in ILI during the first week of winter school break, lasting 4 weeks (which includes the first 2 weeks back in school). The effect was then seen among other age groups, each experiencing a smaller decrease in ILI. These findings are significant and biologically and epidemiologically plausible because the effect of school closures on disease transmission could be expected to begin with students and subsequently move to parents of these students and eventually to older family members. These results are consistent within each of Argentina's 6 regions and across the country as a whole.

Our findings support those of previous studies, suggesting that school closure can be an effective mitigation strategy for limiting the spread of pandemic influenza (*5*–*7*,*9*–*11*,*18*). These findings are comparable to those of Cauchemez et al., who also studied ILI surveillance data from outpatient visits in France and found a 16%–21% decrease in seasonal influenza cases that were attributed to winter breaks in that country (*6*). Our results are also consistent with a study that found a 42% decrease in diagnoses of respiratory infections and a 28% decrease in

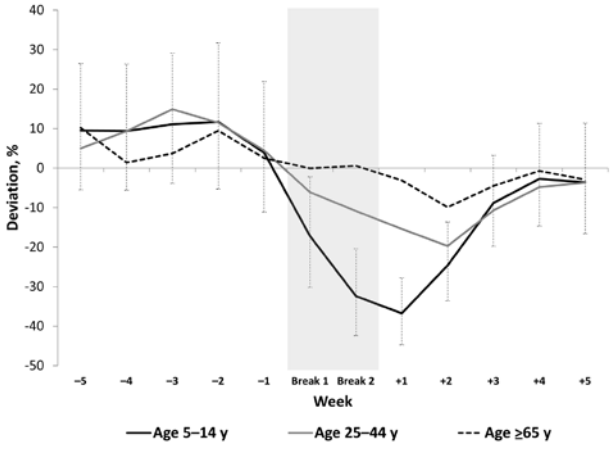

Figure 2. Estimated deviation from predicted incidence rates for influenza-like illness relative to winter break, by week and age group, Argentina, 2005–2008. Dashed lines show the 95% CI for the incidence rate ratios of age group 5–14 years because this is the age group of interest and because it simplifies the display of these results. Statistical significance for the other age groups is shown in Table 2.

Table 3.: Estimated reduction in number of influenza-like illness cases as a result of winter school breaks in Argentina, Argentina, 2005–2008*

Patient age, y	Region, no. cases prevented (% reduction; 95% CI)						
	Argentina	Pampas	Noroeste	Gran Chaco	Mesopotamia	Cuyo	Patagonia
0–4	7,044 (5; –4 to 15)	**9,875 (18; 5 to 32)**	908 (5; –6 to 16)	1,380 (8; –4 to 19)	576 (2; –8 to 13)	141 (2; –20 to 24)	514 (12; –10 to 33)
5–14	**38,916 (33; 19 to 47)**	**19,907 (39; 19 to 59)**	**5,639 (28; 12 to 44)**	**4,776 (33; 15 to 51)**	**4,683 (24; 9 to 39)**	**3,244 (48; 19 to 77)**	**2,293 (44; 19 to 69)**
15–24	**10,064 (14; 1 to 26)**	**4,388 (15; 2 to 29)**	**3,365 (21; 7 to 36)**	**2,126 (27; 10 to 43)**	1,395 (12; –1 to 26)	1,017 (18; –3 to 40)	**1,178 (23; 6 to 39)**
25–44	**16,074 (13; 0 to 26)**	**9,519 (20; 6 to 34)**	**5,715 (21; 9 to 33)**	**3,170 (25; 10 to 40)**	1,235 (9; –4 to 21)	**2,239 (23; 4 to 42)**	**1,236 (13; 1 to 26)**
45–64	6,583 (10; –3 to 23)	**3,711 (13; 1 to 26)**	**4,076 (28; 15 to 41)**	**1,424 (22; 5 to 39)**	518 (6; –7 to 20)	419 (8; –11 to 27)	335 (7; –6 to 21)
≥65	–1,136 (–4; –19 to 11)	1,067 (9; –5 to 22)	263 (5; –10 to 20)	517 (18; –1 to 37)	–164 (–4; –18 to 9)	–136 (–8; –34 to 18)	54 (4; –20 to 27)
Total	77,545 (14)	48,467 (22)	19,966 (19)	13,393 (22)	8,243 (10)	6,924 (20)	5,610 (18)

*Except for the last row, each cell represents a separate regression model. **Boldface** indicates significant changes at the 0.05 confidence level. No confidence intervals are reported in the last row because those cells only represent the sum of other cells in each column.

visits to physicians during a 2-week period of school closure in Israel (*14*).

As an ecologic study that uses a time-trend design, our study is subject to the limitation that our aggregate data cannot be used to make inferences on causality or the effect on individual persons (*19*). Our findings represent reductions in ILI-associated visits to a health care provider, and not laboratory confirmed influenza, although the seasonal increase in ILI among older children and adults is strongly associated with influenza circulation (*20*). Furthermore, the surveillance data we analyzed were obtained primarily from public hospitals and clinics, which account for ≈43% of the health clinics in Argentina and thus might not be representative of the community (*21*). Data are not fully representative, mainly because only 7% of the data reported to the SNVS come from private hospitals, clinics, and providers (*22*). Moreover, our data only included dates of winter breaks in public schools, which account for 77% of all schools in Argentina, because we did not have data on winter school breaks from private schools, which might follow a different school calendar (*23*). We could assume, however, that bias resulting from the incomplete representativeness of the SNVS data and from the school calendar data would move our results toward the null hypothesis.

Another potential limitation of school-closure studies that rely on surveillance data are that observed reductions in disease might be caused by changes in health care–seeking behavior associated with the break and might not represent actual disease reductions. Families might be less likely to seek care during holidays because of travel or other reasons. This limitation is particularly relevant because we analyzed numbers of ILI cases, not ILI rates, as a percentage of all medical visits, as is commonly done to monitor the spread of influenza in the United States (*24*). However, we found that the greatest reductions in ILI were observed in the first week after school reopening, suggesting that the observed decreases in ILI represent true decreases in ILI rather than changes in health care–seeking behavior.

Despite these limitations, a strength of our study is that because the school calendars were set at the beginning of each school year, the timing of winter school breaks was independent of the timing of ILI activity. Furthermore, the dates for winter holidays varied by province and by year, thus allowing for greater differences between a given province's winter school break dates and the province's respective epidemiologic curve. This difference provides our analysis with another source of variation in the explanatory, independent of ILI incidence, resulting in more robust results.

Because of the inherent limitations of ecologic studies, it would be ideal to perform prospective field studies to actually assess the effectiveness of school closures (*25*). Before the emergence of pandemic influenza (H1N1) virus in 2009, most field studies that looked at school closure were in the context of a reactive school closure (i.e., schools were closed because of substantial disease or absenteeism and/or the study group lacked a comparison group), making it difficult to determine the effectiveness of the school closure on disease circulation (*7*,*26*). A study in Israel took advantage of a teachers strike to study the effect of school closure on respiratory diseases and found evidence of an effect on incidence (*14*). A study in Canada found evidence of significant effect of school closures and changes in the weather on the incidence of transmission of pandemic influenza (H1N1) virus (*27*). Another study in a large metropolitan area in the United States took advantage of a natural experiment, in which 1 school district closed its schools for 10 days during the 2009 influenza pandemic, while most schools in a neighboring school district did not close. In that study, the authors found that school closure was associated with fewer self-reported cases of acute respiratory illness and fewer visits to the emergency department for ILI-associated conditions (*28*).

Although school closures might be useful as a mitigation measure during influenza seasons, many additional questions about school closure remain and deserve attention, such as the duration of the closure and the timing with respect to the influenza season. Moreover, closure should probably be accompanied by instructions to not congregate elsewhere. Questions remain about the incidence rate needed to trigger a closure; the social behavior of children when not in school; and the effect of school closure on children, their families, and society. For example, a recent study in Argentina found that the cost of school closure falls disproportionately on the poor (*29*).

Although the effect of winter school breaks was found to be modest, the reduction in disease transmission associated with school closure might slow spread of disease and lessen the effect on hospitals and other health care providers, thus affording extra time to triage limited resources. These factors might be especially crucial when intensive care capacity or antiviral availability are limited, such as in the early stages of a pandemic. Our findings provide additional data for policy makers and public health officials to use when considering such measures to control pandemic influenza.

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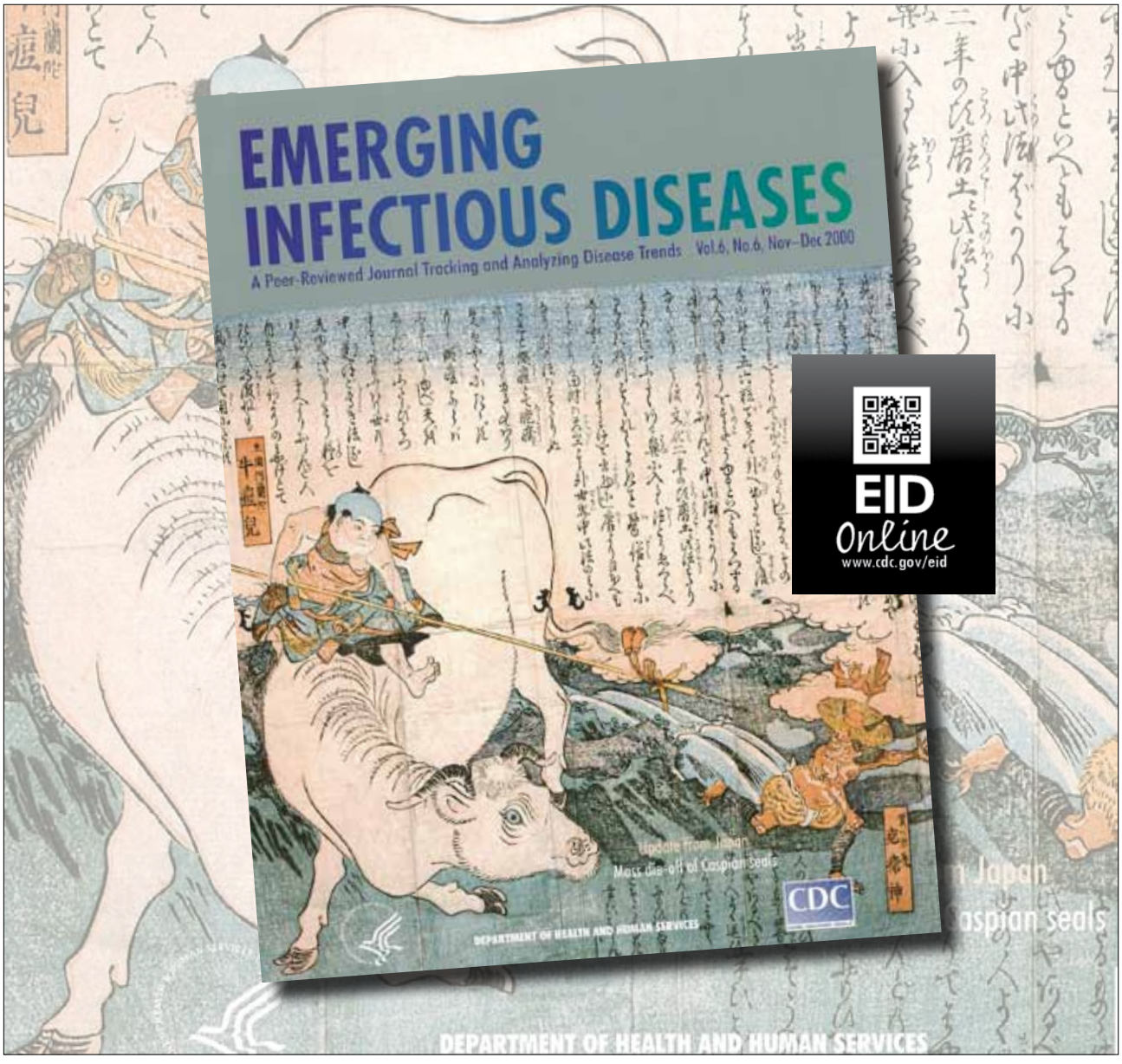

Spatiotemporal Dynamics of Dengue Epidemics, Southern Vietnam

Hoang Quoc Cuong, Nguyen Thanh Vu, Bernard Cazelles, Maciej F. Boni, Khoa T.D. Thai, Maia A. Rabaa, Luong Chan Quang, Cameron P. Simmons, Tran Ngoc Huu, and Katherine L. Anders

An improved understanding of heterogeneities in dengue virus transmission might provide insights into biological and ecologic drivers and facilitate predictions of the magnitude, timing, and location of future dengue epidemics. To investigate dengue dynamics in urban Ho Chi Minh City and neighboring rural provinces in Vietnam, we analyzed a 10-year monthly time series of dengue surveillance data from southern Vietnam. The per capita incidence of dengue was lower in Ho Chi Minh City than in most rural provinces; annual epidemics occurred 1–3 months later in Ho Chi Minh City than elsewhere. The timing and the magnitude of annual epidemics were significantly more correlated in nearby districts than in remote districts, suggesting that local biological and ecologic drivers operate at a scale of 50–100 km. Dengue incidence during the dry season accounted for 63% of variability in epidemic magnitude. These findings can aid the targeting of vector-control interventions and the planning for dengue vaccine implementation.

Dengue is a growing international public health problem for which a licensed vaccine, therapeutic drugs, and effective vector control programs are lacking. The increasing number of cases is associated with an expanding geographic range and increasing intensity of transmission in affected areas (*1*,*2*). The dynamics of dengue in disease-endemic areas are characterized by strong seasonality and multiannual epidemic peaks (*3*), with substantial interannual and spatial heterogeneity in the magnitude of seasonal epidemics (*4*). Extrinsic factors, including climatic and environmental variables, have been hypothesized to drive annual seasonality; intrinsic factors associated with human host demographics, population immunity, and the virus, drive the multiannual dynamics (*5*–*7*). Analyses from Southeast Asia have demonstrated multiannual oscillations in dengue incidence (*8*–*10*), which have been variably associated with macroclimatic weather cycles (exemplified by the El Niño Southern Oscillation) in different settings and with changes in population demographics in Thailand (*11*). In Thailand, a spatiotemporal analysis showed that the multiannual cycle emanated from Bangkok out to more distant provinces (*9*).

Knowledge of spatial and temporal patterns in dengue incidence at a subnational level is relevant for 2 main reasons: it can provide insights into the biological and ecologic mechanisms that drive transmission, and it might facilitate predictions of the magnitude, timing, and location of future dengue epidemics. For both of these reasons, detailed spatial resolution is useful because aggregated datasets can obscure some of the factors that influence the timing and size of individual local epidemics.

In southern Vietnam, dengue occurs year-round; a marked seasonal peak occurs during the rainy months of June–December, and the number of cases has been increasing over the past 15 years (*12*). As in many dengue-endemic settings, the dengue surveillance system in Vietnam relies on passive reporting of clinically diagnosed dengue in hospitalized patients. Vector control is the primary tool available for dengue prevention and control. In Vietnam, vector control is pursued through a targeted approach of low-volume space spraying of households around clusters of reported dengue cases. This strategy faces limitations in timeliness and sensitivity because of the reliance on and response to case reports for hospitalized patients only. A predictive epidemiologic tool that enables prioritization of limited resources for the most cost-effective reduction in cases would be highly valued in dengue-endemic settings.

To investigate spatial and temporal trends for dengue in southern Vietnam, we used a monthly time series of dengue surveillance data over 10 years, disaggregated to the district level. We analyzed the periodicity of dengue incidence, determined whether annual epidemics consistently originate in

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and spread from Ho Chi Minh City (HCMC) or another location, and characterized the differences in the magnitude and timing of epidemics among provinces and districts.

Methods

Study Area and Data Sources

Administrative boundaries for the southern region of Vietnam in 2001 were used for consistency across the study period (2001–2010): this region included 19 provinces, subdivided into 159 districts (Figure 1). In 2009, the total population of the study area was 32.3 million (≈38% of the national population). Demographic data were obtained from the Government Statistics Office (*13*).

As part of the national dengue control program in Vietnam, dengue case notifications in southern Vietnam are aggregated by provincial authorities and reported monthly to the Pasteur Institute, Ho Chi Minh City (PI-HCMC). Only hospitalized dengue patients are reported, and the case definition is a clinical diagnosis of dengue at hospital discharge. A conservative estimate of the specificity of a clinical dengue case diagnosis in Vietnam is ≈50%, based on IgM in 1 serum sample collected from a small proportion (<10%) of patients (PI-HCMC, unpub. data). Most cases are not laboratory confirmed.

The time series used in this analysis included all dengue cases reported from January 1, 2001, through December 31, 2010, from the 19 provinces of southern Vietnam; cases were aggregated by month of hospital admission and district of residence. No identifying personal information was included in the data. The study was approved by the institutional review board of PI-HCMC.

Determining Dengue Periodicity

To explore the periodicity in the dengue incidence time series, we performed continuous wavelet transform, which decomposes the time series into time and frequency components. Calculation of the wavelet power spectrum quantifies the distribution of the variance of the time series in the time–frequency domain (*14*,*15*). The Morlet wavelet was used, and all analyses were performed with MATLAB software version 6.5 (MathWorks Inc., Natick, MA, USA). All time series were square-root transformed and normalized, and the trend was suppressed before analysis by removing periodic components >6 years with a classical low-pass filter (*16*). Significance levels were computed with an appropriate bootstrapping scheme that used the Markov process and preserved the short-term temporal correlation of the raw series, the HMM Surrogate (*17*); 1,000 HMM Surrogate series were used. Significance was set at $p<0.05$.

Quantifying Synchrony

To explore the temporal relationship between dengue time series across the 19 provinces and 159 districts, we subjected each time series to wavelet decomposition as described above. Using the imaginary and the real parts of the wavelet transform in the annual mode (0.8–1.2 years), we computed the phase angles and phase difference between

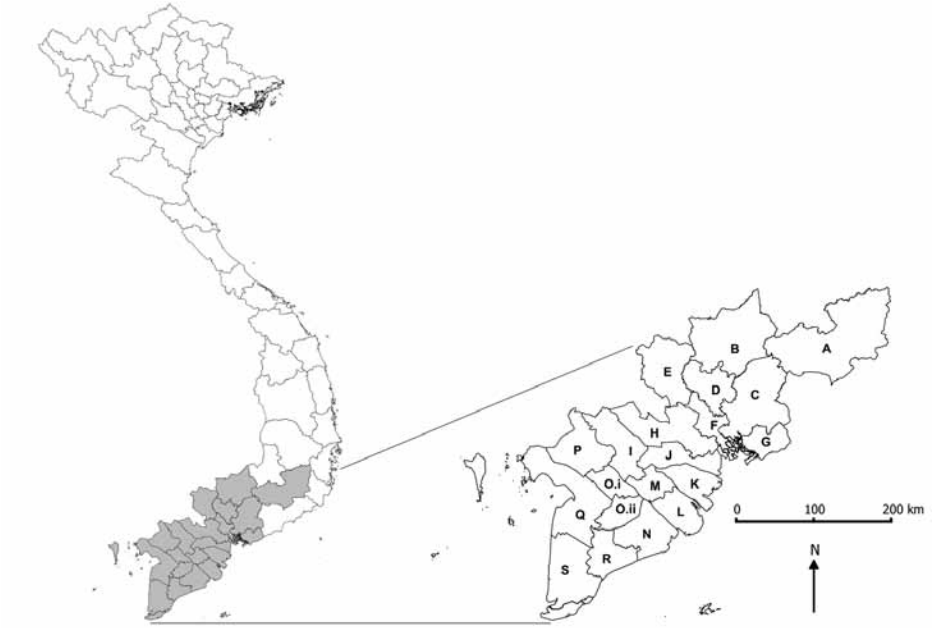

Figure 1. Vietnam and the southern 19 provinces included in this analysis. The map shows current administrative boundaries; for our analysis, we aggregated 2 provinces (Can Tho and Hau Giang) to reflect the administrative boundaries before 2004. A, Lam Dong; B, Binh Phuoc; C, Dong Nai; D, Binh Duong; E, Tay Ninh; F, Ho Chi Minh City; G, Ba Ria – Vung Tau; H, Long An; I, Dong Thap; J, Tien Giang; K, Ben Tre; L, Tra Vinh; M, Vinh Long; N, Soc Trang; O, Can Tho; P, An Giang; Q, Kien Giang; R, Bac Lieu; S, Ca Mau.

2 time series (*18*). The wavelet decomposition was also used as a band-pass filter for filtering the raw time series in the annual mode to obtain the seasonal oscillations, which were used together with the phase differences for computing the pairwise delay (in days) between district and province dengue time series (*8*, equation 8).

We used Spearman and Pearson correlation tests to assess whether larger dengue epidemics were more synchronous, as defined by the variance in pairwise interprovince or interdistrict delays as described above. Additionally, pairwise correlations between district-level and province-level dengue time series were made in 3 transformed datasets: square root–transformed monthly incidence normalized to a mean of 0 and SD of 1 (a correlation in magnitude and timing of dengue epidemics), square root–transformed and normalized annual incidence (a correlation in magnitude only), and phase angles (a correlation in timing only). The relationship between these correlation coefficients and the intervening distance between provinces or districts was assessed by using the nonparametric spline covariance function from the NCF (spatial nonparametric covariance function) package in R (*19*) (R 2.14.2, R Foundation for Statistical Computing, Vienna, Austria) with 1,000 bootstraps to generate 95% confidence bands. We calculated pairwise distances between districts and provinces by using geographic coordinates of district and province centroids in R. To test the hypothesis that similarity in the timing and/or magnitude of dengue epidemics increases with spatial proximity, we performed a Mantel test of the correlation between each matrix of coefficients above and the intervening distance between provinces and districts (in km), by using the NCF package in R (*19*).

Predicting Seasonal Epidemic Magnitude

To determine whether the magnitude of a seasonal dengue epidemic could be predicted by the dengue activity in the previous interepidemic period, we used a linear model of log-transformed dengue incidence during the epidemic period (April–December) in each district or province as a function of the incidence during the preceding dry period (January–March) in the same district or province. These definitions of epidemic and dry periods were decided a priori and were based on scrutiny of the seasonal pattern of dengue across the study period; in HCMC, these periods were shifted (a priori) 1 month later (May–January and February–April, respectively) to account for the fact that the trough in dengue incidence occurred markedly later in HCMC than in other provinces.

Results

Temporal Trends

During 2001–2010, a total of 592,938 dengue cases were reported from the southern 19 provinces of Vietnam; median was 66,608 cases annually (range 22,519–88,311 cases). This finding corresponds to the median annual incidence of 232 cases per 100,000 population (annual range 78–288 cases/100,000 population). Most cases (mean 82%, annual range 74%–92%) were reported during the rainy season, June–December (Figure 2, panel A). Differences

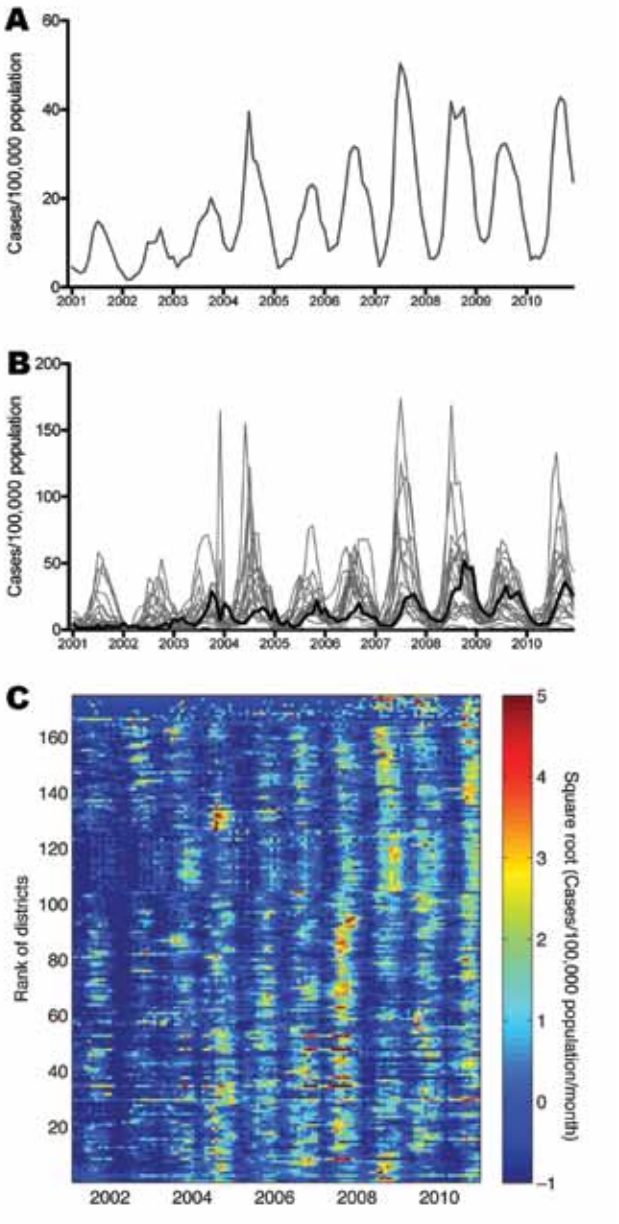

Figure 2. Dengue time series from the 19 provinces and 159 districts in southern Vietnam, 2001–2010. A) Monthly aggregate time series of dengue cases reported from provinces. B) Monthly dengue incidence in each province; **boldface** line indicates Ho Chi Minh City. C) Monthly dengue incidence in each district. Data have been square-root transformed and normalized to zero mean and unit variance. Districts are ordered from north (top) to south (bottom) by first ordering provinces north to south, then ordering districts within each province, according to latitude of district centroid.

in temporal trends between the provinces are apparent in Figure 2, panel B, which shows that in terms of per capita incidence, substantially higher epidemic peaks are reached in provinces outside HCMC than within HCMC. The annual peak also appears consistently later in HCMC than in other provinces. The time series for individual provinces are shown in the online Technical Appendix Figure 1 (wwwnc.cdc.gov/EID/article/19/12-1323-Techapp1.pdf).

A visual comparison of time series between the 159 districts (Figure 2, panel C) suggests overall seasonal synchrony across southern Vietnam but with geographic differences in the timing and magnitude of high-incidence periods at the district level. We explored whether the high dengue incidence outside HCMC represented urban transmission in provincial cities and towns, but we found no parametric or nonparametric correlation between district-level cumulative 10-year dengue incidence and either the proportion of the district population that was rural/urban ($p>0.6$) or the district population density ($p>0.1$).

Dengue Periodicity

Wavelet analysis of the aggregate time series showed a strong annual periodicity but no multiannual cycle (Figure 3, panel A). To investigate spatial differences in dengue periodicity, we performed wavelet analyses for individual province time series (online Technical Appendix Figure 2). An annual cycle was apparent in all provinces but with substantial heterogeneity in the relative strength of the multiannual component. In 3 provinces, a 2–3 year multiannual cycle was either dominant (Bac Lieu and Ca Mau, Figure 3, panels C and D), or of similar intensity as the annual signal (Binh Duong, Figure 3, panel B). In several other provinces, a transient subdominant multiannual cycle was observed, but these cycles are difficult to interpret epidemiologically.

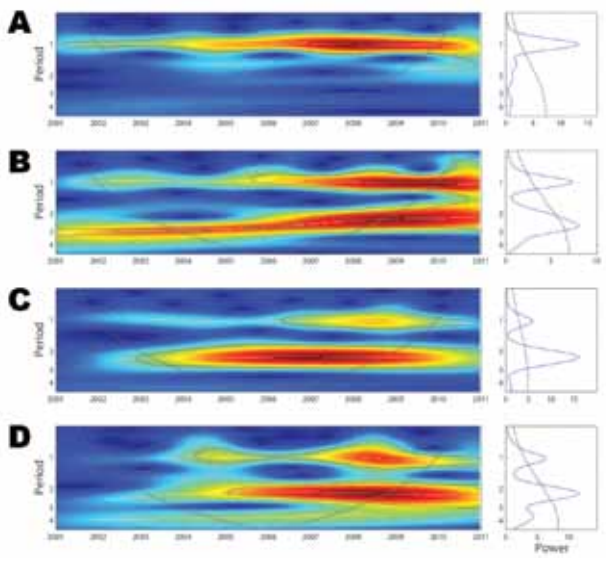

Figure 3. Wavelet analysis of dengue periodicity, 2001–2010. A) Left panel: wavelet power spectrum (WPS) of the aggregate monthly dengue time series for southern Vietnam (square-root transformed, normalized, and trend suppressed). Colors code for increasing spectrum intensity, from blue to red; dotted lines show statistically significant area (threshold of 95% CI); the black curve delimits the cone of influence (region not influenced by edge effects). Right panel: Mean spectrum (solid line) with its threshold value of 95% CI (dotted line) for the aggregate time series. B) WPS and mean spectrum for Binh Duong Province. C) WPS and mean spectrum for Bac Lieu Province. D) WPS and mean spectrum for Ca Mau Province. The wavelet power spectra for Binh Duong, Bac Lieu, and Ca Mau Provinces are shown because they were the only 3 provinces in which a dominant multiannual cycle was detected.

Origins and Spread of Annual Dengue Epidemics

Despite the pronounced seasonality of dengue, we observed substantial heterogeneity in the timing of annual epidemics across the study region and period. The average interval between the province experiencing the earliest and latest dengue epidemic within a given year was 14.2 weeks

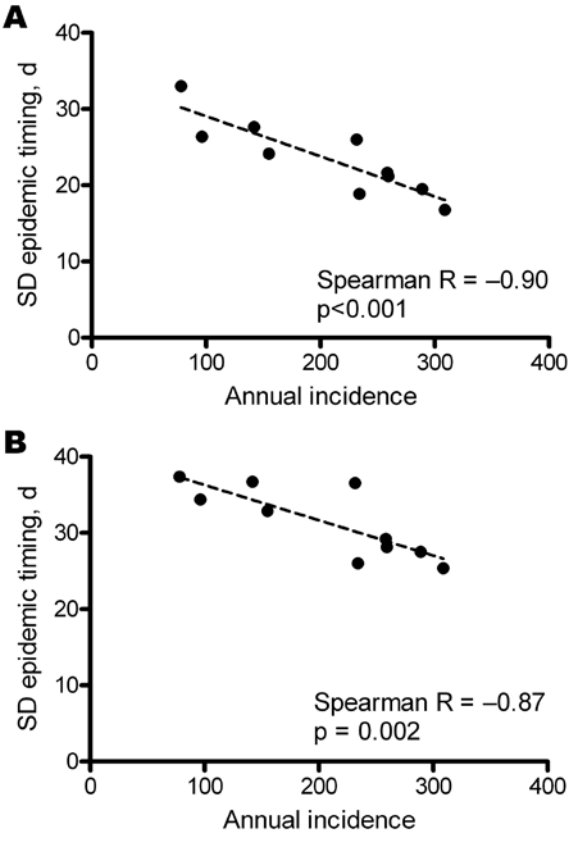

Figure 4. Correlation across provinces (A) or districts (B) between annual dengue incidence and variation in epidemic timing. Epidemic timing represents the pairwise interprovince or interdistrict delay between wavelet transformed annual dengue time series. The variation in epidemic timing is significantly correlated with the overall magnitude of transmission in that year; there is less variation (i.e., more synchrony) in the timing of dengue epidemics across southern Vietnam in high-incidence years than in low-incidence years

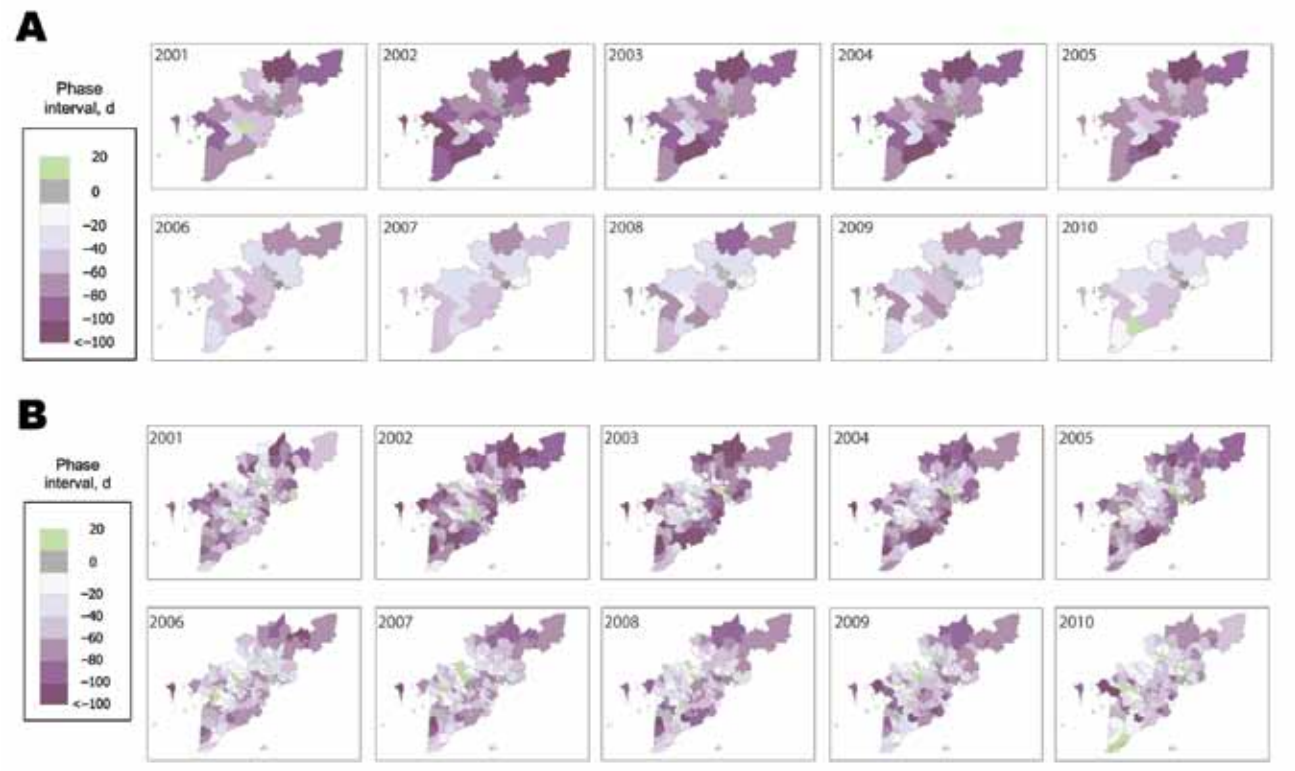

Figure 5. Spatiotemporal patterns in annual dengue epidemics in southern Vietnam. The phase interval (days) between the dengue time series in each province (A) relative to Ho Chi Minh City (HCMC) and each district (B) relative to District 1 in HCMC is shown by year. The largest negative values (dark purple) indicate the earliest locations for the annual dengue epidemics, zero (gray hatched) represents synchrony with the HCMC time series, and positive values (green) indicate dengue epidemics that occurred later than in HCMC.

(annual range 8.9–20.0 weeks). The interprovince and interdistrict lag in the onset of seasonal dengue epidemics was significantly negatively correlated with the overall magnitude of the epidemic (Figure 4); in other words, dengue epidemics are significantly more synchronous throughout the region in years with higher overall incidence than in years with lower incidence.

To explore further the observation that annual dengue epidemics occur later in HCMC than elsewhere, we plotted the phase interval (in days) between the dengue time series in each province relative to HCMC, averaged across the 10-year period (not shown) and in each individual year (Figure 5, panel A). A multifocal origin of seasonal dengue epidemics in southern Vietnam was revealed, in which the epidemic cycle in each of the 18 provinces preceded HCMC by a median of 55 days (range 26–90 days) averaged over the 10-year period. The dengue epidemic occurred later in HCMC than in all other provinces in all but 2 years (2001 and 2010), and in these 2 years, in only 1 province did the dengue epidemic occur later than in HCMC. Figure 5, panel B, shows the equivalent analysis for district-level time series. These analyses indicated that in some locations (Binh Phuoc to the north of HCMC, Lam Dong to the northwest, Soc Trang in the far south, and Kien Giang in the southwest), despite their considerable distance from one another, dengue epidemics were consistently among the earliest each year (Figure 1). However, the earliest epidemics often occur in multiple simultaneous locations, and there is no clear spatial pattern for the movement of the dengue epidemic within a given season. Signals of early epidemics in Lam Dong should be treated with caution because the case numbers for the dry and the rainy seasons were small.

Synchrony in Dengue Dynamics

Overall, dengue epidemics across southern Vietnam were more highly correlated in timing than in incidence (Figure 6, horizontal lines), consistent with the pronounced seasonality of dengue virus (DENV) transmission despite heterogeneities in epidemic magnitude. Coherence in the size of annual dengue epidemics was spatially dependent ($p<0.001$ for provinces and districts; Mantel test). Districts within 100 km of each other were significantly more likely to have concurrent high-incidence and low-incidence years (Figure 6, panel A), and the degree of correlation increased with increasing proximity. This spatial dependence was

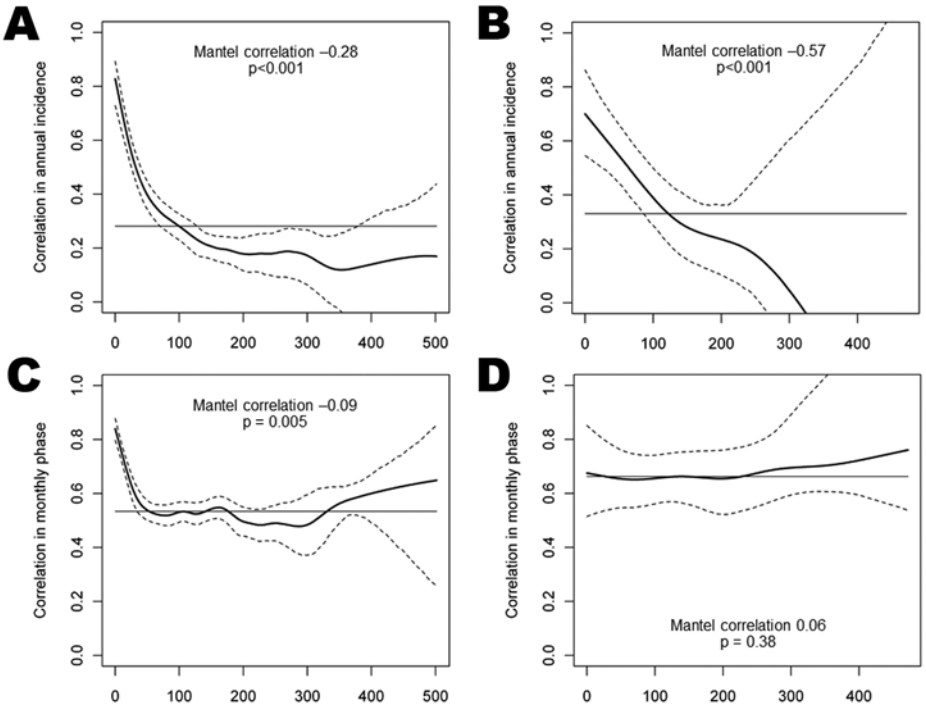

Figure 6. Spatial coherence in the magnitude (A and B) and timing (C and D) of dengue epidemics in southern Vietnam. District data are shown in panels A and C, and province data in panels B and D. Solid lines represent the correlation between provinces/districts as a function of the distance between the centroids of those provinces/districts, in kilometers. Dashed lines represent 95% CIs, and the horizontal line is the overall correlation across southern Vietnam. Coherence in the magnitude and timing of epidemics was measured by pairwise correlation between provinces/districts in their standardized square root–transformed annual incidence and monthly phase series, respectively.

observed also for province-level data (Figure 6, panel B) out to 122 km. The timing of dengue epidemic cycles was less spatially dependent. Nearby districts experienced more synchronous epidemics (p = 0.005), significant out to 52 km (Figure 6, panel C); however, this spatial dependence was not seen at the province level (Figure 6, panel D; p = 0.38). Spatially dependent synchrony out to 101 km was also observed when correlating the raw monthly time series, which takes the timing and the magnitude of dengue epidemics into account (online Technical Appendix Figure 3). Overall, this analysis demonstrates spatial clustering of dengue activity at a scale of up to ≈50–100 km.

Dry Season Dengue as Predictor of Subsequent Epidemic Magnitude

We found a significant positive association between dengue incidence during the dry season and the magnitude of the subsequent dengue epidemic in a given province or district. Using province-level data, we found that an increase of 1 SD above the mean dengue incidence during the dry season was associated with an increase of 0.79 SDs (95% CI 0.70–0.88; p<0.0001) above the mean epidemic magnitude during the subsequent rainy season (Figure 7, panel A). Dry season incidence accounted for 63% of the variation in epidemic magnitude among provinces and years. Stratified by province, this association held for 12 of the 19 provinces (data not shown); this finding might reflect a lack of power to detect such an association with 10 data points. Stratified by year, the association was significant (p≤0.001) for every year during 2001–2010. Using data for 159 districts, we found that the association between dry season and wet season incidence was also highly significant (p≤0.001) overall (Figure 7, panel B) and stratified by province or year (not shown), although the proportion of the total variation in epidemic magnitude accounted for by dry season incidence was lower (45%).

Discussion

In southern Vietnam, dengue exhibits pronounced seasonal peaks that coincide with the rainy season and causes tens of thousands of hospitalizations every year. Within this overall high-transmission setting, substantial spatial and temporal heterogeneity is apparent from our monthly district-level time series analysis. Several characteristics of the epidemic cycle in this setting could help inform public health efforts to prevent and control dengue.

All provinces in southern Vietnam exhibit annual seasonality; however, 2 provinces in the far south (Bac Lieu and Ca Mau) and 1 north of HCMC (Binh Duong) also show evidence of multiannual cycles. These patterns suggest possible differences in the intrinsic and extrinsic drivers of DENV transmission in these provinces; however, interpretation of these findings must take into account the limitations of wavelet analysis, especially within a 10-year time series, in which it is difficult to obtain strong statistical support for long multiannual cycles. Further considerations in the interpretation of our findings relate to the limitations of dengue surveillance data as a proxy for DENV transmission. A variable majority of DENV infections are asymptomatic (*20*), and it is possible that the observed dis-

ease dynamics are an imperfect reflection of the underlying DENV transmission dynamics. Furthermore, case surveillance data for dengue, as for many diseases, have sensitivity and specificity limitations, because of underreporting and a lack of laboratory confirmation, respectively.

Dengue is typically thought of as an urban disease (*1*,*21*–*23*). HCMC is the major urban center in southern Vietnam, but the per capita incidence reached there during seasonal dengue peaks is substantially lower than in most of the other less urban provinces. This finding supports evidence from Cambodia (*24*), Thailand (*25*), and Vietnam (*26*) that dengue presents a health challenge in periurban and rural settings as well as in urban centers. Furthermore, dengue epidemics occur ≈2–3 months later in HCMC than in the surrounding rural provinces, indicating that HCMC cannot act as a source population initiating annual epidemics in other provinces. In fact, the presence of multiple locations with early dengue epidemics indicates that there may not be a consistent year-to-year spatial pattern of DENV transmission and no consistent geographic source from which dengue epidemics emanate. These findings lead directly to new research questions for dengue in southern Vietnam, to explore what factors influence the timing and size of the annual epidemic wave in each province.

Although the dynamics we describe give the appearance of dengue traveling from several early foci to HCMC each season, we think this is unlikely for 3 reasons. First, dengue cases occur throughout the dry season in HCMC as well as in other provinces; hence, re-introduction of DENV is not required to initiate the seasonal increase in cases. Second, we found no correlation between the timing of dengue epidemics in each province or district and their geographic distance from HCMC (analysis not shown); such a correlation would have suggested a traveling wave of infection toward HCMC (*9*,*10*). Third, phylogenetic analyses of DENV-1 (*27*) and DENV-2 (*28*) from southern Vietnam suggest that these viruses disperse from HCMC out to other provinces. Our findings are not necessarily inconsistent with these phylogenetic studies; the former relate to spatiotemporal dynamics in case incidence within 1 dengue season, and the latter describe processes of viral dispersion over several years. Together, these studies suggest that despite a lower per capita incidence, the larger absolute virus population in HCMC exerts an influence on the relatively small virus populations in rural provinces but that other conditions determine the timing and magnitude of the increased transmission during annual dengue epidemics.

Several factors could explain the observed spatial dynamics besides the geographic movement of DENV. First, vector development, survival, and biting behavior and viral replication within the vector are all highly sensitive to climatic conditions including rainfall, temperature, and relative humidity (*29*,*30*), and it is possible that geographic differences in microclimate might contribute to differences in the timing of dengue epidemics across southern Vietnam. Second, the ratio of vectors to human hosts, rather than density of vectors or hosts alone, has been shown to be a key parameter in classical models of vector-borne disease transmission (*31*,*32*) and in epidemiologic studies (*26*). Understanding how this ratio differs between HCMC and lower population-density areas might help explain the lower per capita

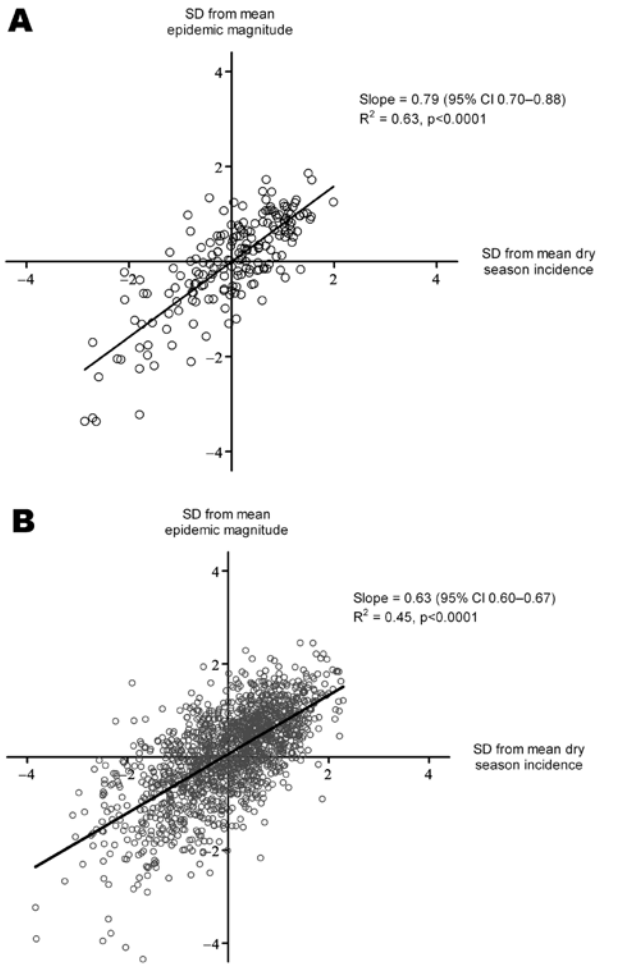

Figure 7. Dry season dengue incidence as a predictor of the magnitude of the subsequent dengue epidemic. Plots show the association between annual epidemic incidence (April–December) and the preceding dry season dengue incidence (January–March). For Ho Chi Minh City (HCMC), these definitions were a priori shifted 1 month later (May–January and February–April, respectively) because of the consistently later occurrence of the dengue epidemic season in HCMC. Each point represents 1 province (A) or district (B) and year, correlating the standard deviation from mean incidence in the rainy season against the standard deviation from mean incidence in the preceding dry season, in the same province or district. The solid line shows fitted values from a linear model of epidemic incidence against dry season incidence. We excluded 71 data points from the district analysis (B) because there were no dengue cases during the dry season.

incidence in HCMC. Third, the later and lower-incidence seasonal epidemics in HCMC are consistent with the higher median age of the population in HCMC (*13*); an older, and thus more immune, population reduces the probability of a vector feeding on a susceptible or infectious person, both of which are necessary to drive transmission.

We demonstrate that the timing and the magnitude of annual dengue epidemics in southern Vietnam are significantly more similar in districts in closer proximity, and this association remains significant out to 50–100 km. This finding suggests a role for local drivers of DENV transmission operating at this spatial scale, possibly including microclimatic and environmental determinants of vector abundance and vector–host contact, population immunity, or human movement patterns at the scale of a district or town. Several studies have demonstrated focal transmission of DENV at a fine spatial scale from 100 m to 1 km, attributable to direct chains of transmission, vector flight distances, human movement, and serotype-specific immune profiles (*33*–*35*). We extended this finding by demonstrating spatial dependence of dengue incidence at an intermediate scale of <100 km, with a weakening association as proximity decreases. This association is comparable with the spatial extent of synchrony (180 km) demonstrated among province-level dengue time series in Thailand (*9*). This finding highlights the need to analyze disease surveillance data at as fine a spatial scale as possible because the spatial dependence of dengue epidemic timing was not apparent in our province-level analysis.

In Vietnam, the public health authority classifies dengue incidence within any administrative boundary as epidemic when cases exceed 2 SDs above the mean incidence in that month and location over the past 5 years (excluding any previous epidemic months). This definition allows little to no lead time for intervention, and the ability to predict further in advance where epidemic thresholds are likely to be crossed could improve the timeliness and possibly the effectiveness of control interventions. Our simple result showing that dengue incidence during the dry interepidemic period accounts for 63% of variability in rainy season dengue epidemic magnitude might help local public health authorities take advantage of dengue's predictable cyclical behavior for informing public health action. This method relies on using data that are available 3–6 months before the peak of DENV transmission, and this extra lead time might be an invaluable resource for targeted dengue intervention planning in years when a season with a large number of dengue cases is expected. Evaluating the performance of this model in real time for forecasting dengue epidemic magnitude is the next challenge in determining its public health utility.

Dengue prevention and control activities in many disease-endemic settings, including Vietnam, currently rely on targeted spraying of adulticides to reduce vector populations in and around the homes of reported patients. These activities are usually complemented with public health outreach and some routine activities to reduce vector breeding sites, within the constraints of limited public health budgets. Future intervention strategies will also incorporate rollout of a dengue vaccine (*36*,*37*) or modified mosquitoes (*38*). Understanding the spatial dynamics and timing of dengue epidemics might enhance the implementation of current and future interventions by improved targeting to avert high-incidence dengue seasons based on dry-season signals and to dampen dengue incidence in neighboring areas.

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Dr Cuong is a staff member of the Training Center of the PI-HCMC and a PhD candidate at the Oxford University Clinical Research Unit, Vietnam. His research is focused on the transmission dynamics of dengue, with the aim of improving dengue control strategies in Vietnam.

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Active Surveillance for Influenza A Virus among Swine, Midwestern United States, 2009–2011

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Veterinary diagnostic laboratories identify and characterize influenza A viruses primarily through passive surveillance. However, additional surveillance programs are needed. To meet this need, an active surveillance program was conducted at pig farms throughout the midwestern United States. From June 2009 through December 2011, nasal swab samples were collected monthly from among 540 groups of growing pigs and tested for influenza A virus by real-time reverse transcription PCR. Of 16,170 samples, 746 were positive for influenza A virus; of these, 18.0% were subtype H1N1, 16.0% H1N2, 7.6% H3N2, and 14.5% (H1N1)pdm09. An influenza (H3N2) and (H1N1) pdm09 virus were identified simultaneously in 8 groups. This active influenza A virus surveillance program provided quality data and increased the understanding of the current situation of circulating viruses in the midwestern US pig population.

Influenza A virus has become a major pathogen, causing epidemics of respiratory disease in humans, which not only result in increased deaths but also raise public health organization alarms regarding the need for further understanding and control of this virus (*1*). Additionally, the ability of the virus to cross species barriers has raised more concern over the probability of reassortment and generation of highly transmissible viruses that might pose a threat to humans (*2*). Despite evidence of reassortment in other species, swine have been most often labeled as the "mixing vessel" because avian- and mammalian-type receptors for influenza A virus have been found in pig tracheas, making swine a potential source of new viruses through reassortment (*3,4*). Because these viruses can infect humans, influenza A virus in swine should be monitored for public health reasons (*5*).

In the United States, influenza A virus has been present in swine for almost a century (*6*). Within the US pig population, 3 major subtypes of influenza A virus (H1N1, H1N2, H3N2) circulate, causing widespread respiratory disease characterized by dry coughing, sneezing, fever, anorexia, rhinorrhea, and lethargy (*7*). Swine influenza viruses have been monitored through seroprevalence studies. Such studies from the 1970s through the 1990s revealed that influenza virus subtypes H1N1 and H3N2 circulated in the US pig population (*8–11*).

At the turn of the 21st century, 2 new viruses were detected in the swine population. These viruses were the result of either double or triple reassortment between human, avian, and swine viruses (*11–13*). Since 1998, circulating influenza viruses in pigs have been able to change because of mutations and the propensity for influenza A virus of swine with the triple reassortant genotype to frequently reassort and generate new genotypes, therefore increasing the diversity of influenza A virus in swine (*14*). Virologic and seroprevalence studies have provided valuable information about influenza A virus in swine, but the epidemiology of influenza A virus in swine is not fully understood.

Newly emerged pathogens can be detected through passive or active surveillance. Passive surveillance is driven by laboratory submission of samples after outbreaks of respiratory disease, whereas active surveillance is based on purposely collecting and screening field samples regardless of clinical status. In Asia, active surveillance for influenza conducted through collection of nasal swabs at slaughter plants has reportedly detected uncommon influenza viruses (i.e., subtypes H3N1, H7N2, H9N2) in the local pig population (*15–17*). In the United States, similar studies, following the same sample collection method, during the early and late 1990s have been reported (*10,18*). However,

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a surveillance program that will identify and report newly emerged viruses in a timely manner is still needed (*14*).

Overall, studies have elucidated epidemiologic features of the virus in swine, such as the constant circulation of influenza A virus in the swine population and sporadic infections with rare subtypes. However, absence of a proactive approach leaves a gap that needs to be filled (*19*). Therefore, the objectives of this study were to 1) conduct an active surveillance program to better characterize the presence of influenza viruses in the swine population and 2) make live viruses available for genetic characterization, potential vaccine, and diagnostic use. Procedures and protocols used in this study were approved by the University of Minnesota Institutional Animal Care and Use Committee

Methods

Farm Selection

Veterinarians who agreed to participate in the study were asked to enroll growing-pig farms (i.e., farms that house pigs 3–30 weeks of age) in the midwestern United States that were representative of modern swine production systems and that were owned by producers interested in participating in the study. Producers were allowed to withdraw from the study at any time.

Sample Collection

From June 2009 through December 2011, participating farms were visited every month for 12–24 consecutive months. At each visit, the investigator would meet with the farm manager/owner to decide which pigs were to be sampled. If pigs were all in 1 age group, samples were collected from that group; if pigs were in >1 age group, samples would be collected from the age group closest to 10 weeks, the group most likely to yield the most influenza A virus–positive pigs, per previous reports (*20*).

A total of 30 nasal swab samples were collected at each visit, enabling us to be 95% confident of detecting at least 1 positive sample when influenza prevalence was at least 10%. Clinically healthy pigs were restrained by a snare, and a nasal swab (Starswab II, Starplex Scientific Inc., Etobicoke, Ontario, Canada) was inserted 2–3 inches into the back of each nostril while being rotated. Nasal swabs were labeled with a specific code containing the farm identification number, month, 2-letter state abbreviation, and nasal swab sample number.

During the visit, the age of the pigs and respiratory clinical signs (absence or presence of sneezing, coughing, and nasal secretion) among the group members were recorded. Nasal swabs and submission sheets were placed into a Styrofoam container with ice packs and shipped overnight to the laboratory for testing.

Sample Testing

All nasal swab samples were tested at the virology department laboratory of St. Jude Children's Research Hospital (Memphis, TN, USA). Nasal swab samples were initially screened for influenza A virus by real-time reverse transcription (RRT-PCR) selective for the matrix gene. Samples that were positive by RRT-PCR underwent further diagnostics for determination of subtype, including the influenza A(H1N1)pdm09 virus and swine H1 and H3 viruses (*21–23*).

Statistical Analyses

A farm was considered positive for a given month if any of the 30 individually collected swab samples tested positive. Farm-level data, such as farm size, were analyzed by repeated measures logistic regression, and differences between farms were accounted for by including farm as a random effect and allowing for an autoregressive effect by month within the farm. Model building was performed by first screening independent variables through univariate analysis. Variables with a p value <0.25 were retained for the multivariable model. All selected variables were forced in the model including 2-way interactions and were sequentially removed if p value was >0.05.

We built 2 models. The first model assessed the relationships between farm status (positive vs. negative) for influenza virus and age, year, clinical signs, and season. The second model assessed the relationship between respiratory clinical signs (presence vs. absence) and subtype and season. Season was included in the models by categorizing the 4 seasons as follows: winter (January–March), spring (April–June), summer (July–September), and fall (October–December). Statistical procedures were performed in SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

Results

Farms were enrolled in the program as agreement to participate (by veterinarians and producers) was obtained; thus, the program started in June 2009 and ended in December 2011. The 33 producers who agreed to participate were located throughout the midwestern United States: 17 farms in Iowa, 4 in Illinois, 8 in Indiana, and 4 in Minnesota. Each of the 33 farms housed 1,000–13,000 pigs. One group of 7 farms in Iowa withdrew from the program in August 2009 after the influenza A(H1N1)pdm09 virus–associated crisis in the swine industry.

Sample Test Results

A total of 16,170 nasal swab samples from 540 groups of growing pigs from the remaining 32 farms were collected. From the total number of samples collected, 746 (4.6%) were positive for influenza A virus by RRT-PCR, and 178 viruses were isolated from these samples. At least

1 positive sample was detected in 117 (21.7%) groups of pigs; thus, these groups were classified as positive. Of the 32 farms, 29 (90.6%) had at least 1 positive group throughout the study. Of the 117 groups with RRT-PCR–positive results for influenza A virus, complete or partial subtype details were obtained for 99 (84.6%) pig groups (Figure 1). Influenza A virus infection with just 1 subtype (H1N1, H1N2, H3N2, or H1N1pdm09) was detected in 21, 19, 9, and 17 groups, respectively. Dual infections were detected in 10 groups, of which 8 concurrently harbored influenza virus subtypes H1N2 and H1N1pdm09, 1 harbored subtype H1N1 and an H3N-untypeable virus, and the remaining group harbored subtype H1N1 and an H1N-untypeable virus. Partial subtyping information was obtained for 16 pig groups, from which 11, 4 and 1 were infected with an H1N-untypeable, H3N-untypeable, and an H1N-untypeable with pandemic matrix gene virus subtype, respectively. In 18 groups, a subtype could not be defined through either RRT-PCR or sequencing. At most farms in our study, groups of pigs were identified as having multiple and different influenza A viruses detected throughout the surveillance period (Figure 1). Viruses were isolated from 178 swab samples that originated from 62 pig groups.

Of the positive groups, the mean and median numbers of samples positive for influenza A virus by RRT-PCR were 6.4 and 2, respectively. The numbers of positive samples ranged from 1 to 30; most groups (n = 48) had 1 positive sample (Figure 2). There were 15, 10, 4, and 2 groups that had 2, 3, 4, and 5 positive samples, respectively. A total of 13 groups had ≥20 positive samples, 2 had 29 positive samples, and 1 had 30 positive samples. Although most samples collected from these 13 groups were positive for influenza A virus by RRT-PCR, pigs in only 7 of these groups exhibited clinical signs of influenza-like illness.

The number of positive groups per farm ranged from 1 to 18. On average, 31% of the groups tested by farm throughout the program were classified as positive (Figure 3). Farms with no influenza A virus–positive results were monitored for ≈1 year but lacked consistency in the testing frequency and time intervals between tests.

The average age of the pigs in the 540 groups was 13.7 ± 5.7 weeks. Influenza virus was detected in pigs as young as 4 weeks and as old as 30–32 weeks of age. Mean age in positive groups was 12.4 ± 5.2 weeks and in negative groups was 13.9 ± 5.8 SD (Figure 4). However, age was not a statistically significant predictor of influenza A virus test status.

Clinical Signs

Respiratory clinical signs were observed in pigs in 187 (34.6%) of 540 groups. From these 187 groups that reportedly exhibited clinical signs, 43 (22.9%) were positive for influenza A virus. From the 353 groups that exhibited no clinical signs, 74 (20.9%) were positive for influenza virus (Table 1). Even when within-group prevalence of influenza A virus was high, such as in the 13 groups in which ≥20 samples were positive for influenza A virus, clinical signs of respiratory disease were low. Indeed, clinical signs of influenza-like illness were noted in only 7 of those 13 groups.

Season

Throughout the study, the numbers (proportions) of groups sampled in each season were similar. In winter, 124 (23%) were sampled; in spring, 137 (25%); in summer, 150 (28%); and in fall, 129 (24%) (Table 2).

Logistic Regression

In the first model, univariate analysis of all variables (age, season, and year) except clinical signs yielded

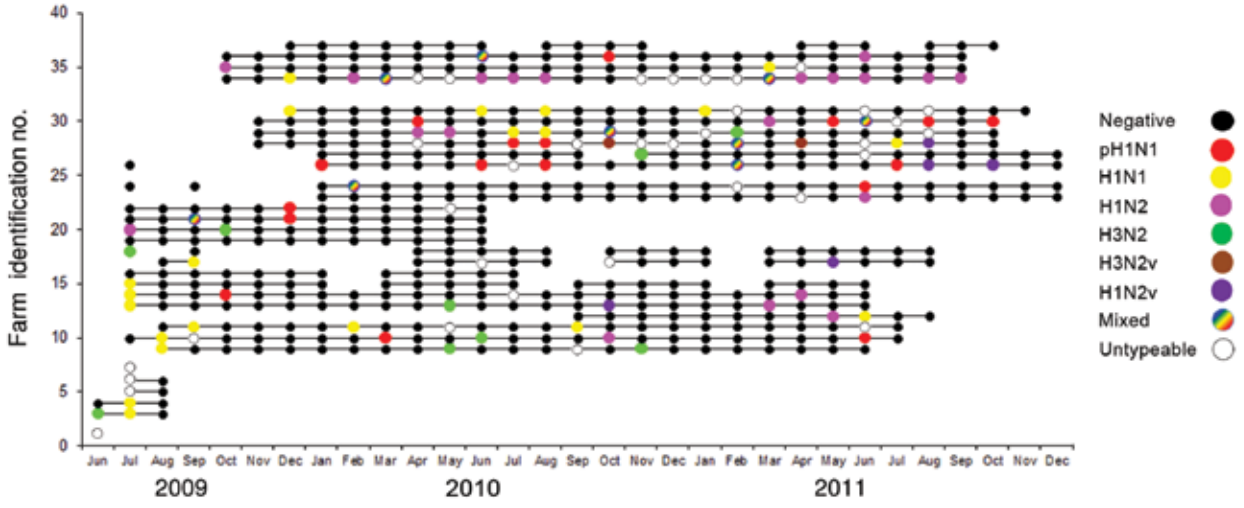

Figure 1. Swine influenza virus group status for 32 pig farms participating in an active surveillance project, midwestern United States, June 2009–December 2011. Each horizontal line represents a farm, each dot represents a sampling event, and colors indicate virus status of the group.

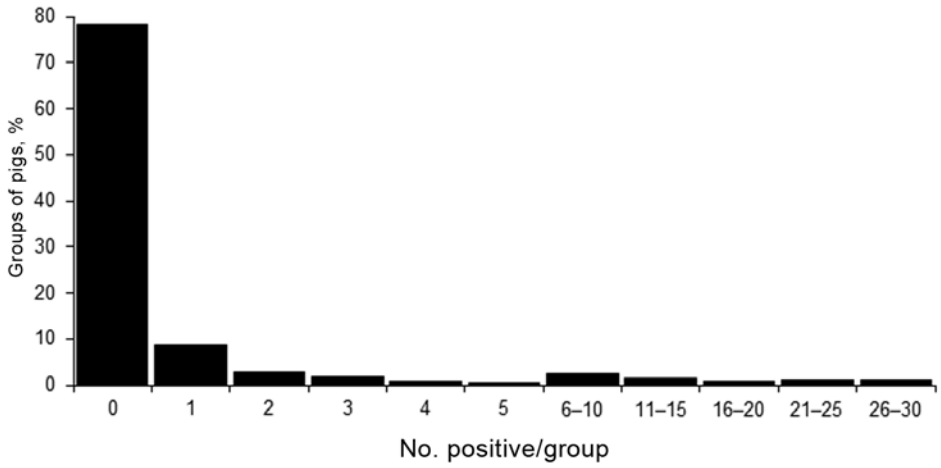

Figure 2. Frequency distribution of number of nasal swab samples positive for influenza virus by real-time reverse transcription PCR, per group (total 540 groups of pigs), midwestern United States, June 2009–December 2011.

p<0.25. The multivariable model retained 2 variables: season and age. Odds of positive results for influenza A virus were 2 (95% CI 1.1–3.8) times and 1.9 (95% CI 1.0–3.5) times higher for groups of pigs tested during the spring and summer, respectively, than for groups tested during the fall. There was no association between the winter season and influenza virus detection. Age was not significantly (p = 0.09) associated with influenza A virus group status; however, the variable was left in the model because of confounding (Table 3).

In the second model, complete subtype information was available for 81 monthly test results. Because neither subtype nor season was significantly associated with presence of clinical signs at the univariate level (Table 4), no attempts were made to build a multivariate model.

Discussion

Active surveillance at 32 farms demonstrated that influenza A virus is commonly present in the nasal secretions of pigs; 29 (90.6%) farms had at least 1 positive group throughout the study. Despite this high group or population prevalence, detection of swine influenza A virus in individual samples was low and thereby compatible with previously published findings (*10,15–18*) in which swine influenza A virus detection rates through either virus isolation or RRT-PCR on individual nasal swab samples was ≤5%, presenting a challenge for surveillance programs. However, new sample collection techniques in swine, such as the collection of pen-based oral fluids (e.g., saliva) for antibody and antigen detection are becoming more commonly used because of their practicality and lower testing costs (*24*); other studies have shown an increased probability of detection, making oral fluid sampling a suitable and essential tool for population surveillance on pig farms (*25,26*). Asymptomatic carriers of influenza A virus can be detected more efficiently through oral fluid sampling of clinically healthy populations than through nasal swab sampling.

Influenza A virus persistence in pig populations is not fully understood. In our study, 41% of the positive groups had only 1 positive nasal swab sample. One possible explanation could be that these groups of pigs were sampled either at the beginning or end of an infection. Another possible explanation could be that underlying passive or active immunity enabled transmission to occur at a rather low rate and that transmission remained continuous in and thus perpetuated the infection within the population. However, our study was not designed to measure transmission. In addition, our study did not obtain information regarding

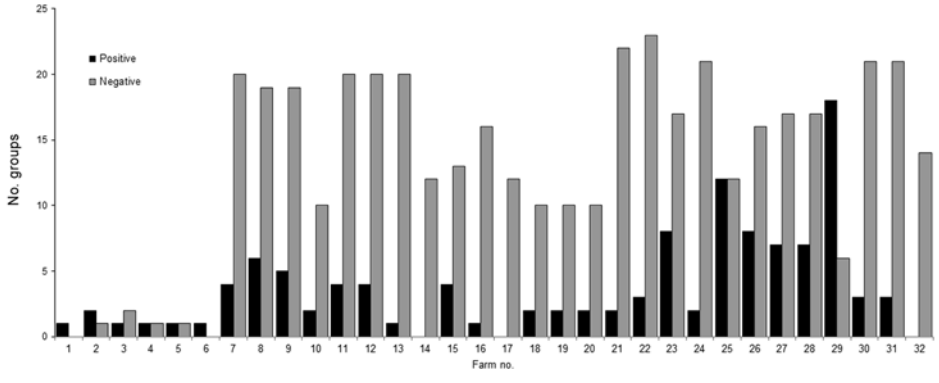

Figure 3. Number of influenza A virus–positive and of influenza A virus–negative groups, by farm, midwestern United States, June 2009–December 2011.

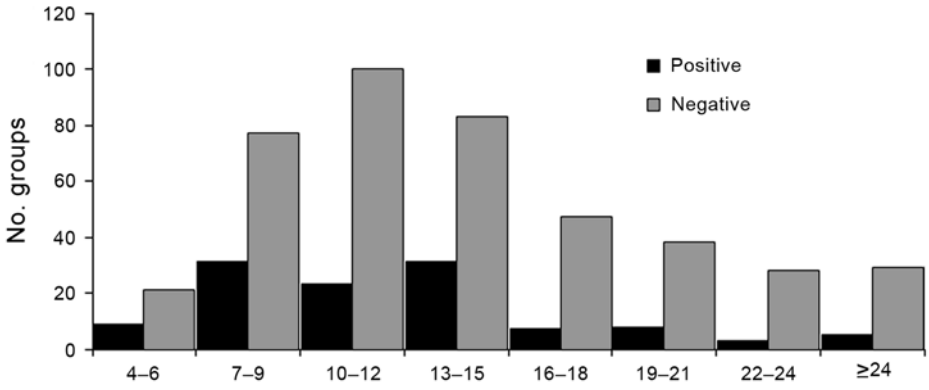

Figure 4. Age distribution of groups of pigs that were positive or negative for influenza A, determined by real-time reverse transcription PCR, midwestern United States, June 2009–December 2011.

the entrance into or exit from the farm by groups of pigs. Therefore, it is possible that the same group of pigs was sampled on consecutive months.

In our study, the absence of respiratory clinical signs in groups of pigs harboring influenza A virus is notable. This lack of signs could be the result of events such as the presence of antibodies conferred by colostrum ingestion (e.g., maternal immunity), vaccination, or previous exposure; other studies have suggested that low levels of exposure to virus might preclude clinical signs in pigs (*27–29*). Subclinical infections in pigs have public health implications because humans can become infected after coming in contact with apparently healthy pigs that are shedding enough infectious viral particles (*5,30–32*). Subclinical infections are perhaps one of the most common routes for influenza A virus entry into a pig farm because replacement breeding stock or recently weaned animals are constantly moved within and between states and countries. In fact, there is evidence that movement of pigs might have been the cause of dissemination of certain virus lineages within the United States, and subclinical infections might have played a role (*33*).

Pathogenicity of swine influenza A virus can vary among strains within the same subtype (*34*). From a clinical signs standpoint, experimental infection with influenza A virus has resulted in great variability (*29*). When swine influenza A virus is part of a co-infection (i.e., with other viruses and/or bacteria), clinical signs are evident (*35,36*); such co-infections might reflect the health status situation of the groups of pigs used in this study because the likelihood of co-infections in the field was high. However, in our study, the lack of association between virus subtype and clinical signs is difficult to explain. More studies are needed to better understand the role that virus subtypes play on the presence of respiratory signs.

Our study demonstrated that influenza A virus is present in growing pigs throughout the year and that groups of pigs are more likely to have positive test results during the spring and summer than in the fall. This finding is contrary to what has been suggested (*7*). The previously suggested seasonal trend could have been based on presence of clinical signs that appear during a time of the year when other factors are present (e.g., cold weather, bad air quality inside barns, co-infections) (*7,37*), which led veterinarians to submit samples to diagnostic laboratories for the detection of influenza A virus. However, as mentioned earlier, subclinical infections might have occurred during warm months, thereby leading to misinterpretation of the information available at that time. Another possible explanation for finding more influenza A virus–positive groups in the spring and summer is the increase of pigs born to primiparous females. This increase in potentially more susceptible growing pigs is a result of increased breeding of gilts (female pigs that have not had their first litter) during late summer, when swine producers often increase the number of gilts bred. These primiparous females often have a lower level of antibody protection to offer to their first litter of piglets. These relatively immunologically naive piglets would often be the group studied in the spring and summer seasons (*38*), providing a source of susceptible individuals in which viruses circulate during these seasons.

A limitation of our study might be the locations of the farms, which were all in the midwestern United States. Weather conditions and seasons in the midwestern United

Table 1. Influenza virus status and respiratory clinical signs among growing pigs, midwestern United States, June 2009–December 2011

	Influenza virus status, no. groups		Total, no.
Clinical signs	Positive	Negative	groups
Present	43	144	187
Absent	74	279	353
Total	117	423	540

Table 2. Influenza virus status among growing pigs, by season, midwestern United States, June 2009–December 2011

	Influenza virus status, no. groups		Total, no.
Season	Positive	Negative	groups
Winter	19	105	124
Spring	37	100	137
Summer	40	110	150
Fall	21	108	129
Total	117	423	540

Table 3. Relationship between group influenza status with age and season in growing pigs tested for influenza virus, midwestern United States, June 2009–December 2011*

Variable	Estimate	Odds ratio (95% CI)	p value
Intercept	–1.330	NA	<0.01
Season			
Fall (referent)	NA	NA	NA
Spring	0.741	2.0 (1.1– 3.8)	0.01
Summer	0.657	1.9 (1.0–3.5)	0.03
Winter	–0.072	0.9 (0.4–1.8)	0.83
Age	–0.041	0.9 (0.9–1.0)	0.09

*Repeated measures logistic regression multivariable analysis.
Generalized χ^2 divided by degrees of freedom = 0.92. NA, not applicable.

States might not accurately reflect conditions in other swine-producing areas of the United States, namely, the southeastern and south-central regions. For logistical reasons, neither of these regions was included in this study.

Surveillance will continue to be a useful tool for infectious disease epidemiology because the data it provides will aid in the understanding of the determinants of infection, enabling scientists and practitioners to work toward generation of disease prevention and control strategies (*39*). Surveillance studies contribute to science by generating data about zoonotic and emerging pathogens (*40*). Such contributions are true for influenza A virus in swine because emerging influenza viruses have been identified through surveillance programs (*15,16*). In summary, our study has led to the following 3 conclusions: 1) different influenza viruses circulate simultaneously within pig populations; 2) influenza is present in pigs of different ages at a rather low prevalence throughout the year; and 3) subclinical infections are frequent among groups of pigs. More studies are needed to add to understanding of influenza A virus.

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Dr Corzo was a PhD student at the University of Minnesota College of Veterinary Medicine, Saint Paul, Minnesota, during the study period . His research interests include the epidemiology of swine influenza viruses.

Table 4. Relationships between clinical signs with subtype and season among pigs tested for influenza virus, midwestern United States, June 2009–December 2011*

Variable	Estimate	Odds ratio (95% CI)	p value
Season†			
Intercept	–0.234	NA	0.65
Fall (referent)	NA	NA	NA
Spring	–0.411	0.6 (0.1–2.5)	0.53
Summer	–0.410	0.6 (0.1–2.4)	0.53
Winter	0.361	1.4 (0.3–6.7)	0.63
Influenza virus subtype‡			
Intercept	1.7	NA	0.95
H1N1 (referent)	NA	NA	NA
H1N2	–1.381	0.5 (0.1–2.0)	0.96
H3N2	–1.476	0.5 (0.09–2.5)	0.95
H1N2v	–2.105	0.2 (0.03–2.0)	0.93
H3N2v	9.541	NA	0.95
H1N1pdm09	–1.582	0.4 (0.1–1.7)	0.95
Mixed infection	–2.210	0.2 (0.04–1.3)	0.93

*Logistic regression univariate analysis; NA, not applicable.
†Generalized χ^2 divided by degrees of freedom = 0.96.
‡Likelihood ratio χ^2 (6) = 6.01 df, p = 0.42.

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Foodborne Botulism in Canada, 1985–2005

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Learning Objectives

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

- Describe the incidence of laboratory-confirmed outbreaks of foodborne botulism occurring between 1985 and 2005 in Canada, based on a study of laboratory databases.
- Describe the implicated pathogens and sources of laboratory-confirmed outbreaks of foodborne botulism occurring between 1985 and 2005 in Canada, based on a study of laboratory databases.
- Describe the outcomes of laboratory-confirmed outbreaks of foodborne botulism occurring between 1985 and 2005 in Canada, based on a study of laboratory databases.

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During 1985–2005, a total of 91 laboratory-confirmed outbreaks of foodborne botulism occurred in Canada; these outbreaks involved 205 cases and 11 deaths. Of the outbreaks, 75 (86.2%) were caused by *Clostridium botulinum* type E, followed by types A (7, 8.1%) and B (5, 5.7%). Approximately 85% of the outbreaks occurred in Native communities, particularly the Inuit of Nunavik in northern Quebec and the First Nations population of the Pacific coast of British Columbia. These populations were predominantly exposed to type E botulinum toxin through the consumption of traditionally prepared marine mammal and fish products. Two botulism outbreaks were attributed to commercial

ready-to-eat meat products and 3 to foods served in restaurants; several cases were attributed to non-Native home-prepared foods. Three affected pregnant women delivered healthy infants. Improvements in botulism case identification and early treatment have resulted in a reduction in the case-fatality rate in Canada.

Foodborne botulism, a notifiable disease in Canada, results from the ingestion of foods contaminated with preformed botulinum neurotoxin types A, B, E, or F, produced by *Clostridium botulinum* groups I and II (*1*). More rarely, outbreaks of foodborne botulism in the United States, India, and China have been caused by neurotoxigenic *C. butyricum* type E (*2*,*3*) and *C. baratii* type F (*4*). In Canada, *C. botulinum* type E has been the most common serotype since the first type E outbreak in 1944 in Nanaimo, British Columbia (reported in 1947) (*5*–*7*).

Six forms of botulism have been described in the literature (*8*), but only foodborne and infant botulism and rare cases of adult colonization have been reported in Canada (*1*,*9*). Regardless of the form or serotypes involved, however, human botulism is a medical emergency that requires rapid intervention. Because prompt administration of antitoxin can reduce the severity of the disease (*10*), the decision for treatment is based on clinical diagnosis and epidemiologic information, without laboratory confirmation.

Investigation of foodborne botulism incidents provides useful information regarding implicated foods and conditions resulting in toxin formation. The last epidemiologic review on foodborne botulism in Canada was done for the 1971–1984 period (*7*). Since then, annual summaries of botulism cases were inconsistently published through disease surveillance reports (e.g., *11*,*12*). Here, we summarize reports of all laboratory-confirmed cases of foodborne botulism in Canada during 1985–2005.

Materials and Methods

Data Sources

Two independent laboratory databases, maintained by the Botulism Reference Service at Health Canada, Ottawa, Ontario, and the British Columbia Public Health Reference Microbiology Laboratory, Vancouver, British Columbia, were examined for cases of foodborne botulism confirmed during 1985–2005. Information regarding the number of clinical cases, age and sex of patients, implicated food, case history, laboratory analysis, date, and location of the outbreak were extracted.

To ensure consistency in data recording and analysis throughout the study period, we used the 2009 national case definition for confirmed cases of foodborne botulism (*13*). A case of foodborne botulism is confirmed on the basis of clinical evidence and a positive laboratory specimen (i.e., detection of botulinum neurotoxin in serum, feces, gastric aspirate, or food or isolation of *C. botulinum* from feces or gastric aspirate). In addition to cases with laboratory confirmation, persons with botulism who were epidemiologically linked to a laboratory-confirmed case were considered to have confirmed, and thus reportable, cases. Detection of botulinum neurotoxin and isolation of viable *C. botulinum* from foods and clinical specimens were performed according to Health Canada method MFHPB-16 (*14*). Data on length of hospitalization were retrieved from the Hospital Morbidity Database (HMDB) of the Canadian Institute for Health Information (www.cihi.ca); all records that listed botulism in the first 3 suspected diagnostic codes were extracted for the years 1994–2005 (all years currently available). These records were then matched to the laboratory records by age, sex, date of admission, date of sample, and province of residence. Only cases with laboratory confirmation were included in the analysis of the HMDB data.

Data Analysis

To provide a descriptive epidemiology of outbreaks of foodborne botulism in Canada during 1985–2005, we analyzed data from laboratory-confirmed outbreaks (in Canada, because of the urgency of the disease, 1 case of botulism constitutes an outbreak) in terms of case numbers and rates, demographic characteristics of patients, length of hospitalization, food types, outbreak settings, serotype, and circumstances of occurrence. The rates of disease were calculated by using census data from Statistics Canada and other sources (*15*,*16*). Statistical analysis of the HMDB data was done in SPSS version 19 (IBM, Armonk, NY, USA). The Mann-Whitney test was done to compare central tendency of the data; relationships were considered significant at $p<0.05$.

Results

Demographics and Incidence

Of 91 laboratory-confirmed outbreaks (a total of 205 cases), *C. botulinum* type E was implicated in 75 (86.2%), followed by types A (7, 8.1%) and B (5, 5.7%). Median patient age was 45 years (range 3–80 years); 93 (48.4%) were male. Three cases in the Nunavik region were recorded as repeated episodes of type E botulism, with the second episodes occurring 10–20 years after the initial intoxication. Overall, the number of outbreaks of foodborne botulism did not decrease during the study period, with a mean of 4.3 outbreaks/year. Outbreaks involved 1–37 cases, and 78% of outbreaks involved 1 or 2 cases. Mean annual incidence was 0.03 cases/100,000 population. The annual number of cases was marked by peaks associated with large outbreaks (Figure 1). The number of cases appeared to decrease

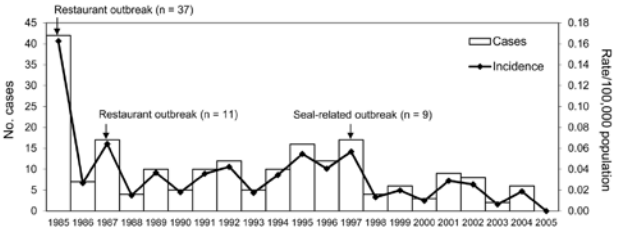

Figure 1. Number of cases of foodborne botulism and disease incidence (rate/100,000 population), Canada, 1985–2005.

during the last 5 years of the study period; lower numbers of outbreaks with multiple cases were reported (Table 1).

Morbidity and Mortality

A total of 11 deaths were reported (case-fatality rate 5.4%). Within the 21-year study period, the case-fatality rate declined from 8.8% in 1985–1989 to 2.4% in 1990–1994 and then remained below 3.6% during the remaining 5-year intervals (Table 1). However, because mortality data were derived from the laboratory databases that do not officially keep mortality records, these data should be interpreted with caution.

Three of the reported cases occurred in pregnant women. Case history revealed that 2 of these women were exposed to type E botulinum neurotoxin and 1 to type B botulinum neurotoxin. No apparent effect on pregnancy was reported.

Severity of illness was assessed by using HMDB data for 1994–2005. The length of hospitalization was obtained for 70 patients with type E botulism (Table 2); the low number of type A and type B botulism cases recorded in HMDB did not enable valid data reporting. Although HMDB does not list deaths, the authors are aware of 1 fatal type E case from among these patients. Of botulism type E case-patients with known length of hospitalization, 37 (52.9%) were female and 33 male, which was not significantly different ($p = 0.633$). The median length of hospitalization was 7 days for female (mean 14.8 days) and male (mean 8.7 days) patients; these findings were not significantly different ($p>0.05$). The mean for the female group was influenced by 1 fatal case in which the patient was hospitalized for 225 days. When type E case-patients were subdivided based on age, age groups differed in length of hospitalization, but a trend was not apparent (Table 2).

Hospital procedure codes were extracted from HMDB for 54 patients with type E botulism. These 54 cases represent only a subset of the total type E cases, and all were laboratory confirmed and matched with laboratory records. Intubation alone was recorded for 16 (30%) patients, antitoxin alone for 21 (39%) patients, and both for 5 (9%) patients; 12 (22%) patients received neither antitoxin nor ventilation.

Table 1. Foodborne botulism outbreaks, cases, deaths, and case-fatality rates, by 5-year intervals, Canada, 1985–2005

Period	No. outbreaks	No. cases	No. deaths	Case-fatality rate
1985–1989	20	90	7	8.8
1990–1994	22	42	1	2.4
1995–1999	27	55	2	3.6
2000–2005	22	28	1	3.4
1985–2005	91	205	11	5.4

For the group comprising all patients that received antitoxin (n = 26), the median length of hospital stay was 5 days (mean 15.1 days, SD 43.2), significantly ($p = 0.003$) shorter than for the group that did not receive antitoxin (n = 28), which had a median length of hospital stay of 11 days (mean 13.3 days, SD 8.32). For the group comprising all patients that were intubated (n = 21), the median length of hospital stay was 13 days (mean 24.5 days, SD 46.6), significantly ($p<0.001$) longer than for the group that did not undergo intubation (n = 33), which had a median length of hospital stay of 5 days (mean 7.58 days, SD 6.24). Intubation is likely a marker of severity of symptoms and is not thought to be the cause of increased length of hospitalization. No determination for the elapsed time period between symptom onset and the administration of antitoxin or ventilation was available.

Geographic Distribution

Most (85, 93.4%) confirmed botulism outbreaks originated in Quebec, British Columbia, Nunavut, and the Northwest Territories. Reported cases predominantly occurred in Quebec and British Columbia, with 89 and 71 cases, respectively; these cases accounted for 78% of the total number of cases (Table 3). Of 51 outbreaks in Quebec, 45 (88%) occurred in the Nunavik region of northern Quebec; 91% of these were clustered in 3 villages of southern Ungava Bay (Kuujjuaq, Kangiqsualujjuaq, and Tasiujaq), which are inhabited by an Inuit population of 2,587 (Figure 2). In British Columbia, 9 (64%) of 14 outbreaks (20 cases) occurred in First Nations communities located along the Pacific Coast. The high number of cases recorded in the province was primarily because of 2 large restaurant-associated outbreaks in Vancouver that affected 37 persons (*17*) and 11 persons (*18*). In Ontario, 3 outbreaks were recorded, with 1 affecting 3 persons and causing 1 death. No

Table 2. Length of hospitalization for botulism type E case-patients, by age group, Canada, 1985–2005

Age group, y	No. cases	Length of hospitalization, d		
		Mean	Median	Range
<30	5	47.6	2	1–225
30–39	8	4.6	4	1–8
40–49	18	10.8	9.5	3–29
50–59	23	6.0	4	1–18
60–69	10	15.3	15.5	3–30
≥70	6	12.0	6.5	2–37
All	70	11.9	7	1–225

Table 3. Foodborne botulism outbreaks, cases, and deaths, by province/territory, Canada, 1985–2005

Province/territory	No. (%) outbreaks	No. (%) cases	No. (%) deaths
Newfoundland and Labrador	2 (2.2)	3 (1.5)	1 (9.1)
Quebec*	51 (56.0)	89 (43.4)	2 (18.2)
Ontario	3 (3.3)	5 (2.4)	1 (9.1)
British Columbia	14 (15.4)	71 (34.6)	3 (27.3)
Yukon	1 (1.1)	3 (1.5)	1 (9.1)
Northwest Territories	12 (13.2)	16 (7.8)	2 (18.2)
Nunavut	8 (8.8)	18 (8.8)	1 (9.1)
Canada†	91	205	11

*Forty-five outbreaks occurred in the Nunavik region of Northern Quebec
†No laboratory-confirmed cases of foodborne botulism were reported from Alberta, Saskatchewan, Manitoba, New Brunswick, Nova Scotia or Prince Edward Island.

botulism cases were reported in the provinces of Alberta, Saskatchewan, Manitoba, New Brunswick, Nova Scotia, and Prince Edward Island during the study period.

Traditional Native Foods

Other than a single outbreak in Nunavut caused by consumption of fermented fish heads, all type E outbreaks with known food sources in northern Canada were linked to marine mammals, including beluga whales, seals, and walruses (Table 4). Of the 41 botulism outbreaks in Nunavik, 32 (78%) were caused by food products of seal origin; aged meat (*igunaq*) and aged flippers (*utjaq*) were most frequently implicated foods in Nunavik, accounting for 15 (47%) seal-related outbreaks. Beluga whale was involved in 16 (80%) of 20 outbreaks in Nunavut and the Northwest Territories. Eleven of these outbreaks were caused by *muktuk*, which is aged pieces of skin (with fat and meat) of the beluga whale.

In the First Nations communities of British Columbia, 9 outbreaks of type E botulism were associated with the consumption of aged fish products. Fermented salmon eggs (stink eggs) have been the primary source of botulism, accounting for 8 of 9 (89%) outbreaks in British Columbia coastal communities. Because no carbohydrates are available in these eggs for a fermentative transformation into organic acids, the aging process involves putrefaction rather than fermentation.

Restaurant Food

The 1985 type B outbreak involving garlic-in-oil at a Vancouver restaurant affected 37 persons; 24 were hospitalized, and none died (*17*) (Table 4). The outbreak occurred in 2 clusters, with 11 cases in the first cluster during July 28–August 2, 1985, and 26 cases in the second cluster during August 29–September 5, 1985. In this incident, garlic-in-oil used in sandwiches was kept at room temperature

Figure 2. Distribution of outbreaks of foodborne botulism by serotype, Canada, 1985–2005. Circles represent type E outbreaks, triangles type A outbreaks, pentagons type B outbreaks, and squares outbreaks of unknown serotype. Circle sizes are proportionate to the number of outbreaks occurring in a given location.

Table 4. Foodborne botulism outbreaks, cases, and deaths, by food source and year, Canada, 1985–2005

Food source and year	Implicated food source	No. outbreaks	No. cases	No. deaths	Toxin serotype
Commercial retail foods					
1995	Pâté	1	2	0	B
2000	Cooked boneless pork	1	1	0	A
Restaurant foods					
1985	Chopped garlic in oil	1	37	0	B
1987	Bottled chanterelle mushrooms	1	11	0	A
2002	Baked potato	1	1	0	A
Home-prepared foods					
1985	Home-canned mushrooms	1	1	0	B
1988	Homemade sausages	1	1	0	E
1989	Bean soup	1	1	0	A
1991	Home-canned asparagus	1	3	1	A
1993	Homemade beef and vegetable soup	1	1	0	A
1995	Marinated and smoked fish	1	3	1	E
2000	Spaghetti sauce containing home-canned sausage	1	1	0	A
2001	Fish	1	1	0	B
Native foods					
1985–2005	Marine mammal products				
	Seal meat and fat	37	70	5	E
	Walrus meat	3	9	0	E
	Beluga meat and skin*	19	31	1	E
	Meat and fat	3	3	0	E
1985–2001	Fish products				
	Salmon eggs	8	19	3	E
	Fish and fish heads	2	2	0	E
Unknown food source*					
1990–2005	Unknown	6	7	0	B, E

*Serotype was not confirmed for 2 outbreaks involving *muktuk* and for 1 outbreak with an unknown food source.

for 8 months. In a 1987 botulism outbreak at a hotel restaurant in Vancouver, 11 persons became ill after consuming bottled chanterelle mushrooms contaminated with type A botulinum neurotoxin (*18*). The mushrooms were grown on Vancouver Island and bottled at the hotel using an in-house heating process. One jar recovered during the investigation was found to contain 4,000 minimal lethal dose/mL of type A neurotoxin in the liquid phase.

A third outbreak associated with a restaurant occurred in Ontario in 2002 and involved a single case of type A botulism linked to a baked potato (*19*). This patient began to show gastro-intestinal and neurologic symptoms 12 hours after eating the potato, the remains of which had been discarded and was unavailable for testing. The patient was hospitalized for >6 months and released with long-term sequelae; the patient continued to experience weakness and vision problems 4 years after the incident.

Commercial Foods

Two unrelated incidents involving commercial ready-to-eat meat products were reported in Quebec in 1995 and 2001 (Table 4). In both instances, products that were intended to be refrigerated were stored at room temperature, which enabled growth of *C. botulinum* and toxin production. In the 1995 incident, *C. botulinum* type B was isolated from fecal samples of 2 persons and from a commercial country-style pâté. In the 2001 incident, *C. botulinum* type A was isolated from a cooked boneless pork product and from fecal and gastric liquid samples from a patient. The cooked boneless pork product was recalled as a precautionary measure.

Home-prepared Foods

Several incidents during the 21-year study period involved non-Native home-prepared foods (Table 4). Home-canned mushrooms were linked to a type B botulism incident that affected 1 person in Quebec. Home-canned asparagus was involved in 1 type A botulism incident in Ontario; 3 persons required intensive care, and 1 died. In this incident, 8 of 10 glass jars of whole-stalk asparagus showed evidence of odorous gas production during the investigation. One additional type A case was linked to the consumption of home-canned vegetable and beef soup.

A variety of noncanned home-prepared foods, including bean soup, spaghetti sauce, sausages, and fish, were also implicated in botulism outbreaks, although most of the incidents could not be laboratory confirmed because no remaining food was available. Home-smoked fish was responsible for 3 cases of type E botulism, with 1 fatality, in the Yukon in 1995. Diagnosis and treatment with antitoxin was delayed until 3 days after the onset of symptoms. Another incident of fish consumption affected 1 tourist traveling in Quebec in 2001, but the mode of preparation was not recorded. Home-prepared spaghetti sauce made with home-canned sausage caused botulism in 1 person from

Quebec in 2000. Another type A case was associated with the consumption of homemade bean soup, but its preparation was not documented.

Laboratory Findings

Of 91 total confirmed outbreaks in Canada during the study period, 77 (84.6%) were confirmed by detection of botulinum neurotoxin only or by detection of neurotoxin and isolation of *C. botulinum* from laboratory specimens. The remaining 14 outbreaks were confirmed by detection of *C. botulinum* in gastric contents and/or fecal samples only, without detection of neurotoxin in clinical samples. Food was recovered in 69 (75.8%) of the outbreak investigations. The detection rate for botulinum neurotoxin in foods (78.3%) was found to be higher than for serum (34.9%), feces (34.5%), or gastric contents (11.0%) (Table 5). *C. botulinum* was also more frequently detected in foods (83.3%), followed by gastric contents (51.3%) and feces (40.3%). Two botulism cases were confirmed by the detection of *C. botulinum* in liver tissue during autopsies.

Discussion

The annual mean of 4.3 confirmed botulism outbreaks during 1985–2005 was identical to that reported for the 1971–1984 period (*7*); however, the case-fatality rate dramatically decreased during 1985–2005, from 17% to 5.4%. This decrease could reflect the increased awareness of symptoms in high-risk communities, which leads to prompt medical attention and the administration of antitoxin. Similar to 1971–1984, most cases occurred in Quebec, British Columbia, Nunavut, and the Northwest Territories, which is likely a reflection of the rate of consumption of higher-risk traditional foods in these provinces and territories. The annual mean number of outbreaks for British Columbia decreased from 0.9 to 0.7, whereas the rate for Quebec remained unchanged at 2.4 outbreaks per year. The Northwest Territories, Nunavut, and Yukon combined had 21 outbreaks during the study period, an annual average of 1 outbreak per year, unchanged from 1971–84 (*7*).

Botulism remains a public health challenge in many communities where Native foods are persistently incriminated (*7*). The mean incidence rate for all of Canada is low and is similar to that of the United States (*20*), but the rate among the Native population of Nunavik, where type E botulism linked to aged marine mammal products is endemic, is >1,600 times higher than for the rest of Canada (0.03/100,000 population for Canada vs. 50.5/100,000 population for Nunavik). Outbreaks of botulism associated with Native foods in Canada account for 83.5% of all outbreaks; in the United States, 36.3% of all outbreaks were associated with Native foods in Alaska (*20*).

Previous epidemiologic studies have identified the food types and the modes of preservation of foods frequently associated with type E botulism in northern Canada (*6*,*7*). Aged marine mammals and fish were the predominant vehicles of type E botulism before 1985 and remain responsible for most type E botulism cases in Canada. The risk for contamination of marine mammal meat during the butchering process performed under field conditions is high because of the ubiquitous presence of the organism in the coastal environment (*21*,*22*). Measures to minimize field contamination of marine mammal tissues with type E spores during the butchering and preparation of meat, fat, or skin have recently been developed (*23*). In collaboration with regional health authorities, studies directed at controlling the growth of *C. botulinum* type E in aged marine mammal products have indicated that storage temperatures <3°C will prevent toxin production during the aging process of Native foods (*23*,*24*).

Food service establishments are potential risk settings where widespread public exposure to contaminated foods may occur. The garlic-in-oil incident in 1985 was the first reported Canadian botulism outbreak associated with a food service establishment and was the largest ever reported botulism outbreak in Canada. This incident, along with a similar incident that occurred in the United States in 1989, led to regulatory changes requiring inclusion of acidifying agents in commercial garlic-in-oil products (*25*). The restaurant outbreak involving bottled

Table 5. Results of laboratory analyses of clinical and food specimens submitted for botulism investigation, Canada, 1985–2005

Specimen type	Test type	No. outbreaks	No. specimens	No. (%) specimens with positive results
Serum	BoNT	82	212	74 (34.9)
Gastric contents	BoNT	48	73	8 (11.0)
	Clostridium botulinum	47	78	40 (51.3)
Feces	BoNT	57	84	29 (34.5)
	C. botulinum	60	139	56 (40.3)
Liver				
Blood	BoNT	1	2	0 (0.0)
Tissue	*C. botulinum*	1	2	2 (100)
Cerebrospinal fluid	BoNT	1	1	0 (0.0)
Food	BoNT	69	69	54 (78.3)
	C. botulinum	66	66	55 (83.3)

*BoNT, botulinum neurotoxin.

chanterelle mushrooms in 1987 led the Canadian Restaurant and Foodservices Association to modify its sanitation code to recommend that in-house canned or bottled foods not be served in restaurants.

The number of botulism incidents associated with foods sold at retail remained low, with only 2 outbreaks causing illness to 3 persons. Non-Native home-prepared foods were implicated in 8 outbreaks in Canada; 2 of these were caused by home-canned vegetables. Continuous education is needed to inform consumers of the potential risks of botulism from eating home-prepared foods and to promote the use of a pressure canner according to manufacturer's instructions and proper food storage temperatures in the home.

In addition to the classic diagnostic samples (i.e., serum, gastric content, feces, and suspect foods), the confirmation of 2 cases in 1985 was completed with the detection of viable *C. botulinum* in liver tissue during postmortem examination. Detection of viable *C. botulinum* in postmortem liver samples has been reported previously (*7*,*26*).

We found that 3 pregnant women who were diagnosed with botulism had no reported pregnancy complications. One of the patients had persistent toxemia for ≥10 days during the first trimester of pregnancy and later delivered a normal infant. Five previous cases of botulism during pregnancy have been reported; none of the infants appeared to have clinical botulism at birth (*27–31*). No evidence shows that botulinum toxin can cross the placenta (*32*) or that the neuromuscular effects of botulinum toxin on the mother would affect the development of the fetus (*28*).

Type A botulism is recognized to be more severe than type E botulism because of the higher proportion of patients who require intubation (*33*). Hughes et al. reported the mean length of hospitalization as 63 days for type A and 21 days for type B botulism (*34*), longer than the 11.9 days determined in this study for type E botulism. Administration of antitoxin has been shown to reduce risk for death and shorten the course of type A botulism (*10*). The data in this study showed that antitoxin shortened the median length of hospitalization for patients with type E botulism from 11 days to 5 days.

The overall case-fatality rate from botulism in Canada has decreased from 17.2% to 5.4%, approaching the rate in United States (*20*), which can likely be attributed to the early recognition and medical management of type E botulism cases in First Nations and Inuit communities. Most foodborne botulism outbreaks in Canada continue to occur among First Nations and Inuit people, a trend that has not changed since a 1974 comprehensive review of botulism cases, which attributed most cases of type E botulism to aged marine mammal and fish products (*6*). However, extensive progress has been made in improving case identification and early treatment, which has led to a substantial decrease in the case-fatality rate.

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Novel *Mycobacterium tuberculosis* Complex Isolate from a Wild Chimpanzee

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Tuberculosis (TB) is caused by gram-positive bacteria known as the *Mycobacterium tuberculosis* complex (MTBC). MTBC include several human-associated lineages and several variants adapted to domestic and, more rarely, wild animal species. We report an *M. tuberculosis* strain isolated from a wild chimpanzee in Côte d'Ivoire that was shown by comparative genomic and phylogenomic analyses to belong to a new lineage of MTBC, closer to the human-associated lineage 6 (also known as *M. africanum* West Africa 2) than to the other classical animal-associated MTBC strains. These results show that the general view of the genetic diversity of MTBC is limited and support the possibility that other MTBC variants exist, particularly in wild mammals in Africa. Exploring this diversity is crucial to the understanding of the biology and evolutionary history of this widespread infectious disease.

Tuberculosis (TB) is caused by closely related acid-fast bacteria known as the *Mycobacterium tuberculosis* complex (MTBC) (*1*). MTBC includes the typical human-associated pathogens *M. tuberculosis* and *M. africanum* (*2*); *M. canettii* and other so-called "smooth TB bacilli" (*3*), the actual host range of which remains unknown; and several lineages adapted to different mammal species that include *M. bovis*, *M. microti*, *M. caprae*, *M. orygis*, and *M. pinnipedii* (*4*–*6*). Because of the wider host range of animal-associated MTBC, the common view until a decade ago was that human TB strains had evolved from *M. bovis*, the typical agent of bovine TB. Recent comparative genomic analyses have challenged this view by showing that animal MTBC strains nest within the genetically more diverse human MTBC strains (*4*,*7*–*9*). These results not only contradict the hypothesis of an animal origin for human MTBC but also promote an alternative scenario for a human origin of animal MTBC (*10*). However, little is known about MTBC diversity in domestic animals, and even less about MTBC diversity in wildlife, including our phylogenetically closest relatives, the great apes. Of note, novel members of MTBC affecting wild mammals in Africa have recently been discovered (*11*,*12*), a finding that suggests animal MTBC is more diverse than previously thought.

We report microbiologically confirmed MTBC infection in a wild chimpanzee. We show that this infection was caused by a divergent MTBC strain that does belong to the clade that includes *M. bovis* and all other animal-associated members of MTBC but is more closely related to human-associated lineage 6 (also known as *M. africanum* West Africa type 2 [WA2]). This finding highlights critical gaps in knowledge of MTBC diversity and indicates that African wildlife, and more particularly nonhuman primates, are potential hosts of novel MTBC variants.

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Materials and Methods

Investigation of Wild Chimpanzee Death

In the course of a long-term study comprising behavioral observations and disease investigations of wild chimpanzees habituated to humans in Taï National Park, Côte d'Ivoire, necropsies are performed routinely on any chimpanzee or other mammal found dead. Detailed analyses are performed to identify the causes of death of every animal (*13*).

On August 5, 2009, an adult female chimpanzee of one of the study communities was found dead; lesions on the throat and alarm calls by other members of the community under observation at the time indicated that the animal had been killed by a leopard. The chimpanzee was one of the oldest females of the group (estimated age 52 years), and her body condition had deteriorated over the years. Necropsy was performed and tissue samples were frozen and fixed in formalin. Frozen organ material was submerged in 70% ethanol, rinsed twice in 0.85% NaCl, shredded with a scalpel, and streaked onto Löwenstein-Jensen PACT agar (Oxoid, Cambridge, UK). Bacteria were then cultivated on Middlebrook 7H11 agar supplemented with OADC or in Middlebrook 7H9 broth supplemented with OADC (Becton Dickinson, Franklin Lakes, NJ, USA) without shaking at 37°C.

Investigation of MTBC in Other Chimpanzees

To investigate the possible presence of MTBC strains in other chimpanzees, samples were collected from 28 chimpanzees, many from the same community, that died in the same area within the previous 10 years. DNA was extracted from 115 tissue samples (lung, spleen, liver, lymph nodes, and small intestines) from these 28 chimpanzees by using the QIAGEN DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany). All tissues were tested in duplicate by using the primers MTC_IAC Fw and MTC_IAC Rv and MTC Probe as described (*14*). We performed the PCR-RFLP of *gyr*B using the primers MTUB-f (5′-TCGGACGCGTATGCGATATC-3′) and MTUB-r (5′-ACATACAGTTCGGACTTGCG-3′) and an annealing temperature of 65°C for the PCR. We used the DreamTaq DNA Polymerase Kit and Fermentas restriction enzymes (Thermo Scientific, Waltham, MA, USA).

Genome Sequencing of MTBC Isolates

Mycobacterial DNA was isolated by using the CTAB method as described (*15*). The DNA was used to generate libraries for 454 and Illumina sequencing (Illumina, Inc., San Diego, CA, USA). For both libraries, the DNA was sheared to a size of 400–500 bp by using a Covaris S2 (Covaris, Inc., Woburn, MA, USA). The 454 library was generated by using the Rapid Library Kit and sequenced with Titanium chemistry on a 454 FLX instrument (Roche, Penzberg, Germany). The paired-end library for Illumina sequencing was generated by using the TruSeq DNA Sample Preparation Kit (Illumina). Cluster generation was done by using TruSeq PE Cluster Kit version 2.5 (Illumina) on a cbot. Sequencing was performed on a HiScanSQ instrument and TruSeq SBS Kit–HS chemistry (Illumina) to generate 2 × 100 bases long paired-end reads.

Mycobacterial strains (as defined in [*7*,*16*,*17*]) were cultured from single colonies. Genomic DNA was extracted by using a standard kit (QIAGEN) and sequenced with an Illumina Genome Analyzer. Sequencing libraries were constructed by using standard kits from Illumina, according to the manufacturer's instructions. Libraries for each strain were loaded into a single lane of a flow cell. SYBR green assays were used to test flow cells for optimal cluster density.

Single-nucleotide Polymorphism Calling and Genome Assembly

Illumina Sequencing Reads

We used BWA (*18*) to map Illumina reads from the 10 genome sequences published in this study (www.ebi.ac.uk/ena/data/view/ERP001571) and 24 genomes published previously (*18*) or available in public databases (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/article/19/6/12-1012-Techapp1.pdf) against the MTBC reference genome. The reference genome used was an inferred common ancestor of all MTBC lineages (*19*). BWA outputs were analyzed with SAMtools (*20*). We applied heuristic filters to remove problematic positions and set Phred-scaled probability at 20. SNP lists for individual strains were combined in a single, nonredundant dataset, and the corresponding base call was recovered for each strain. After excluding single-nucleotide polymorphisms (SNPs) in genes annotated as PE/PPE, integrase, transposase, or phage and SNPs that showed an ambiguous base call, we kept 12,920 high-confidence variable positions for downstream analysis. Lineage 6 strains showed an average sequencing depth of between 80 and 204-fold whereas the chimpanzee strain was sequenced at 4428-fold coverage.

454 Sequencing Reads

A combined mapping and de novo assembly was performed on the 454 reads obtained during the initial sequencing. Mapping of reads to the genome of *M. tuberculosis* strain CCDC5180 resulted in reference coverage of 98.39% and a total of 90 contigs. Mapping to the genome of strain H37Rv resulted in reference coverage of 98.3% and 87 contigs. Newbler 2.5 (Roche) and MIRA 3.0.0 (*21*) were used for de novo assembly. The parameters (minimum overlap identity and length, seed length, step and count, and alignment difference and identity scores for newbler; minimum

overlap identity and length; and clip length and stringency for MIRA) were automatically optimized for contig length by using a genetic algorithm with the default parameters used as starting points, a population size of 10, and 10 generations. The best set of parameters resulted in 2,538 contigs with a maximum length of 14,183 bp and a mean length of 1,738 bp. Reassembly of the contigs obtained from both mappings and from the de novo assemblies was performed by using Geneious 5.0 (Biomatters Ltd., Auckland, New Zealand) and yielded 33 contigs with a maximum length of 435,720 bp, a mean length of 130,500 bp, and a total length of 4,306,842 bp, compared with the ≈4.4 Mbp of the reference strains. The raw reads were mapped against these contigs to eliminate assembly errors.

A total of 33 contigs resulting from assembling 454 reads were aligned respective to the MTBC reconstructed ancestor genome using MAUVE (*22*). SNP lists obtained from Illumina sequencing were verified with the 454 contig sequences.

Phylogenetic Analysis

Phylogenetic analysis was performed on the basis of 13,480 high-confidence variable positions, specifying *M. canettii* as the outgroup (Figure 1). Both coding and noncoding SNPs were included. The SNPs were used to infer the phylogenetic relationships between strains by using neighbor-joining (Figure 1), maximum-likelihood (ML; online Technical Appendix Figure 2), and Bayesian (online Technical Appendix Figure 2) methods. Because of the low number of homoplasies expected (*18*), a neighbor-joining tree was obtained by using MEGA5 (*23*), with observed number of substitutions as a measure of genetic distance. We used the Akaike information criterion as implemented in jModelTest version 0.1 (*24*) to select the best-fit model of nucleotide substitution for the ML and Bayesian analyses. The ML tree was obtained by using PhyML version 3 (*25*), assessing branch robustness through bootstrapping (1,000 pseudo-replicates). The Bayesian summary tree was obtained by summarizing posterior tree samples generated along two 1 million generation–long Metropolis-coupled Markov chain Monte Carlo runs of 4 chains, which were performed in MrBayes version 3.1 (*26*). Convergence of the chains was assessed visually in Tracer version 1.5 (http://tree.bio.ed.ac.uk/software/tracer), and all parameters were checked to have an effective sample size of ≥100 in the combined run. Branch robustness was assessed through their posterior probabilities (i.e., the proportion of trees in the posterior sample in which the considered branches appeared).

Spoligotyping, Deletion, and Principal Component Analyses

Spoligotyping was performed as described and compared with data published in SITVITWEB (*27*). Deleted regions in the chimpanzee genome with respect to H37Rv genome were inferred as regions showing a mean coverage of <50 (1% mean coverage of the genome) by using awk scripts. Principal component analysis was conducted by using BioNumerics 6.6 (www.applied-maths.com/bionumerics) with the 13,480 high-confidence variable positions used for the phylogeny. The first 3 principal components accounted for 28%, 15%, and 8% of the variability and were used to generate a 3-dimensional scatter plot (online Technical Appendix Figure 4).

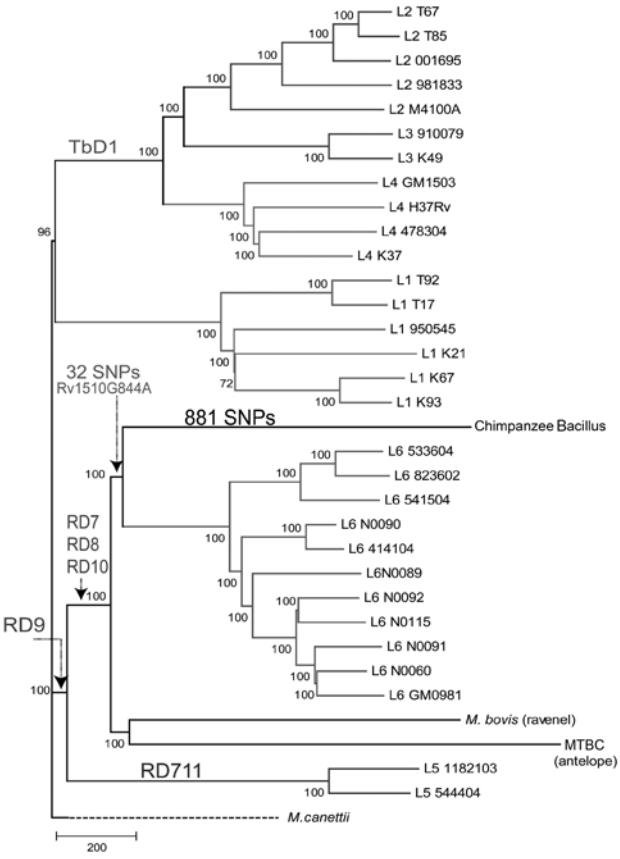

Figure 1. Neighbor-joining phylogenic tree constructed on the basis of 13,480 variable common nucleotide positions across 36 human and animal *Mycobacterium tuberculosis* complex (MTBC) genome sequences, including 21 previously published genomes (*18*) and the MTBC strain isolated from an adult female chimpanzee that was found dead in Taï National Park, Côte d'Ivoire, on August 5, 2009 (Chimpanzee Bacillus). The tree is rooted with *M. canettii*, the closest known outgroup. Node support after 1,000 bootstrap replications is indicated. Genomic deletions identified in (*7*) are indicated. The number of single-nucleotide polymorphisms (SNPs) exclusive of the chimpanzee strain is indicated in the respective branch, and the number of SNPs shared with the most closely related group of strains is indicated in the common branch. Scale bar indicates number of SNPs. This tree is congruent with the maximum-likelihood phylogeny shown in Technical Appendix Figure 2 (wwwnc.cdc.gov/EID/article/19/6/12-1012-Techapp1.pdf).

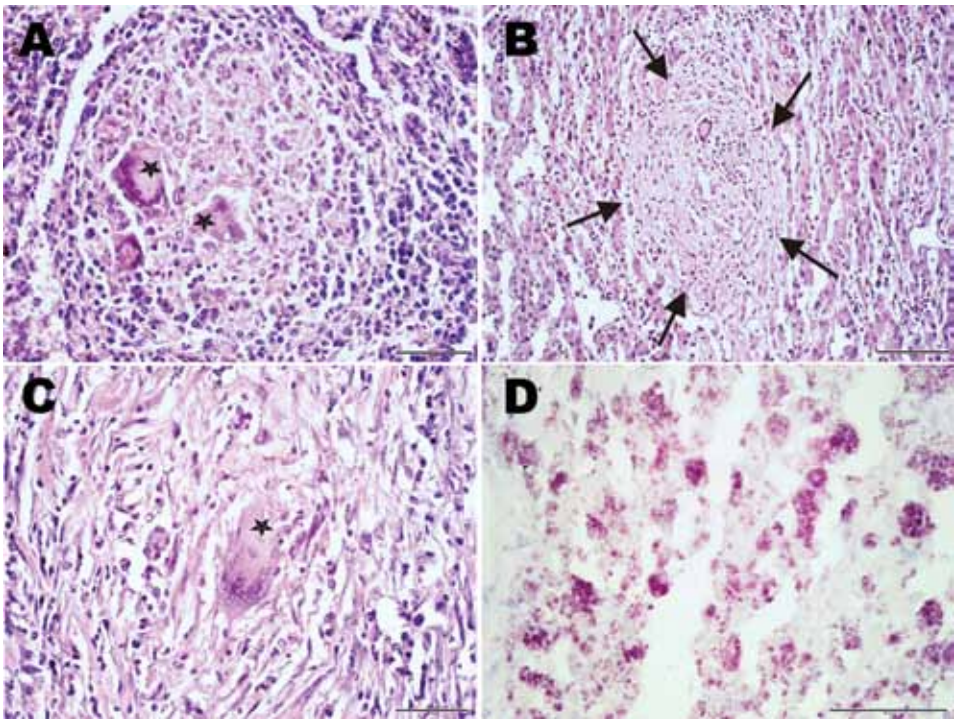

Figure 2. Histopathologic examination of tissue samples from adult female chimpanzee that was found dead in Taï National Park, Côte d'Ivoire, on August 5, 2009. A) Hematoxylin and eosin (H&E) stain of the spleen shows focal granulomatous inflammation with central accumulation of multinucleated Langhans giant cells (stars). B, C) H&E stain of the liver shows focal granulomatous inflammation within liver parenchyma (B, arrows) and large granulomatous alteration demar-cated by fibrous connective tissue infiltrated by Langhans giant cells (C). D) Ziehl-Neelsen stain of the liver shows aggregates of acid-fast bacilli within a large granuloma. Results were consistent with *Mycobacterium tuberculosis* complex infection.

Results

Necropsy of a wild chimpanzee found dead in Taï National Park, Côte d'Ivoire revealed a large, yellow-white granuloma of 5 × 6 × 3 cm in the liver and several smaller ones in the spleen parenchyma and the mesenterial lymph nodes. All other organs appeared unaffected; the lungs could not be evaluated in full because the leopard had consumed most of the tissue. 16S rDNA testing of the frozen tissue samples from the dead wild chimpanzee indicated the presence of a *Mycobacterium* sp. in various tissues. PCR–restriction fragment length polymorphism analysis confirmed MTBC in DNA preparations from spleen and mesenterial lymph nodes (*28*). MTBC was also confirmed by real-time PCR (*29*) in lung, spleen, liver, and colon abscesses.

Histopathologic examination of the liver, spleen, and lymph nodes revealed a chronic granulomatous inflammation within the altered tissues (Figure 2, panels A–C). Multiple unencapsulated granulomas of varying sizes were observed in the spleen (Figure 2, panel A) and liver (Figure 2, panel B). These lesions were composed of epitheloid macrophages, few granulocytes, and multinucleated Langhans giant cells. Larger tuberculoid lesions in the liver and the lymph nodes contained a prominent central necrotic core surrounded by epitheloid cells and a few scattered Langhans giant cells. The periphery of the granulomas was demarcated by variable amounts of fibrous connective tissue and infiltrates of lymphocytes interspersed with few Langhans giant cells (Figure 2, panel C). Both intra- and extracellular acid-fast bacilli were present in the lesions (Figure 2, panel D). Taken together, these lesions were characteristic of TB and indicative of hematogenous spread and generalization of the disease.

After 23 days' incubation, the lymph node preparations yielded typical mycobacterial colonies. The isolated MTBC strain exhibited a slow growth on Middlebrook 7H11 agar and yielded colonies after 43 days, compared with 27 days for *M. tuberculosis*. The rough surface of the colonies and the irregular spreading margins were typical features of MTBC (online Technical Appendix Figure 1).

Whole-genome sequencing was conducted by using the 454 and Illumina platforms (online Technical Appendix Table 1). Phylogenetic reconstruction using previously published MTBC genomes representative of the MTBC's global diversity (*18*) confirmed that the chimpanzee strain belonged to MTBC but not to any of the known phylogenetic lineages (Figure 1). Specifically, the chimpanzee strain grouped with strains from the human-associated lineage 6, sharing 32 SNPs with this lineage, but was separate from the lineage leading to most of the animal-adapted MTBC. To further test whether the chimpanzee strain represented a new lineage rather than a variant within lineage 6, we sequenced the genome of 9 lineage 6 clinical strains from TB patients originating from different West-African countries (online Technical Appendix Table 1). Our phylogenomic analysis revealed that the chimpanzee strain harboured 881 exclusive SNPs, not found anywhere else in the global MTBC phylogeny, even when including these additional

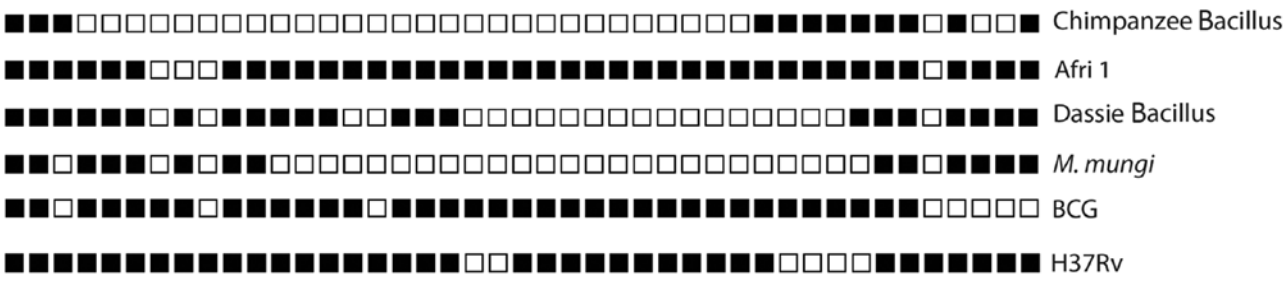

Figure 3. Comparison of the spoligotype of the *Mycobacterium tuberculosis* complex chimpanzee strain isolated from an adult female chimpanzee that was found dead in Taï National Park, Côte d'Ivoire, on August 5, 2009 (Chimpanzee Bacillus), with the Afri 1 spoligotype found in the most closely related human strain and the Dassie Bacillus and *M. mungi* spoligotypes described in (*12*). Spoligotypes are also shown for *M. bovis* strain BCG and human lineage 4 strain H37Rv.

lineage 6 strains. Moreover, the maximum number of SNPs between the 2 most divergent lineage 6 strains was only about half (783 SNPs) of the minimum number of differences between the chimpanzee strain and the most closely related lineage 6 strain (1,405 SNPs). When all MTBC lineages were considered, pairwise SNP distances among any 2 strains belonging to a particular lineage were always markedly lower than the minimum number of differences between the chimpanzee strain and the most closely related lineage 6 strain (online Technical Appendix Figure 3).

To investigate the distinctiveness of the chimpanzee strain we isolated, we used a principal component analysis as an additional clustering method. The first 3 principal components were used to generate a 3-dimensional scatter plot (online Technical Appendix Figure 4). This analysis confirmed that the chimpanzee strain did not group with lineage 6. Taken together, these results strongly suggest that the chimpanzee strain belongs to a distinct MTBC population, separate from the human-associated lineage 6.

To further confirm the uniqueness of the chimpanzee strain, we compared the chimpanzee spoligotype (Figure 3) with 2 large international databases that encompass >8,702 spoligotyping profiles corresponding to >58,187 MTBC isolates from global sources [*33*]; www.mbovis.org). We found that none of the spoligotyping patterns included in these databases (including 64 spoligotyping profiles from Côte d'Ivoire) matched the pattern of the chimpanzee strain (*30*) (Figure 3).

Large-sequence polymorphisms have been used as phylogenetic markers for MTBC (*4*,*7*,*8*). We found that the region of difference (RD) 9 was absent in the chimpanzee strain (*4*) (Figure 1). In addition, this strain harbored deletions in RD7, RD8, and RD10, which supports its phylogenetic relationship with lineage 6 (online Technical Appendix Table 2 and Figure 2). However, the chimpanzee strain harbored only 1 of 14 lineage 6–specific deletions and did not contain the lineage 6–specific region RD900 (online Technical Appendix Table 3) (*29*,*31*). Hence, this deletion-based analysis also supports a related, yet separate, phylogenetic position of the chimpanzee strain relative to lineage 6.

To investigate the possible presence of MTBC in other chimpanzees, 115 tissue samples from 28 chimpanzees that died in the same area (many from the same community) within the previous 10 years were tested by real-time PCR (14). However, no test results were positive. Consistent with the molecular analyses, necropsies and pathological evaluation of these animals revealed no signs suggestive of TB.

Discussion

Chimpanzees are known to be susceptible to TB, and MTBC strains have been reported previously in captive chimpanzees (*32*,*33*). These 2 studies concluded that the infecting strains belonged to *M. africanum* and *M. tuberculosis*, respectively. Direct comparison of those isolates was not possible because of the limitations of typing techniques at that time. However, close contact with humans (i.e., the persons caring for these animals) suggests those captive chimpanzees were infected with human strains, as reported for other captive nonhuman primates.

By contrast, several lines of evidence support the view that the chimpanzee strain we report was not acquired from humans. First, its position on the MTBC phylogeny strongly suggests it belongs to a novel lineage. This notion is sustained by the fact that the minimum genetic distance between the chimpanzee strain and any of the nearest human strains (i.e., lineage 6) was larger than the corresponding distance between any 2 strains from the same lineage. Second, our PCA analysis showed that the chimpanzee strains did not group with lineage 6. Third, spoligotyping revealed a novel pattern among 58,187 clinical isolates from 102 countries, including Côte d'Ivoire. Fourth, genome deletion analyses corroborated the distinct phylogenetic position of the chimpanzee strain compared with known MTBC lineages. Fifth, researchers and their assistants who are in proximity of the chimpanzees at Taï National Forest are regularly screened for TB, but none has ever had a positive test result.

Two other animal-associated members of MTBC are known to cluster with lineage 6 rather than with the classical animal-adapted lineages: *M. mungi* and the Dassie Bacillus, which infect African mongooses and hyraxes, respectively (*11*,*12*). Whole genome analyses are not available for these organisms, but genomic deletion data have been reported (*34*). The chimpanzee strain we isolated did not harbor any of the specific deletions found in *M. mungi* or Dassie Bacillus (online Technical Appendix Table 2) (*34*). Moreover, spoligotyping confirmed that the chimpanzee strain was distinct from *M. mungii* and Dassie Bacillus and from any other MTBC strain genotyped to date (*30*). However, 1 of the 32 SNPs shared between the chimpanzee genome and the lineage 6 strains also occurred in *M. mungii* or Dassie Bacillus. On the basis of this 1 SNP in Rv1510, which has been reported before (*5*), one could hypothesize that the chimpanzee strain and *M. mungii* and Dassie Bacillus might be related. However, genome-wide data will be necessary to define the exact phylogenetic position of *M. mungii* and Dassie Bacillus, and their relationship with the chimpanzee strain, in the global MTBC tree.

Even though chimpanzees maintain close social contacts with other members of their group, extensive necropsies and molecular screening of 28 chimpanzees from the same region yielded no additional case of TB infection, which suggests TB is rare in this chimpanzee population. This low prevalence could have several explanations. While we can likely disregard a human origin of the chimpanzee strain described here, we cannot exclude the possibility that this strain was acquired from another unidentified animal host, including other primates; chimpanzees are known to hunt other animals, including monkeys and small antelopes. The chimpanzee strain we isolated shared ≥1 SNP with *M. mungi* and the Dassie Bacillus, which are pathogens of 2 other small African mammals. On a more speculative note, and if it is assumed that the MTBC strain described is indeed chimpanzee-specific, this MTBC variant might be relatively attenuated and only marginally affect a chimpanzee's health and longevity. This would enable sustained transmission and persistence of the pathogen in small host populations (*35*).

Although more work is needed to establish the prevalence, diversity, and clinical outcome of MTBC infection in wild chimpanzees and other great apes, from a conservation point of view, MTBC may join Ebola virus, *Bacillus cereus* biovar *anthracis*, and simian immunodeficiency viruses as a microorganism capable of threatening great apes in the wild (*36*). Our results suggest the effect of this MTBC strain on chimpanzee populations might be limited, but small outbreaks or single deaths can have a strong influence on the viability of isolated populations, particularly in great apes, which exhibit a slow reproductive rate and a high juvenile mortality rate (*37*).

Our study also sheds new light on the overall diversity of MTBC with implications for understanding the evolution of this pathogen. Together with other recent reports (*11*,*12*), our work suggests wider MTBC diversity, particularly among African mammals. Moreover, these data indicate that the theory that MTBC originally evolved as a human pathogen and jumped into animals is overly simplistic and may apply mainly to domestic animals. These data indicate 1 possible model could be that the common ancestor of MTBC was a generalist capable of infecting many mammals, including humans. From here, only few descendants spread around the world through human and animal migrations, creating the human-dominated phylogenetic picture we see today. We may well expect to find a much higher diversity of this MTBC, extending well outside the human-associated MTBC strains that infect various species, represented mainly through wildlife, including our closest relatives, the great apes. *M. canettii* and the other smooth TB bacilli show a high genetic diversity and are largely limited to the Horn of Africa, although whether these bacilli should be formally considered part of MTBC is controversial (*3*). Hence, they have been proposed to be part of the mycobacterial population that gave rise to the classical members of MTBC. Together with the apparent lack of human-to-human transmission of *M. canettii* (*38*), this suggestion would be consistent with a wider host range and/or environmental reservoir for the original ancestor of MTBC.

In conclusion, we report a microbiologically confirmed case of TB in a wild chimpanzee. Our molecular data show that the chimpanzee strain described here belongs to a novel lineage, more closely related to human-associated lineage 6 than to the other classical animal MTBC and possibly related to *M. mungii* and the Dassie Bacillus. This strain could represent a chimpanzee-specific pathogen or an MTBC variant acquired from another source. Because of our limited understanding of the ecology of this microbe, we propose at this stage to name it "Chimpanzee Bacillus" rather than to develop a dedicated species or subspecies name. Further studies are warranted, not only to better understand the natural history of TB in great apes and the biology of the Chimpanzee Bacillus, but also to estimate a possible risk for transmission of new types of MTBC to humans (e.g., through hunting and consumption of bushmeat). Moreover, further characterization of MTBC diversity will be crucial for understanding the origins of TB and the potential for the emergence of new strains through proximity between humans and wildlife.

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Endemic Norovirus Infections in Children, Ho Chi Minh City, Vietnam, 2009–2010

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We performed a case–controlled investigation to identify risk factors for norovirus infections among children in Vietnam. Of samples from 1,419 children who had diarrhea and 609 who were asymptomatic, 20.6% and 2.8%, respectively, were norovirus positive. Risk factors included residential crowding and symptomatic contacts, indicating person-to-person transmission of norovirus.

Norovirus (NoV) is a leading cause of acute gastroenteritis in children <5 years of age (*1*). The epidemiology of NoV in industrialized countries has been intensively investigated, yet the contribution of this pathogen to the effects of diarrheal disease in low- and middle–income countries is not well characterized (*1,2*). Gaining insight into the epidemiology of NoV infections of children in such countries is essential for disease control, particularly considering that several vaccine candidates are in advanced-stage clinical trials (*3*). To address the lack of data on risk factors for endemic NoV infections in low-income countries, we conducted a prospective case–control study among hospitalized children in a major urban location in southern Vietnam.

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The Study

This study was conducted in 3 hospitals (Children's Hospital 1, Children's Hospital 2, and the Hospital for Tropical Diseases) in Ho Chi Minh City, Vietnam, during May 2009–December 2010. Written informed consent from a parent or legal guardian was mandatory for participation. Children <5 years of age who resided in Ho Chi Minh City, who had acute diarrhea on admission (≥3 loose stools or ≥1 bloody loose stool within a 24-hour period), and were given no antimicrobial drug treatment 3 days before hospitalization, were invited to participate during May 2009–April 2010. To collect control data, during March–December 2010, we enrolled children who were attending outpatient and inpatient clinics in the nutrition or gastroenterology departments for routine health checks or conditions unrelated to gastroenteritis. Children in this control group met the same demographic criteria, did not have diarrhea, and had not received antimicrobial drugs during the preceding 3 weeks.

Stool specimens were collected from case-patients on the day of admission (n = 1,419) and from control participants while they were attending the clinic (n = 609). All stool samples were cultured by using classic microbiologic methods to detect *Shigella, Salmonella*, *Campylobacter,* and *Yersinia* spp. and were microscopically examined for *Entamoeba, Cryptosporidium,* and *Giardia* spp. Methods are described in the online Technical Appendix (wwwnc.cdc.gov/EID/article/19/6/11-1862-Techapp1.pdf). Conventional reverse transcription PCR was performed on RNA extracted from stool samples to detect rotavirus (*4*) and NoV genogroups I (GI) and II (GII) (*5*), followed by direct sequencing of the amplicons for genotyping.

After rotavirus (46.6%; 661/1,419), NoV was the second most common pathogen detected in symptomatic case-patients (20.6%; 293/1,419); diarrheal bacteria and parasites were cumulatively found in 14.5% (online Technical Appendix Table). The prevalence of NoV was higher than in a pooled international estimate (*1*) and than in previous studies performed in Ho Chi Minh City (*6–8*), yet was lower than that found in a study conducted in northern Vietnam (*9*). The frequency of NoV detected in control participants was 2.8% (17/609), similar to a pooled international estimate (*1*). The majority of NoV-positive case-patients experienced nonbloody, nonmucoid watery diarrhea, vomiting, and fever. These symptoms were comparable to those in previous studies of diarrheal infections in children in Vietnam (*7,9*).

NoV was detected throughout the study period (online Technical Appendix Figure). There was a positive linear correlation between NoV infections and monthly rainfall ($R = 0.550$, $p = 0.029$), but no similar correlation with temperature (range 22.1°C–37.8°C) ($R = 0.308$, $p = 0.330$). This association of NoV infections with the tropical rainy

Table 1. Baseline characteristics of NoV-positive and NoV-negative case-patients and control participants, Vietnam, 2009–2010*

Characteristic	Case-patients		Controls	
	NoV positive, n = 241	NoV negative, n = 1,126	NoV positive, n = 15	NoV negative, n = 592
Male sex	147 (61.0)	724 (64.3)	8 (53.3)	314 (53.0)
Mean age, mo (range)	13.3 (2–45)	15.8 (1–59)	15.8 (2.3–52)	16.8 (0–60)
Age groups, mo				
≤6	24 (10.0)	165 (14.7)	3 (20.0)	98 (16.6)
7–12	102 (42.3)	375 (33.3)	4 (26.7)	208 (35.1)
13–18	76 (31.5)	245 (21.8)	3 (20.0)	113 (19.1)
19–24	25 (10.4)	147 (13.1)	3 (20.0)	56 (9.5)
24–60	14 (5.8)	194 (17.2)	2 (13.4)	117 (19.8)
Poor Z score†	18 (7.5)	73 (6.5)	1 (6.7)	75 (12.7)
Breastfed	187 (77.6)	790 (70.2)	11 (73.3)	452 (76.4)
Daily activity				
Day care/nursery school	30 (12.5)	194 (16.4)	4 (26.7)	89 (15.2)
Home	211 (87.6)	938 (83.6)	11 (73.3)	498 (84.8)

*Values are no. case (%) unless otherwise specified. Case-patients indicate patients who had diarrhea; controls indicate asymptomatic (diarrhea-free) children. Study dates span May 2009–December 2010. NoV, norovirus; WHO, World Health Organization.
†Weight-for-age Z score calculated based on WHO Child Growth Standards guidelines (www.who.int/childgrowth/standards/technical_report/en/); Z score below −2 was considered to indicate that a child was malnourished.

season may reflect differential transmission between different climatic regions because NoV infections are typically associated with the winter season in industrialized countries in temperate regions (*10*).

GII NoV was detected in 239 (99.1%) of 241 and 11 (73.3%) of 15 NoV-positive stool samples from the symptomatic and asymptomatic enrollees, respectively. The remaining children were infected with NoV GI (GI.3, GI.4, GI.5); 1 enrolled case-patient was infected with 2 genotypes: NoV GI.3 and GII.4. Of the GII strains, GII.4 was the most prevalent genotype, comprising 201 (84.1%) of the 239 samples. The next most prevalent was GII.3: 24 (10.0%) were identified in the symptomatic and asymptomatic groups. Other GII genotypes (GII.2, GII.6, GII.7, GII.9, GII.12, and GII.13) were found in <3% of NoV-positive samples.

Socioeconomic and behavioral data were obtained from all enrollees by using a questionnaire and analyzed by using Stata Version v9.2 (StataCorp LP, www.stata.com) (Table 1). We used χ^2 and Fisher exact tests to compare proportions between groups and Mann-Whitney U tests for nonparametric data. Univariate analyses were performed to assess factors associated with symptomatic NoV infections. Factors found to be significantly associated with infection in the univariate analysis, in addition to a-priori factors of age, sex, and income level, were then included in a multivariate logistic regression model to simultaneously control for confounding effects. Two-sided p values <0.05 were considered significant throughout (Table 2).

NoV infections are commonly associated with outbreaks in enclosed environments (*2*), yet we found attendance in daycare centers and nursery schools was not common; the majority of children remained at home during the day. However, several factors were significantly and independently associated with symptomatic NoV infections. Demographic risk factors included younger age (in months) (adjusted odds ratio [aOR] 0.96, 95% CI 0.94–0.98, p<0.001) and household crowding (≥3 children in the house) (aOR 1.70, 95% CI 1.0–2.9, p = 0.052). Living in a household where food was regularly purchased from outdoor markets added a significant risk (aOR 4.99, 95% CI 3.1–7.9, p<0.001). Unpredictably, we found that consuming bottled water, rather than pipeline water (aOR 2.18, 95% CI 1.4–3.4, p< 0.001), was a risk factor and did not correlate with household income. However, those drinking municipal water also reported boiling or filtering water before consumption, and those drinking bottled water did not. This association suggests that bottled water in this location may be of poor quality. A further unexpected finding was the protective nature of outdoor toilets (aOR 0.22, 95% CI 0.1–0.4, p<0.001), which may be a result of the sterilizing capabilities of sunlight or of containing fecal contamination outside the residence, possibly protecting children during the period of infancy before they can use toilets. We found that the greatest risk factor for symptomatic NoV infections (aOR 26.14, 95% CI 10.4–65.9, p<0.001) was contact with a person who recently had a diarrheal infection. This finding is consistent with previous investigations showing that person-to-person transmission is predominant during sporadic outbreaks (*11–14*).

This study has several limitations. First, passive case detection limits generalizability because health care–seeking behavior may depend on disease severity and income in this setting. Second, the control participants may not be entirely representative of the population from which the case-patients arose because a large proportion of the control participants were visiting the hospital for nutritional advice, which may have an effect on diarrheal disease risk (*15*). Yet, a limited sensitivity analysis comparing NoV-positive case-patients to NoV-negative control participants and NoV-negative case-patients to NoV-negative control participants demonstrated

Table 2. Univariate and multivariate analysis of risk factors for symptomatic NoV infections, Vietnam, 2009–2010*

Risk factor	NoV-positive case-patients	NoV-negative control participants	OR	95% CI	aOR	95%CI
Mean age, mo (range)	13.3 (2–45)	16.8 (0–60)	**0.97**	**0.96–0.99**	**0.96**	**0.94–0.98**
Male sex (%)	147 (61.0)	314 (53.0)	**1.38**	**1.0–1.9**	1.38	0.9–2.0
Poor Z-score	18 (7.5)	75 (12.7)	**0.56**	**0.3–0.9**	0.61	0.3–1.1
Low income†	150 (62.2)	335 (56.6)	1.26	0.9–1.7	0.89	0.6–1.3
≥5 adults in hh	72 (29.9)	158 (26.7)	1.17	0.8–1.6	NI	NI
≥3 children in hh	36 (14.9)	58 (9.8)	**1.62**	**1.0–2.5**	**1.70**	**1.0–2.9**
Refrigerator in hh	187 (77.6)	506 (85.5)	**0.59**	**0.4–0.9**	0.73	0.5–1.2
Consumes market food	201 (84.1)	345 (58.4)	**3.77**	**2.6–5.5**	**4.99**	**3.1–7.9**
Household water source						
Pipeline‡	132 (54.8)	347 (58.6)	1.00	NA	NI	NI
Well	96 (39.8)	220 (37.2)	1.15	0.8–1.6	NI	NI
Other§	13 (5.4)	25 (4.2)	1.37	0.7–2.8	NI	NI
Drinking water source	NI	NI	NI	NI	NI	NI
Pipeline‡	116 (48.1)	334 (56.4)	1.00	NA	1.00	NA
Bottled water	69 (28.6)	122 (20.6)	**1.63**	**1.1–2.3**	**2.18**	**1.4–3.4**
Well	42 (17.4)	109 (18.4)	1.11	0.7–1.7	0.94	0.6–1.5
Other§	14 (5.8)	1.49	0.8–2.9	0.25	1.45	0.6–3.2
Toilet type						
Indoor‡	213 (90.6)	446 (75.9)	1.00	NA	1.00	NA
Outdoor	22 (9.4)	142 (24.2)	**0.32**	**0.2–0.5**	**0.22**	**0.1–0.4**
Hand washing¶	130 (85.0)	291 (89.5)	0.66	0.4–1.2	NI	NI
Attends day care/nursery school	30 (12.5)	89 (15.2)	0.80	0.5–1.2	NI	NI
Contact with symptomatic persons	38 (16.5)	8 (1.4)	**14.23**	**6.5–31.0**	**26.14**	**10.4–65.9**
Rural residence#	36 (14.9)	75 (12.7)	1.21	0.8–1.9	NI	NI

*Values are no. case (%) unless otherwise specified. Values in **boldface** indicate statistical significance at p≤0.05. NoV, norovirus; OR, odds ratio; aOR, adjusted OR; NA, not applicable; NI, not included in multivariable analysis; hh, household.
†Classified as making less than the Gross National Income ($232/mo) according to World Bank (http://data.worldbank.org/indicator/NY.GNP.PCAP.CD).
‡Reference group.
§Rain water, water from a truck provided by the government, or other water source.
¶Washing of children's hands, either by an adult or the child, after the child uses the toilet.
#Binh Chanh, Can Gio, Cu Chi, Hoc Mon, and Nha Be districts.

several differences in risk factors, suggesting that the identified risk factors are associated with NoV rather than health care–seeking behavior (online Technical Appendix Table).

Conclusions

This epidemiologic investigation showed that 20.6% of hospitalized children with acute diarrhea in Ho Chi Minh City tested positive for NoV, compared with 2.8% of diarrhea-free control participants. We conclude that young age, residential crowding, use of bottled water, and recent contact with a symptomatic individual are key risk factors for symptomatic NoV infection in this location. Because most children did not attend day care, potential preventative measures for NoV infection in Ho Chi Minh City should be focused on improving local hygiene standards to prevent person-to-person transmission within the home.

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Human Melioidosis, Malawi, 2011

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A case of human melioidosis caused by a novel sequence type of *Burkholderia pseudomallei* occurred in a child in Malawi, southern Africa. A literature review showed that human cases reported from the continent have been increasing.

Melioidosis is widely distributed in tropical and subtropical regions, but data are lacking on this disease in sub-Saharan Africa. We report a case of melioidosis caused by a novel sequence type in a 16-month-old boy from rural Malawi. Increasing reports of melioidosis from Africa indicate a need for further investigation.

Case Report

A 16-month-old boy was seen at Queen Elizabeth Central Hospital (QECH), Blantyre, Malawi, in March 2011 with fever of 18 days' duration, poor feeding, subcutaneous lesions of 15 days' duration, and edema of the hands and feet. He had received intravenous (IV) benzylpenicillin and gentamicin for 4 days at a local health center before being referred for persistent fever.

The family lived in a remote village in the Shire Valley near the Malawi–Mozambique border at a latitude of –15°S; daytime temperature average was 27°–29°C, and natural flooding occurs from October through April. The boy's parents were subsistence maize farmers and kept goats and pigs.

On arrival at QECH, the child was irritable and pale; temperature was 38.7°C, weight 9.8 kg (weight-for-age z score –1.14), and height 79 cm (weight-for-height z score –1.06). He had bilateral dactylitis with arthritis of the metacarpophalangeal and interphalangeal joints of the lateral 3 fingers. Numerous rubbery, tender subcutaneous nodules of ≈2 cm diameter were palpable on the face, thorax, and limbs. Overlying hyperpigmentation and weepy ulcerations occurred over some nodules. There was cervical and inguinal lymphadenopathy but no hepatosplenomegaly. Symmetric bipedal pitting edema extended to the knees. Neurologic, cardiovascular, and respiratory examinations revealed no abnormalities.

Laboratory results were as follows: blood glucose 7.8 mmol/L (reference 3.5–7.7 mmol/L), hemoglobin 4.6 g/dL (reference 9.7–15.1 g/dL), leukocyte count 31.9 × 10^3/μL (reference 3.9–10.7 × 10^3/μL), and erythrocyte sedimentation rate 95 mm/h (reference 3–13 mm/h). Blood smear showed poikilocytes with some tear drops and reticulocytes and was negative for malaria parasites. HIV test (Unigold; Trinity Biotech, Bray, Ireland) and VDRL (Venereal Disease Research Laboratory) test for syphilis were negative. Radiographs of the hand showed bilateral osteolytic reactions in the lateral 3 fingers. Chest radiograph and abdominal ultrasound indicated no abnormalities.

Culture (BacT/Alert PF; bioMérieux, Marcy l'Etoile, France) of blood taken on admission and aspirate of pus from a subcutaneous nodule grew white, oxidase-positive colonies of gram-negative rods, and the biochemical profile (API 20NE; bioMérieux) strongly suggested *Burkholderia pseudomallei* (1556575: *B. pseudomallei* [98.3% identity]). The API profile from the pus isolate (1156154) initially suggested *Chromobacterium violaceum*, a recognized misidentification of *B. pseudomallei*, by API profiling (*1*). Antimicrobial susceptibility by disk diffusion indicated resistance to gentamicin and susceptibility to co-amoxiclav; colistin disk testing was unavailable. Because *B. pseudomallei* has not been reported from Malawi, we sought to confirm the isolate by real-time PCR, targeting the highly specific type III secretion system (*2*). DNA was extracted by using a Wizard Genomic Purification kit (Promega, Madison, WI, USA), and real-time PCR was performed on an Applied Biosystems 7900HT (Applied Biosystems, Foster City, CA, USA) by using a technique modified for SYBR green detection (*2*). This PCR confirmed the identity of the organism as *B. pseudomallei*. Whole-genome sequencing (WGS) was performed by using the MiSeq Personal Sequencer (Illumina, San Diego, CA, USA), which enabled multilocus sequence typing (MLST) (*3*) and revealed a novel allelic combination (1,3,3,1,5,1,1). This sequence type (ST) has been submitted to the MLST database (http://bpseudomallei.mlst.net/) and has been assigned MLST ST1008, part of clonal complex 1.

The boy was given chloramphenicol for empiric treatment of systemic bacterial infection before the isolate was

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Table 1. Melioidosis in animals, Africa

Year (reference)	Country	Animal	Clinical characteristics	Method of identification
1936 (*6*)	Madagascar	Pig	Lymphadenopathy	Passaged through guinea pig (tissue from submaxillary gland)
1960 (*7*)	Chad	Goat	Lymphadenopathy	Isolated from mesenteric ganglia
1960 (*8*)	Africa*	Camel	Retropharyngeal abscess	Inoculation and sacrifice of guinea pig
1972 (*8*)	Niger, Burkina Faso	Pigs (>100 cases)	Abscesses in liver, spleen, and lung in apparently healthy pigs	Not described
1995 (*9*)	South Africa	Goat	Mammary gland and renal abscesses	Biochemical and phenotypic characteristics

*Specific country of acquisition was not detailed.

identified. In light of the anthropometric values, anorexia, fecal morphology, and symmetric pedal edema, acute kwashiorkor was diagnosed, and nutritional rehabilitation was begun. Pedal edema and anorexia improved after 48 hours. At 96 hours, *B. pseudomallei* infection was diagnosed, and treatment was changed to IV ceftazidime. Fever abated by day 7, and after 30 days of IV ceftazidime, the nodules had involuted and the dactylitis and arthritis had resolved. At the family's request, the child was discharged on a 6-month regimen of cotrimoxazole, rather than the planned 6-week IV regimen for osteomyelitis.

Four weeks after discharge, the child remained well with no fevers and no new lesions; clinical anemia had resolved, and repeat radiographs showed that the hands were within normal limits. He was then lost to follow-up.

Conclusions

Melioidosis is acquired through the skin or possibly by inhalating the environmental organism *B. pseudomallei*. It causes a wide spectrum of clinical disease–from localized skin infection to severe acute septicemia–but progresses to disease in only a small proportion of exposed persons (*4*). *B. pseudomallei* is endemic in Southeast Asia and Northern Australia and typically is distributed from latitude 20°N to latitude 20°S, particularly in association with wet soil (*4*).

Sporadic cases have been documented in all inhabited continents, but a lack of diagnostic microbiological facilities and systematic studies in many low-income regions limit knowledge of the true distribution of the disease (*5*), a particular problem in rural sub-Saharan Africa. Although the relationship between human and animal

Table 2. Melioidosis in humans, Africa*

Year (reference)	Country where acquired (diagnosed)	Details of infected persons	Clinical characteristics	Diagnostic method (source of isolation)	Definitive treatment (duration, wk)	Outcome
1982 (*10*)	Kenya (Denmark)	Adult	Sepsis	Culture (blood, urine, sputum)	OTC (4); cotrimoxazole TMP-SXT (8)	Complete recovery
1985 (*11*)	Sierra Leone (Gambia)	Child	Cutaneous abscesses, osteomyelitis	Culture; indirect hemagglutination (pus)	CHL, TET	Improved; lost to follow-up
2004 (*12*)	Mauritius (Mauritius)	Adult with SLE	Sepsis, cellulitis	Culture; API 2ONE (blood)	None	Died (d 9)
2004 (*13*)	Madagascar (La Réunion)	Adult smoker, alcoholic	Sepsis, respiratory distress	Culture (blood, BAL fluid)	CAZ	Complete recovery
		Adult with CPI	Septicemia	Culture (blood, BAL fluid)	CAZ; cotrimoxazole (20)	Not described
		Adult with SLE	Septicemia	Culture (blood, BAL fluid)	CAZ; cotrimoxazole (20)	Not described
2006 (*14*)	Madagascar (La Réunion)	Adult smoker	Pneumonia	Culture; API 20NE; PCR (BAL fluid);	IPM (2); cotrimoxazole (20)	Resolution at 3 mo
2010 (*15*)	Africa† (France)	Adult	Mycotic aneurysm	Culture (blood, arterial tissue)	IPM + CIP (5); cotrimoxazole (20)	Resolution at 6 mo
2011 (*16*)	Gambia (Spain)	Adult with diabetes mellitus	Pyomyositis, pneumonia	Culture; PCR (pus, sputum)	CAZ + cotrimoxazole (5); DOX + cotrimoxazole (20)	Complete recovery
2011 (*17*)	Nigeria (UK)	Adult with diabetes mellitus	Localized lymphadenopathy	Culture; chromatography; PCR (blood)	Meropenem (1); cotrimoxazole (12)	Lost to follow-up
2011 (*18*)	Africa† (Spain)	Adult	Sepsis	Culture (blood)	CAZ + DOX	Not described

*OTC, oxytetracycline; TMP-SXT, trimethoprim–sulfamethoxazole; CHL, chloramphenicol; TET, tetracycline; SLE, systemic lupus erythematosus; BAL, bronchoalveolar lavage; CAZ, ceftazidime; CPI, chronic pulmonary insufficiency; IPM, imipenem; CIP, ciprofloxacin; DOX, doxycycline; MER, meropenem.
†Exposure was in multiple countries on the continent or the specific country of acquisition was not detailed.

infection is not precisely understood, infections of animals have been recognized throughout the continent (Table 1) (*6–9*). During the past 30 years, 11 cases of human melioidosis acquired in Africa (1 in a child) have been reported in the literature (Table 2 [*10–15*; *16–18* in online Technical Appendix, wwwnc.cdc.gov/EID/article/19/6/12-0717-Techapp1.pdf]). Three of these cases were PCR confirmed. In many earlier reports, identification was not confirmed by methods that would satisfy modern taxonomists; thus, the true distribution of melioidosis in Africa remains uncertain.

We used eBURST software (*19* in online Technical Appendix) to model the relationship between ST1008 and the global MLST database (online Technical Appendix Figure 1). The founder of this branch of clonal complex 1 is ST916 (online Technical Appendix Figure 2), which was isolated from Cambodia. The other STs on this branch, ST186 and ST250, were isolated in Thailand. Although none of the other Africa *B. pseudomallei* isolates of known MLST are predicted to be in the same subgroup, 2 isolates from human infections that are thought to have occurred in Kenya (ST5 and ST9; online Technical Appendix Figure 2) are in the adjacent subgroup. Thus, these Malawi and Kenya strains might share a recent common ancestor. We have submitted WGS data from our sample to a project that is undertaking WGS on a large number of *B. pseudomallei* isolates from around the world. This approach is anticipated to offer superior resolution of the global phylogeny.

Unfamiliarity with the culture characteristics of *B. pseudomallei* often has resulted in delays in recognition, identification, diagnosis, and treatment (*1*). The organism exhibits considerable interstrain and media-dependent variability in colonial morphology (*1*); its wrinkled appearance in older colonies may result in their dismissal as contaminants. Even relatively expensive biochemical test kits, such as the API20NE, may result in misidentification, as with the pus isolate here, which raises the question about whether the infrequency of the diagnosis is due to rarity of the disease or lack of capacity to identify it. *B. pseudomallei* might be more widespread than recognized in Malawi and ecologically similar areas of sub-Saharan Africa where the environment is conducive to its growth. Health care in Malawi, as in most of sub-Saharan Africa, is delivered frequently without use of even basic diagnostic facilities, which leads to overdiagnosis of malaria and tuberculosis (*20* in online Technical Appendix). In this environment, patients with septicemic melioidosis could have died before all locally available empiric treatments had been tried. *B. pseudomallei* is resistant to penicillin, gentamicin, and many other antimicrobial drugs used to treat sepsis in the tropics, so diagnosis is necessary for appropriate antimicrobial therapy.

Melioidosis therefore could be underestimated in Malawi and throughout the region. Environmental microbiology and seroprevalence studies are required to gauge the extent of this infection and to guide local and regional health care policy.

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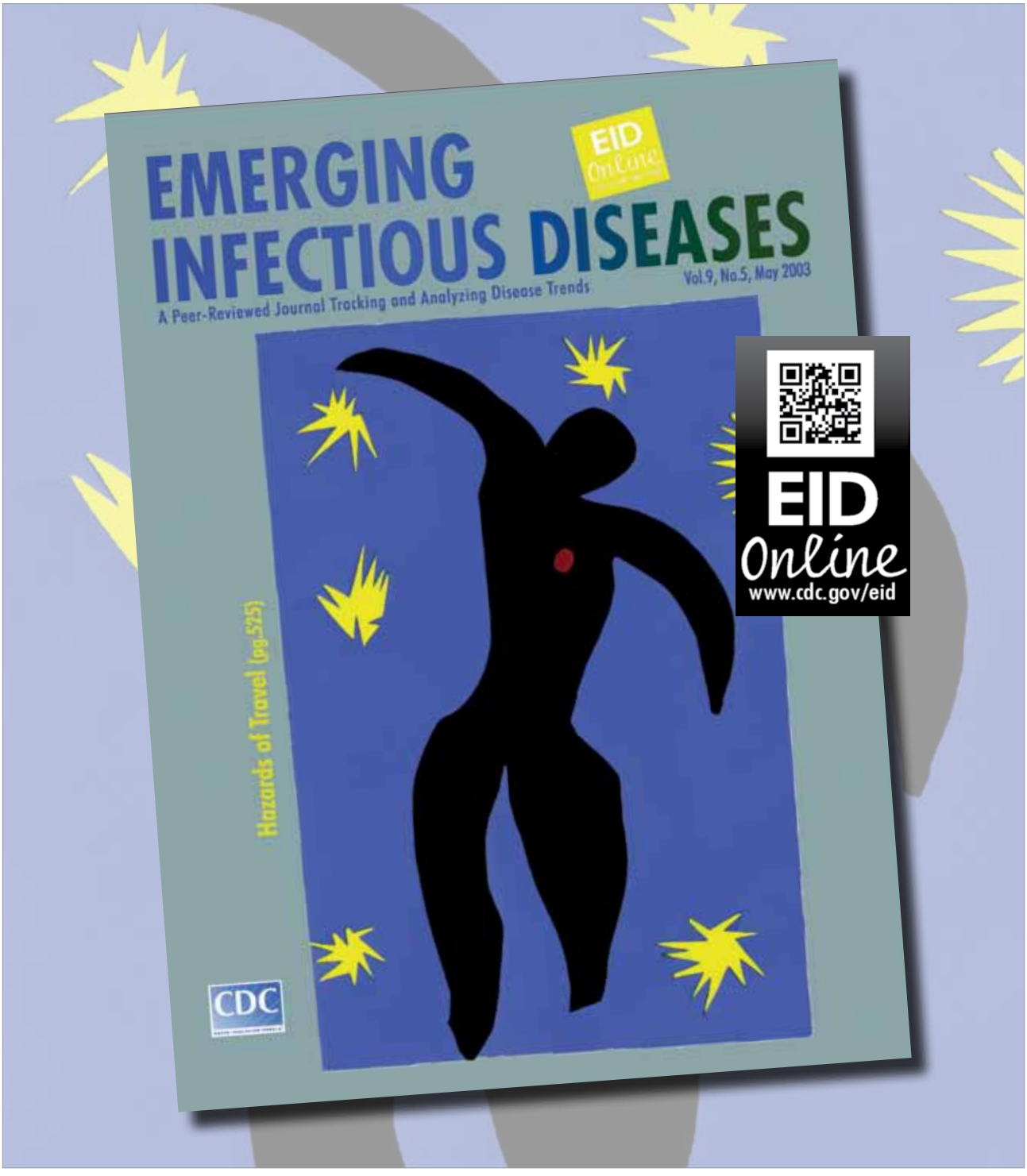

BSE-associated Prion-Amyloid Cardiomyopathy in Primates

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Prion amyloidosis occurred in the heart of 1 of 3 macaques intraperitoneally inoculated with bovine spongiform encephalopathy prions. This macaque had a remarkably long duration of disease and signs of cardiac distress. Variant Creutzfeldt-Jakob disease, caused by transmission of bovine spongiform encephalopathy to humans, may manifest with cardiac symptoms from prion-amyloid cardiomyopathy.

Human prion diseases are progressive neurologic disorders that include sporadic, genetic, and acquired forms of Creutzfeldt-Jakob disease (CJD) (*1*). A key step in disease initiation is conversion of PrP^{C} into PrP^{Sc}, which is partially resistant to proteolytic digestion and an essential part of prion infectivity. Transmission of bovine spongiform encephalopathy (BSE) to humans has led to a novel form of acquired CJD, termed variant CJD (vCJD) (*2*). The pathogenesis of vCJD differs substantially from sporadic CJD with remarkable colonization of non–central nervous system regions with infectious prions and PrP^{Sc} (*3*).

Although risk reduction measures have been introduced to limit transmission from BSE-diseased cattle to humans, vCJD has occurred in several hundred instances (www.eurocjd.ed.ac.uk). Most clinically affected vCJD patients are homozygous for methionine on polymorphic codon 129 on the gene coding PrP (*PRNP*), and the clinical presentation of vCJD in these patients is uniform (*4*). The occurrence of atypical clinical features in persons with vCJD that encodes methionine and valine on *PRNP* codon 129 and human-to-human transmission of vCJD through blood transfusion have raised concern about atypical clinical features and alternative distribution of PrP^{Sc} in vCJD (*5*). We report on the novel clinicopathologic characteristics of vCJD as prion-amyloid cardiomyopathy in 1 of 3 macaques inoculated with BSE.

The Study

In 2002, three rhesus macaques were inoculated with BSE intraperitoneally (10 mL of a 10% homogenate of brain from BSE-diseased cattle). As controls, 2 rhesus macaques received saline (10 mL) and 1 was untreated. All procedures involving rhesus macaques were performed at the Institute of Neuropathology, University Medical Center Hamburg-Eppendorf (Hamburg, Germany), in accordance with the German Animal Welfare Act and the Council Directive 86/609/EEC (Permit 33.42502/08–08.02 LAVES, Lower Saxony, Germany). Animals were observed for clinical signs of prion disease and, when signs of terminal prion disease became evident, were euthanized and underwent autopsy. In all 3 BSE-challenged macaques and none of the controls a progressive neurologic disease developed 49, 59, and 61 months postinoculation. Examination of brain by using hematoxylin and eosin staining showed typical neuropathologic features of vCJD (data not shown) and abundant deposits of PrP^{Sc} in the cortex, basal ganglia, and cerebellum in paraffin-embedded tissue blots performed as described by using 12F10 monclonal antiprion antibody (*6*) (Figure 1, panel A). The mobility of the unglycosylated PrP^{Sc} band and the glycoform ratio of proteinase K–digested PrP^{Sc} were similar to those in BSE when assessed by Western blot analysis by using monoclonal POM-1 antiprion antibody as described (*7*) (Figure 1, panel B).

Besides lymphoreticular tissues, the muscular compartment is targeted by prions (*7*,*8*). Thus, we assessed presence of PrP^{Sc} in skeletal and heart muscle by Western blot analysis with sodium phosphotungstic acid precipitation for enrichment of PrP^{Sc} and protein misfolding cyclic amplification by using published protocols (*3*). We could not detect substantial amounts of PrP^{Sc} in skeletal muscle (Figure 2, panel A). One macaque showed abundant PrP^{Sc} (≈1/100 of PrP^{Sc} found in brain) in heart in Western blot and protein misfolding cyclic amplification (Figure 2, panels A, B). Paraffin-embedded tissue blot analysis of this heart showed PrP^{Sc} as amyloid, occupying considerable stretches of heart tissue, mainly in the septum (Figure 2, panel C), whereas no PrP^{Sc} could be seen in hearts of other macaques (data not shown). These findings were confirmed by strong Congo red–positive patch-like depositions in cardiomyocytes in the heart of this monkey (Figure 2, panel D). The primate with cardiac PrP^{Sc} showed the longest disease duration (4 months, compared with 4 weeks for other BSE-infected monkeys), signs of cardiac affection

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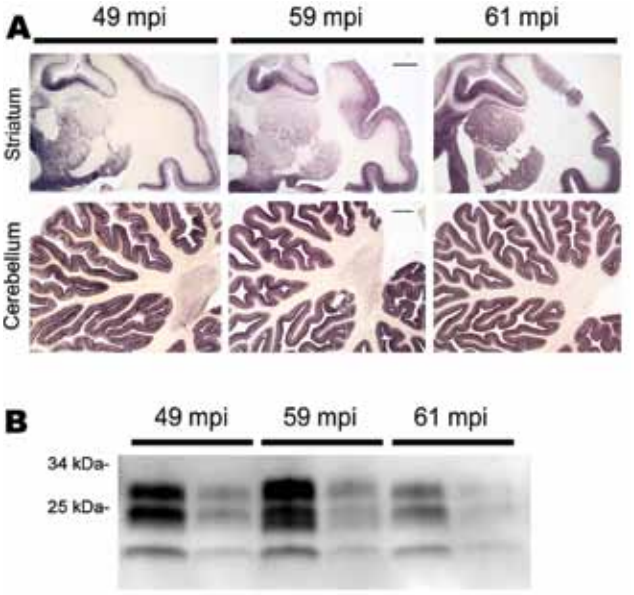

Figure 1. PrP^{Sc} distribution and content in brain of bovine spongiform encephalopathy (BSE)–infected rhesus macaques. A) Paraffin-embedded tissue blot of striatum and cerebellum show a typical BSE-like deposition pattern of PrP^{Sc} with no differences between individual BSE-diseased monkeys at 49, 59, and 61 months postinoculation (mpi). Scale bars = 1 mm. B) Western blot analysis for PrP^{Sc} in brain of BSE-infected monkeys with incubation times of 49, 59, and 61 mpi. PrP^{Sc}-type is as expected for BSE prions, and no major differences in PrP^{Sc} load were detected. All samples were proteinase K–digested; loading amount was 0.5 and 0.1 mg fresh wet tissue for each sample

when assessed by relevant makers of cardiac hypertrophy and of cardiac distress–associated inflammation, and only this macaque showed clinical signs of fatigue and signs of cardiac distress (i.e., venous congestion) on autopsy (Table, online Technical Appendix Table, wwwnc.cdc.gov/EID/article/19/6/12-0906-Techapp1.pdf). Histologic examination of heart tissue with hematoxylin and eosin staining and immunohistochemical stainings against B and T cells (CD20 [not shown] and CD3) did not provide evidence for toxic cardiomyopathy (i.e., fibrosis or vacuolization), nor did we find signs of inflammatory reaction (Figure 2, panel D).

Conclusions

Although the vCJD epidemic is declining, considerable concern exists that clinical characterastics of vCJD will shift. The most important genetic risk factor for development of vCJD is homozygosity for methionine on *PRNP* codon 129, and all but 1 patient with clinical vCJD carry this polymorphism (*5*). Thus, future cases of vCJD with longer incubation times are likely to comprise more patients with alternative codon 129 polymorphisms than methionine homozygosity. Data from rodent experiments indicate that clinical features of vCJD may differ in these patients (*9*). Thus, the next decades may see a shift in vCJD phenotypes. Further uncertainty for atypical cases in humans results from the possibility of secondary transmission of vCJD through blood products from subclinical carriers, which may lead to development of nonclassical vCJD phenotypes (*5*).

We showed that BSE infection of primates may occur as prion-amyloid cardiomyopathy. Because prion-amyloid cardiomyopathy developed in only 1 of 3 macaques, host-encoded factors, such as genetic makeup, probably influence development of this cardiac phenotype. All macaques are homozygous for methionine on *PRNP* codon 129; thus, prion-amyloid cardiomyopathy cannot be related to polymorphic codon 129 in our study (*10*). Cardiac involvement has been observed in a patient with sporadic CJD and is prominent in prion-diseased mice expressing PrP^{C} lacking its membrane anchor (*11*,*12*). We considered the possibility that preexisting pathology, such as spontaneous cardiomyopathy or inflammation of the heart, might have contributed to cardiac PrP^{Sc}, and the fact that we did not find any evidence for toxic cardiomyopathy or inflammation in the primate does not exclude this possibility. Because the macaque with abundant PrP^{Sc} deposition in heart had longer disease duration, it is also possible that longer disease duration, which favors centrifugal spread of prions to peripheral tissues, contributed to cardiac affection in this primate (*7*). Peripheral deposition of PrP^{Sc} in vCJD is well studied (*3*). We were surprised by the amount and deposition type of PrP^{Sc} in heart, reaching 1/100 of the amount seen in brain and deposited as amyloid across large stretches of heart tissue. Skeletal muscle of prion-diseased patients and nonhuman primates routinely harbor minimal amounts of PrP^{Sc} (<1/1000 that found in brain), and PrP^{Sc} in muscle is virtually impossible to detect by in situ methods (*6*,*8*,*13*). To our knowledge, PrP^{Sc} has not been detected in heart of vCJD-diseased persons or in patients with systemic amyloidosis, although primates orally exposed to BSE show

Table. Characteristics of 3 rhesus macaques in study of BSE-associated prion-amyloid cardiomyopathy*

Primate	Age at inoculation	Time to clinical disease, mo	Disease duration, wk	Cardiac PrP^{Sc}	Signs of cardiac distress at autopsy
BSE inoculated	8 y	49	4	Neg	Neg
	5 y	59	18	Pos	Pos
	1 y	61	4	Neg	Neg
Control	8 mo	NA	NA	Neg	Neg
	17 y	NA	NA	Neg	Neg
	19 y	NA	NA	Neg	Neg

*BSE, bovine spongiform encephalopathy; Neg, negative; Pos, positive; NA, not applicable.

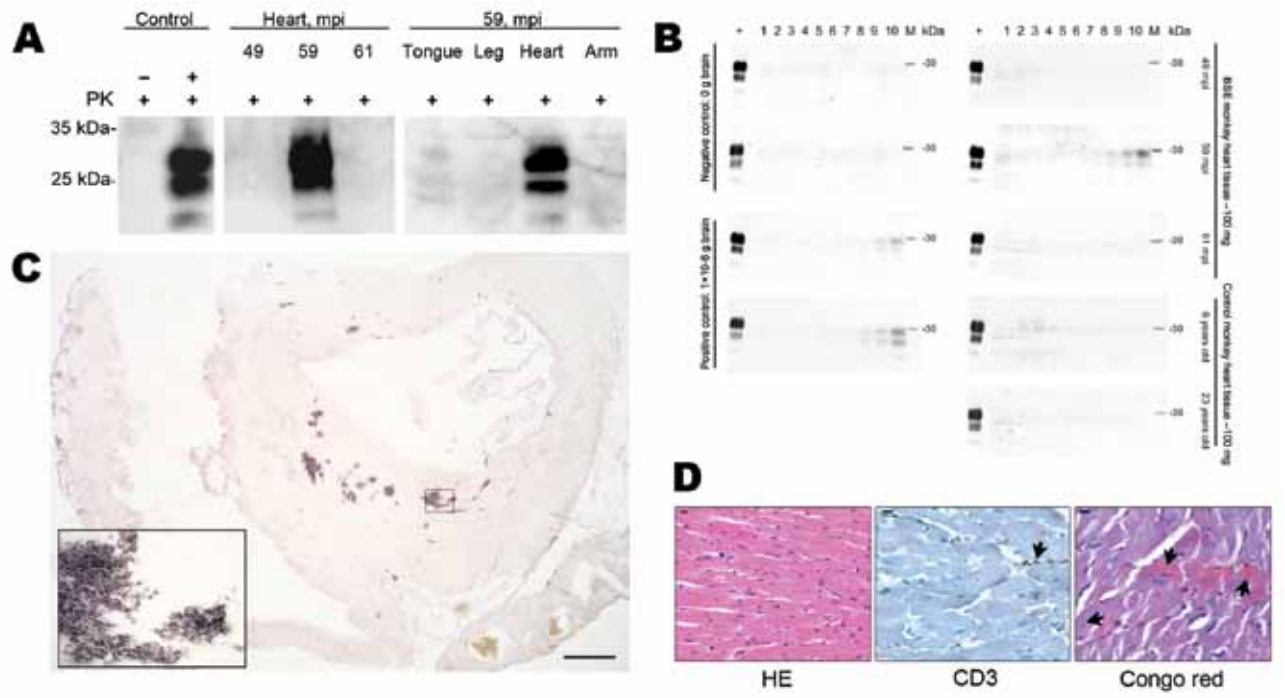

Figure 2. Abundant PrP^{Sc} in heart of 1 bovine spongiform encephalopathy (BSE)–infected rhesus macaque. A) In sodium phosphotungstic acid precipitation of PrP^{Sc}, followed by Western blotting, highly abundant PrP^{Sc} was demonstrated in the heart of 1 BSE-infected primate. In this monkey, only the heart contained PrP^{Sc}. Controls include cardiac muscle spiked with minimal amounts brain of a healthy (–) and prion-diseased (+) primate. All analyses were prepared from 50 mg of tissue except the heart of 1 monkey 59 months postinoculation (mpi) (20 mg). PK, proteinase K. B) In protein-misfolding cyclic amplification, PrP^{Sc} was amplified only from the heart of 1 monkey 59 months postinoculation (mpi). As a positive control, brain tissue from a BSE-diseased monkey was used, and tissue from an uninfected control monkey served as a negative control. PK–digested hamster PrP^{Sc} (263 K) served as loading and digestion control for PrP^{Sc}. C) Paraffin-embedded tissue blotting of the entire heart of the 59 mpi monkey showed abundant deposition of PrP^{Sc}, mainly in the septum of the heart. Inset confirms the deposition pattern of PrP^{Sc} as amyloid. Scale bar = 0.25 mm. D) Histologic and immunohistochemical examination of heart tissue of the 59-mpi monkey by using hematoxylin and eosin (HE) staining and immunohistochemical staining against T-cell marker CD3 showed regularly configured cardiomyocytes and only single T-cells associated with blood vessels (arrow). Congo red staining showed Congo red–positive material in cardiomyocytes in a patch-like deposition pattern (arrows). Scale bar = 10 μm.

very low amounts of cardiac PrP^{Sc} (*8*,*14*,*15*). The lack of cardiac PrP^{Sc} in vCJD may result from small cohorts investigated. Because the spectrum of vCJD is likely to change, broad application of current clinical criteria for vCJD in clinical practice may lead to underreporting of vCJD, missing atypical cases of vCJD.

In conclusion, we showed that BSE-infection of primates may lead to prion-amyloid cardiomyopathy. These data should be considered when vCJD surveillance is conducted.

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The overall study was conceived and designed by M.G., A.A., F.J.K., and S.K. Animal care, housing, and observation were conducted by F.J.K., W.B., and W.S.S. Experiments were performed by S.K., G.M., E.K., K.W., W.S.S., and M.N. Data were analyzed by S.K., G.M., M.B., A.A., and M.G. S.K. and M.G. wrote the paper with substantial contributions from G.M. and A.A.

Dr Krasemann is a research scientist at the Institute of Neuropathology of the University of Hamburg working on prion spread. Her primary research interests are factors involved in spread and clearance of prions.

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etymologia

Shewanella haliotis

[shoo″ə-nel′ə hā″lĭ-o′tĭs]

From the Greek halios (marine) and ōtos (ear), abalones, genus Haliotis, were first mentioned ≈2,500 years ago by Aristotle, who wrote of "the wild limpet (called by some the 'sea ear')." In D'Arcy Thompson's translation of Aristotle, he notes that "wild limpet" is "commonly attributed to Fissurella graecea ... and conceals a forgotten name for Haliotis." The "sea ear" was familiar to the Greeks and was named otia (little ear) by Pliny.

Shewanella haliotis, a species of rod-shaped, gram-negative, facultatively anaerobic bacteria, was first isolated from the gut microflora of abalones collected from the ocean near Yeosu, South Korea, by Kim et al. in 2007. The genus *Shewanella* had been previously named in 1985 by MacDonell and Colwell in honor of Scottish microbiologist James M. Shewan, for his work in fisheries microbiology.

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Novel SARS-like Betacoronaviruses in Bats, China, 2011

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To clarify the evolutionary relationships among betavoronaviruses that infect bats, we analyzed samples collected during 2010–2011 from 14 insectivorous bat species in China. We identified complete genomes of 2 novel betacoronaviruses in *Rhinolophus pusillus* and *Chaerephon plicata* bats, which showed close genetic relationships with severe acute respiratory syndrome coronaviruses.

The 2003 outbreak of severe acute respiratory syndrome (SARS) was caused by a novel betacoronavirus and rapidly spread globally, causing ≈8,000 cases and nearly 900 deaths (*1*,*2*). In June 2012, a novel betacoronavirus (called human coronavirus EMC [HCoV-EMC]) also was isolated from the sputum of a patient from Saudi Arabia who died of pneumonia and renal failure (*3*). Similar viruses were detected in 2 additional patients who had severe pneumonia in Qatar in September 2012 and in Saudi Arabia in November 2012 (*4*,*5*). The clinical picture was remarkably similar to that of SARS and illustrates the epidemic potential of a novel coronavirus (CoV) to threaten global health. SARS-CoVs and HCoV-EMC were suspected of spreading from bats to humans because these CoVs were most closely related to bat CoVs (*1*,*4*). To clarify the evolutionary relationships among betavoronaviruses that infect bats, we analyzed samples collected during 2010–2011 from 14 insectivorous bat species common in 8 provinces in China.

The Study

We obtained pharyngeal and anal swab specimens of 414 insectivorous bats. Samples of each species were pooled and then processed with a viral particle–protected nucleic acid purification method (*6*). The extracted RNA and DNA were amplified by sequence-independent PCR. The amplified viral nucleic acid libraries of the bat species were then sequenced with the Illumina/Solexa GAII sequencer (Illumina, San Diego, CA, USA). Those reads generated by the Illumina/Solexa GAII with length of 80 bases were directly aligned to the protein sequences in the National Center for Biotechnology Information nonredundant protein database by the blastx program in the BLAST software package, version 2.2.22 (www.ncbi.nlm.nih.gov/blast) with parameters "-e 1e-5 -F T -b 10 -v 10." No assembly was performed before alignment. Sequence similarity–based taxonomic assignments were conducted as described (*7*). We found 1,075 reads of betacoronavirus in *Rhinolophus pusillus* bats in Shaanxi and 92 reads of betacoronavirus in *Chaerephon plicata* bats in Yunnan.

We estimated the approximate locations of those reads on the CoV genome and their relative distances on the basis of alignment results exported with MEGAN 4–MetaGenome Analyzer (http://ab.inf.uni-tuebingen.de/software/megan/). The located reads were then used for reads-based nested PCR to identify genomic sequences. We established the complete genome sequences of 2 betacoronaviruses (Bat Rp-coronavirus/Shaanxi2011 and Bat Cp-coronavirus/Yunnan2011), which are 29,484 nt and 29,452 nt, respectively. The G+C content of Bat Rp-coronavirus/Shaanxi2011 and Bat Cp-coronavirus/Yunnan2011 is 41.6% and 40.9%, respectively.

We conducted complete genome comparison and phylogenetic analysis on the basis of polymerase and spike protein. Pairwise genome sequence alignment was conducted by using EMBOSS Needle software (www.ebi.ac.uk/Tools/psa/emboss_needle/) with default parameters. The overall nucleotide sequences between Bat Rp-coronavirus/Shaanxi2011 and Bat Cp-coronavirus/Yunnan2011 indicated 88.7% nt identity. They shared 87.4%–89.5% nt identity with SARS-CoV, 88%–89.9% nt identity with the bat SARS-like CoV (bat SARS-CoV Rm1), and 87.6%–89.6% nt identity with the civet SARS-like CoV (civet SARS-CoV SZ16). On the other hand, comparison between the betacoronavirus genomes and human betacoronavirus (HCoV-OC43) showed only 49.9%–50.4% nt overall identity, whereas the betacoronavirus genomes and HCoV-EMC showed 52.1% nt overall identity.

The RNA-dependent RNA polymerase (RdRp, the 12th nonstructural protein codified to open reading frame 1a,b) is a highly conserved gene of CoVs, which is frequently used for phylogenetic comparison (*8*,*9*). MEGA5.0 (www.megasoftware.net) was used to construct

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the phylogenetic trees on the basis of the nucleotide sequences and deduced amino acid sequences. First, we used the MUSCLE package and default parameters (www.megasoftware.net/) to construct the alignment. The best substitution model was then evaluated with the Model Selection package implemented in MEGA5. Finally, we used the maximum-likelihood method with an appropriate model to process the phylogenetic analysis with 1,000 bootstrap replicates. We constructed a phylogenetic tree based on the nucleotide sequences of the *RdRp* gene to show the evolutionary relationship between these 2 betacoronaviruses and other CoVs (Figure 1). Reference CoV genome sequences were downloaded from GenBank and aligned with the fragments of the newly discovered CoVs. The *RdRp* genes of Bat Rp-coronavirus/Shaanxi2011 and Bat Cp-coronavirus/Yunnan2011 were highly similar, sharing 93.1% nt identity. The phylogenetic analysis demonstrated that betacoronaviruses and the bat SARS-like CoVs in our study are clustered (93.1%–93.4% nt identity) and are close in distance to SARS-CoVs (92.9%–94.8% nt identity) and civet SARS-like CoVs (93.1%–94.8% nt identity) but that bat CoV (BtCoV-HKU9) and HCoV-OC43 are placed among the relatively distant groups (65.8%–65.9% and 62.9%–63.5% nt identities with the betacoronaviruses, respectively). Therefore, collectively we called these betacoronaviruses and bat SARS-like CoVs the bat SARS-like cluster of CoVs. Bat Rp-coronavirus/Shaanxi2011 and Bat Cp-coronavirus/Yunnan2011 showed little genetic similarity (<66.2%–67.3% nt identity) to HCoV-EMC.

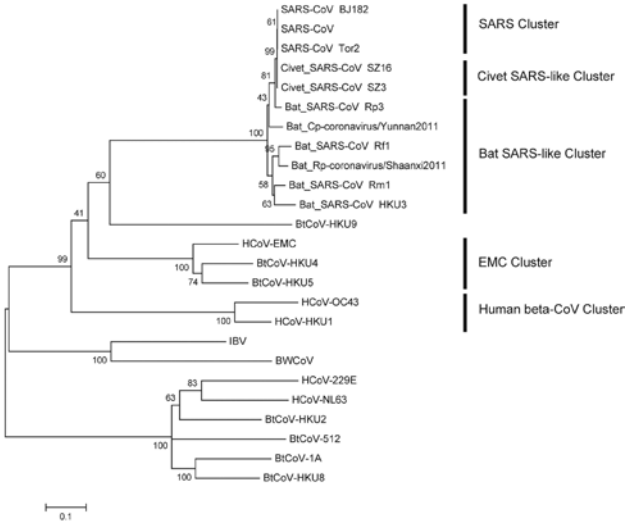

Figure 1. Phylogenetic tree of novel betacoronaviruses based on the nucleotide sequence of the *RdRp* gene. The following coronaviruses (CoVs) and GenBank accession numbers were used: bat severe acute respiratory syndrome CoV Rm1 (bat SARS-CoV Rm1; DQ412043), bat SARS-CoV Rp3 (DQ071615), bat SARS-CoV Rf1 (DQ412042), bat SARS-CoV HKU3 (DQ022305),SARS-CoV isolate Tor2/FP1–10895 (SARS-CoV Tor2; JX163925), SARS-CoV BJ182–12 (SARS-CoV BJ182; EU371564), SARS-CoV (NC004718), civet SARS-CoV SZ3 (AY304486), civet SARS-CoV SZ16 (AY304488), bat CoV HKU9 (BtCoV-HKU9; EF065513), bat CoV HKU4 (BtCoV-HKU4; EF065505), bat CoV HKU5 (BtCoV-HKU5; EF065509), human betacoronvirus 2c EMC/2012 (HCoV-EMC; JX869059), human CoV OC43 (HCoV-OC43; NC005147), HCoV-HKU1 (NC006577), bat coronavirus HKU2 (BtCoV-HKU2; NC009988), bat coronavirus 1A (BtCoV-1A; NC010437), HCoV-229E (NC002645), HCoV-NL63 (NC005831), bat CoV HKU8 (BtCoV-HKU8; NC010438), scotophilus bat CoV 512 (BtCoV-512; NC009657), avian infectious bronchitis virus (IBV; NC001451), beluga whale CoV SW1 (BWCoV; NC010646). Scale bar indicates genetic distance estimated by using TN93+G+I model implemented in MEGA5 (www.megasoftware.net).

The spike proteins of CoVs are responsible for receptor binding and host species adaptation, and their genes therefore constitute one of the most variable regions within CoV genomes (*10*,*11*). The phylogenetic tree based on the amino acid sequences of spike protein (Figure 2) suggests that the selected betacoronaviruses were mainly divided into 5 clusters: SARS cluster; bat SARS-like cluster; civet SARS-like cluster; human betacoronavirus cluster; and EMC cluster. Bat Rp-coronavirus/Shaanxi2011 and Bat Cp-coronavirus/Yunnan2011 shared 89.4% aa identity in spike proteins, which consisted of 1,240 aa and 1,241 aa, respectively. The spike proteins of the CoVs in our analysis have 89.8%–92.7% aa identity with those of bat SARS-like CoVs, with substantial similarity in the receptor-binding domain. The close relationship also was observed with the SARS-CoVs (79.2%–79.4% aa identity) and civet SARS-like CoVs (78.9%–79.1% aa identity). In contrast, the human betacoronaviruses and EMC cluster formed separate clusters distinct from SARS-related CoVs that showed only 27.8%–29.4% aa and 28.8%–30.5% aa identities with the betacoronaviruses, respectively, in our analysis. The genome sequences reported here have been deposited into GenBank (accession nos. JX993987–JX993988).

Conclusions

The recent fatal human infection caused by HCoV-EMC has boosted interest in the discovery of novel CoVs in humans and animals. HCoV-EMC is a novel betacoronavirus, and its closest known relatives are BtCoVs HKU4, and HKU5, which have been detected in Hong Kong only in bats (*12*), the same animal from which SARS is believed to have originated. Bats are increasingly recognized as natural reservoirs of CoVs and may serve as intermediate hosts for interspecies transmission of SARS-CoVs (*10*,*13*). Different bat populations from various countries harbor diverse CoVs that have a high frequency of recombination and mutation rates that enable them to

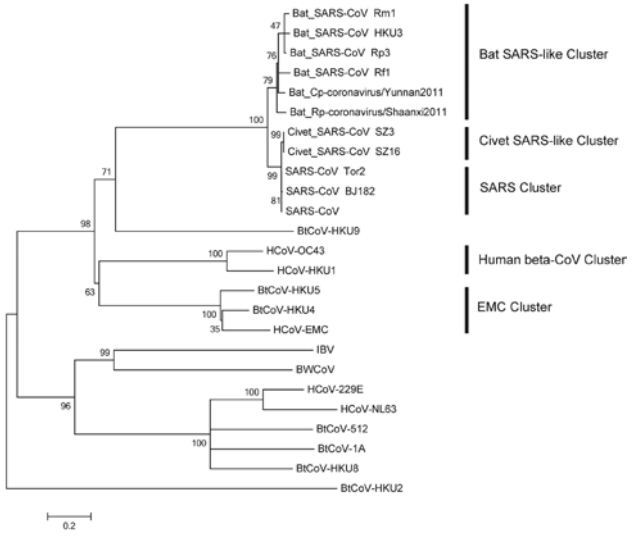

Figure 2. Phylogenetic tree of novel betacoronaviruses based on the deduced amino acid sequence of spike protein. SARS, severe acute respiratory syndrome; CoV, coronavirus; HCoV, human CoV; BtCoV, bat CoV; BWCoV, beluga whale CoV; IBV, avian infectious bronchitis. Scale bar indicates genetic distance estimated by using WAG+G+I+F model implemented in MEGA5 (www.megasoftware.net).

adapt to new hosts and ecologic niches (*14*,*15*). Therefore, continuous studies of CoVs from different bat species and different countries would help better prevent the new global pandemics resulting from novel viral infection.

We detected and characterized 2 novel betacoronaviruses–Bat Rp-coronavirus/Shaanxi2011 in *R. pusillus* bats and Bat Cp-coronavirus/Yunnan2011 in *C. plicata* bats–in China. The high similarity shown by phylogenetic analysis confirmed the close genetic relationship among the CoVs (SARS-like CoVs and SARS-CoVs) that we analyzed. In contrast, Bat Rp-coronavirus/Shaanxi2011 and Bat Cp-coronavirus/Yunnan2011 showed little genetic similarity with human betacoronaviruses and HCoV-EMC. Although several CoVs are found in horseshoe bats (*Rhinolophus* spp.), to our knowledge, the SARS-like CoVs in *R. pusillus* and *C. plicata* bats in China have not been identified. The description presented here will further the understanding of CoVs distribution in different bat species found in human habitats and provide clues for rapid response to potential public health threats.

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Human Papillomavirus Genital Infections among Men, China, 2007–2009

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To determine prevalence of genital human papillomavirus (HPV) infection among men in rural China, we analyzed genital swab specimens. Among 2,236 male residents of rural Henan Province, HPV infection prevalence was 17.5%. The most common oncogenic and nononcogenic types were HPV-16 and HPV-3, respectively. Infection was associated with younger age and multiple sex partners.

Human papillomavirus (HPV) is an etiologic agent of cervical cancer. Among men, genital HPV infection plays a role in the development of anogenital cancer (*1*). Estimates of HPV infection prevalence among heterosexual men, mostly from North America and Europe, vary substantially (3.5%–45%) (*1*,*2*). In the People's Republic of China, estimated HPV prevalence among women varies by geographic location (*3*), but no such estimate is available for men. Therefore, we conducted this cross-sectional study to determine the prevalence of genital HPV infection in a large population of men in a rural province of China and to evaluate relevant factors.

The Study

In 2007–2009, a population-based esophageal cancer cohort study was initiated in 9 villages in rural Anyang, Henan Province, China (*4*). This HPV investigation was added to the original cohort study in 6 of the 9 villages. Eligibility criteria were as follows: 1) male sex; 2) permanent residency in the target villages; 3) age 25–65 years; and 4) no history of cancer, cardiovascular disease, or mental disorder. Of 3,571 eligible men, 3,172 (89%) were enrolled. The main reason why the other 399, who were substantially younger, did not participate was loss of contact because they were employed outside Anyang.

Participants were interviewed, and exfoliated cells were collected from the penile shaft, glans penis, coronal sulcus, and scrotum by using saline-soaked swabs (*5*). The sampling procedure was identical for circumcised and uncircumcised men. To assess the adequacy of the specimens, we tested each specimen by PCR for the β-globin gene. Positive specimens were subsequently tested for 13 oncogenic and 37 nononcogenic types of HPV by using PCR-based direct sequencing with a pair of SPF1/GP6+ primers (*6*). Samples with ambiguous HPV typing signals were subjected to further cloning and sequencing.

To evaluate the associations between exposure variables and the presence of HPV DNA, we used logistic regression analysis with stepwise backward elimination at $p>0.1$. To examine the association across the ordered categorical variables, we used a trend test.

Of 3,172 specimens tested, 2,236 were positive for β-globin and were included in the analysis (median participant age 42 years; interquartile range 35–52 years). We excluded from the study men who were older and reported less risky sexual behavior than those who were included (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/6/11-1597-Techapp1.pdf).

Of the 40 HPV types we tested for, we detected 36, including 13 oncogenic types. Overall prevalence of HPV infection was 17.5% (95% CI 16%–19%). Oncogenic HPV was detected in 140 (6.3%; 95% CI 5.3%–7.3%) specimens, and nononcogenic HPV was detected in 251 (11%; 95% CI 9.9%–13%) specimens. Among 15 HPV-positive specimens that had ambiguous direct sequencing signals and were further cloned and resequenced for genotyping, 3 had a second type and minor types were ignored. Among these infections (Table 1), the most common oncogenic type detected was HPV-16 (17.4%), followed by HPV-18 (7.2%). The most common nononcogenic type was HPV-3 (16.4%), followed by HPV-57 (7.9%).

Prevalence of infection of any or nononcogenic HPV types decreased significantly with participant's increasing age (Table 2). Risk for infection with any HPV type was associated with being unmarried, having had multiple sex partners, and having had oral and anal sex. When the outcome was stratified by oncogenicity of HPV type, the association remained statistically significant for having had multiple sex partners.

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Table 1. Type-specific proportions of HPV infection among 2,236 men from rural Henan Province, China, 2007–2009*

Genotype†	Genus and species	Positive, no. (%)‡
Oncogenic type§		140 (35.8)
HPV-16	*Alpha-9*	68 (17.4)
HPV-18	*Alpha-7*	28 (7.2)
HPV-58	*Alpha-9*	13 (3.3)
HPV-33	*Alpha-9*	8 (2.0)
HPV-45	*Alpha-7*	5 (1.3)
HPV-52	*Alpha-9*	4 (1.0)
HPV-66	*Alpha-6*	4 (1.0)
HPV-35	*Alpha-9*	3 (0.8)
HPV-68	*Alpha-7*	3 (0.8)
HPV-31	*Alpha-9*	1 (0.3)
HPV-51	*Alpha-5*	1 (0.3)
HPV-56	*Alpha-6*	1 (0.3)
HPV-59	*Alpha-7*	1 (0.3)
Nononcogenic type¶		251 (64.2)
HPV-3	*Alpha-2*	64 (16.4)
HPV-57	*Alpha-4*	31 (7.9)
HPV-87	*Alpha-3*	18 (4.6)
HPV-81	*Alpha-3*	17 (4.3)
HPV-11	*Alpha-10*	15 (3.8)
HPV-67	*Alpha-9*	12 (3.1)
HPV-90	*Alpha-14*	12 (3.1)
HPV-43	*Alpha-8*	11 (2.8)
HPV-75	*Beta-3*	10 (2.6)
HPV-54	*Alpha-13*	9 (2.3)
HPV-91	*Alpha-8*	9 (2.3)
HPV-94	*Alpha-2*	8 (2.0)
HPV-6	*Alpha-10*	6 (1.5)
HPV-30	*Alpha-6*	6 (1.5)
HPV-27	*Alpha-4*	5 (1.3)
HPV-40	*Alpha-8*	5 (1.3)
HPV-10	*Alpha-2*	4 (1.0)
HPV-62	*Alpha-3*	2 (0.5)
HPV-74	*Alpha-10*	2 (0.5)
HPV-84	*Alpha-3*	2 (0.5)
HPV-7	*Alpha-8*	1 (0.3)
HPV-29	*Alpha-2*	1 (0.3)
HPV-77	*Alpha-2*	1 (0.3)
Total		391 (100)

*HPV, human papillomavirus; *Alpha*, *Alphapapillomovirus*; *Beta*, *Betapapillomavirus*.
†Of 391 HPV-positive specimens, 15 displayed ambiguous typing signals by direct sequencing of PCR product. Infection with >1 HPV type was detected in only 3 of 15 specimens that were tested by cloning and sequencing; the predominant type is shown.
‡Proportion.
§Oncogenic types tested in this study included HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -66.
¶On the basis of latest published literature and our knowledge of the detection method of HPV DNA adopted in this study (SPF1/GP6+ mediated PCR and sequencing), nononcogenic types that could be tested for were HPV-3, -6, -7, -10, -11, -26, -27, -29, -30, -32, -37, -40, -42, -43, -44, -53, -54, -55, -57, -61, -62, -67, -68, -69, -70, -72, -74, -75, -77, -81, -82, -84, -85, -87, -90, -91, and -94.

Conclusions

This study of genital HPV in a large sample of adult men from rural China addresses the paucity of data on male genital HPV infection in Asia. Prevalence rates for any type of HPV and oncogenic HPV were lower among these men in China than among heterosexual men elsewhere (Asia–Pacific area and globally) (*2*,*7*). This discrepancy might be partly explained by the relatively more conservative sexual behavior and higher median age of participants in this study.

Among populations of similar age, prevalence of a specific HPV type is usually lower among men than among women (*1*). However, the 17.5% prevalence found in this study exceeds estimates for married women 15–59 years of age in China (14.8%–16.8%) (*8*). This finding is consistent with that of another study, which also reported higher HPV prevalence among men than among women (*9*). These inconsistent findings might be explained by differences in HPV type distribution between male genitalia and the female cervix and by variability of type-specific sensitivity in detection methods.

Although most studies of HPV infection in men worldwide have found no clear trend with regard to age (*1*), we found that infection mildly decreased with increasing age. A potential explanation for this age-related trend might be that in this population, young adults more commonly work for long periods outside the rural home area than do older adults (data not shown), and the increased mobility might be associated with more unprotected sexual behavior and consequent increased exposure to HPV (*10*).

Table 2. Selected demographic and behavior variables for genital HPV infection among 2,236 men from rural Henan Province, China, 2007–2009*

Patient variable	Any type HPV infection		Oncogenic HPV infection		Nononcogenic HPV infection	
	Crude OR (95% CI)†	Adjusted OR (95% CI)‡	Crude OR (95% CI)†	Adjusted OR (95% CI)‡	Crude OR (95% CI)†	Adjusted OR (95% CI)‡
Age, y						
25–35	1.0		1.0		1.0	
36–45	0.8 (0.6–1.0)		0.7 (0.4–1.1)		0.8 (0.6–1.1)	
46–55	0.8 (0.6–1.1)		0.9 (0.6–1.5)		0.8 (0.5–1.1)	
56–65	0.7 (0.5–1.0)		0.8 (0.5–1.3)		0.6 (0.4–1.0)	
p value for trend§	0.039		0.498		0.030	
Education level						
Illiterate, <1 y	1.0		1.0		1.0	
Primary school, 1–6 y	0.7 (0.4–1.4)		0.9 (0.3–2.4)		0.7 (0.3–1.4)	
Secondary school, 7–12 y	0.9 (0.5–1.6)		0.9 (0.3–2.2)		0.9 (0.4–1.9)	
College or above, >12 y	1.5 (0.5–4.3)		1.8 (0.4–8.3)		1.3 (0.4–4.8)	
p value for trend†	0.238		0.969		0.132	
Marital status						
Married or cohabiting	1.0	1.0	1.0	1.0	1.00	1.0
Never married, divorced, separated, or widowed	1.7 (1.1–2.6)	1.6 (1.0–2.5)	1.8 (1.0–3.5)	1.8 (0.9–3.4)	1.6 (0.9–2.7)	1.6 (0.9–2.8)
Type of employment						
Farming at home	1.0		1.0		1.0	
Working in local area	1.2 (0.9–1.5)		1.1 (0.7–1.6)		1.3 (0.9–1.8)	
Working outside local area	1.2 (0.9–1.6)		0.9 (0.6–1.5)		1.3 (0.9–1.9)	
Other	1.2 (0.6–2.4)		1.1 (0.4–3.1)		1.3 (0.6–2.9)	
p value for trend§	0.238		0.876		0.100	
Cigarette smoking						
Never	1.0		1.0		1.0	
Ever	1.0 (0.8–1.3)		0.9 (0.6–1.3)		1.1 (0.8–1.4)	
Alcohol consumption						
Never	1.0		1.0		1.0	
Ever	1.0 (0.8–1.3)		1.1 (0.8–1.6)		1.0 (0.7–1.3)	
Lifetime no. sex partners						
0–1	1.0	1.0	1.0	1.0	1.00	1.0
2	2.4 (1.6–3.6)	2.2 (1.5–3.4)	1.3 (0.6–2.7)	1.2 (0.6–2.6)	3.1 (2.0–4.8)	3.0 (2.0–4.7)
≥3	2.0 (1.5–2.9)	1.8 (1.3–2.6)	1.9 (1.1–3.2)	1.9 (1.1–3.1)	2.1 (1.4–3.2)	1.9 (1.3–2.9)
p value for trend§	<0.001	<0.001	0.015	0.020	<0.001	<0.001
Oral or anal sex						
Never	1.0	1.0	1.0		1.0	
Ever	1.7 (1.2–2.4)	1.5 (1.0–2.1)	1.5 (0.9–2.6)		1.8 (1.2–2.6)	
Wash genitalia before sex						
Occasionally or never	1.0		1.0		1.0	
Frequently or every time	1.2 (0.9–1.6)		0.8 (0.5–1.4)		1.5 (1.1–2.1)	

*HPV, human papillomavirus; OR, odds ratio.
†Crude ORs and 95% CIs derived by univariate logistic regression analysis.
‡Adjusted ORs and 95% CIs derived by multivariate logistic regression models including all the listed variables; backward method was used to select significant variables on the 0.10 level.
§p values for trend derived by logistic regression analyses, treating categorical variables as continuous variables.

Our finding that HPV-16 and -18 were the most commonly detected oncogenic HPV types is in keeping with findings of previous studies (*2*,*7*,*11*,*12*). However, our finding that HPV-3 and HPV-57 were the predominant nononcogenic types is in contrast to the findings of other studies that HPV-6 and HPV-11 were the most predominant (*2*,*11*,*13*). This discrepancy might partly be explained by the fact that in our in-house evaluation, the primer set SPF1/GP6+ was more sensitive for HPV-57 but less sensitive for HPV-11 than was GP5+/GP6+. Completely opposite to our findings, Dai et al. reported that among women in neighboring rural Shanxi Province, China, oncogenic types were more commonly detected than were nononcogenic types (*14*). One possible explanation is that in our study, a significant portion of the exfoliated cells were collected from skin tissue. Therefore, a number of cutaneous HPV types, which are nononcogenic for mucosal lesion (cervical cancer), could be detected. We believe this might have led to the higher proportion of nononcogenic HPV than oncogenic HPV detected in our study. Another possible explanation is that sequencing methods used in our study can detect more nononcogenic types, which escape identification in studies that use hybridization with preassigned probes.

In contrast to previously reported rates of infection (*7*,*12*), in our study, infection with multiple types was rare. The previous studies used hybridization, an efficient way to identify co-infections, for genotyping. However, we used

sequencing instead of hybridization to maximize demonstration of the spectrum of HPV types. Because minor types would probably be covered by the predominant type in the sequencing process, sequencing would probably have resulted in underestimation of infection with multiple types.

The low proportion of β-globin positivity might have partly resulted from the lower efficiency of cell collection by use of a saline-moistened swab as opposed to other methods such as emery paper (*15*). Another possible explanation is that a certain proportion of cells collected from male genitalia are keratinized and contain less nucleated human DNA than cells collected from mucosal organs (e.g., the cervix) (*15*).

The age range of the male participants in this large study was broad. However, the biases potentially imposed by the nonparticipation of ≈400 younger men (because of mobility) and 936 older men (because of specimen inadequacy) must be noted. This nonparticipation of younger men might have neutralized the age-related association to some extent. Although circumcision is extremely rare in rural China, the lack of accurate data for this variable is another study limitation, which rendered subgroup analysis by circumcision status impossible.

As reported in other studies (*1*), having had multiple sex partners was associated with HPV infection in this population. This finding indicates that men with higher mobility (i.e., higher risk for multiple sex partners and unprotected sexual behavior) should receive more attention with regard to future HPV control in this region.

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Treatment of Tularemia in Pregnant Woman, France

Charlotte Dentan, Patricia Pavese, Isabelle Pelloux, Sandrine Boisset, Jean-Paul Brion, Jean-Paul Stahl, and Max Maurin

A pregnant woman who had oropharyngeal tularemia underwent treatment with azithromycin and lymph node resection and recovered without obstetrical complication or infection in the child. Azithromycin represents a first-line treatment option for tularemia during pregnancy in regions where the infecting strains of *Francisella tularensis* have no natural resistance to macrolides.

Tularemia is a bacterial zoonotic disease caused by a gram-negative coccobacillus, *Francisella tularensis*; the most virulent subspecies, *tularensis* (type A), is found in North America, but subspecies *holarctica* (type B) occurs throughout the Northern Hemisphere (*1*–*4*). In France, human cases are most often sporadic and occur predominantly in the eastern, southeastern, and western parts of the country (*1*).

F. tularensis has several animal reservoirs, including rodents and lagomorphs (hares and rabbits). Domestic animals may be infected with *F. tularensis* through contact with the wildlife fauna and, occasionally, transmit the disease to humans. Human contamination occurs following direct contact with infected animals, through contaminated environments or arthropod bites (*2*).We describe the diagnosis and treatment of tularemia in a pregnant woman in France.

The Case

In late February 2006, a 27-year-old pregnant woman, at 6 weeks' gestation, was referred to Grenoble University Hospital, Grenoble, France, for investigation of persistent left cervical lymphadenopathy with fever. She had no history of severe infection or underlying illness. The patient lived in a farm in the French Alps. The lymphadenopathy occurred 3 weeks earlier, along with a sore throat, and persisted despite 10 days' treatment with amoxicillin (3 g daily).

At admission, the patient was febrile (38°C) and had a tender, swollen, submaxillary cervical lymph node on the left side. Examination of the oral cavity showed no abnormalities. Magnetic resonance imaging of the left cervical region showed a large mass extending from the parotid region to the submandibular region, with hypo- and hypersignals in T1- and T2-weighted imaging, respectively.

Lymph node tissue was obtained by needle aspiration, and examination revealed nonspecific lesions of lymphadenitis. Laboratory test results showed moderate inflammatory syndrome (C-reactive protein 67 mg/L). Serologic results were negative for HIV, hepatitis B and C viruses, rubella virus, *Treponema pallidum* (syphilis), *Coxiella burnetii* (Q fever), and *Borrelia*, *Bartonella*, *Brucella*, and *Legionella* spp. and showed only residual IgG-type antibodies against cytomegalovirus, Epstein-Barr virus, herpes simplex virus, parvovirus, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*.

Because we suspected severe *Streptococcus pyogenes* infection, a second course of amoxicillin was administered for 13 additional days. After initial clinical improvement, the patient relapsed, and the lymphadenopathy evolved to suppuration and necrosis. In March 2006, a large amount of pus was surgically drained from the site, and inflamed lymph nodes were removed. These unusual clinical features led us to suspect tularemia.

The patient denied receiving any tick bite but reported that she regularly fed domestic rabbits in cages. Her symptoms began a few days after she killed and skinned rabbits, which were then kept frozen but not eaten by the family.

Examination of the removed lymph node samples confirmed the presence of nonspecific lymphadenitis. Routine cultures and mycobacterial cultures remained sterile, but *F. tularensis* DNA was detected in lymph node samples by using specific real-time PCR targeting the gene encoding a 23-kDa surface protein (*5*). PCR amplification and sequencing of the 16S rDNA–23S rDNA intergenic spacer region (*5*) directly from lymph node tissue confirmed the presence of DNA from *F. tularensis* subsp. *holarctica*. Serum samples collected in late February and on March 15, 2006, were tested by using an immunofluorescence assay and a homemade *F. tularensis* antigen (*5*). Both samples were positive, with IgM and IgG titers of 320 (cutoff titers of ≥160).

Because the patient was pregnant and tularemia in France has been caused only by biovar 1 strains of *F. tularensis* subsp. *holarctica* (*2*), which are naturally susceptible to macrolides, she was treated with azithromycin (500 mg/d for 6 weeks). She recovered, with no complications for herself or her baby.

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The patient reported that the rabbits were reared outdoors, in floor-level, wire-mesh cages; therefore, we suspected the animals were infected with *F. tularensis* through contact with wild fauna. Attempts to detect *F. tularensis* in pieces of the frozen rabbits by culture and PCR tests were unsuccessful. All family members of the patient also tested negative for *F. tularensis*.

Conclusions

Clinical symptoms of tularemia are primarily related to the portal of entry of bacteria, the *F. tularensis* strain virulence, and the immune status of the patient. The incubation period is usually 1–3 days but may last up to 15 days (*3*,*6*). The primary clinical forms are glandular and ulceroglandular (skin inoculation), oculoglandular (conjunctival inoculation), oropharyngeal (oral contamination), pneumonic (inhalation of an infected aerosol), and typhoidal (various modes of infection) (*2*,*7*). Tularemia may be severe and even fatal; patients with lymphadenopathy may experience lymph node suppuration in 30% of cases (*1*). There have been no reports of human-to-human transmission.

Our patient's symptoms were fever, pharyngitis, and cervical lymphadenopathy; treatment with a β-lactam drug did not improve her condition, which rapidly evolved to local suppuration, a sign that should prompt physicians to consider oropharyngeal tularemia in disease-endemic regions. Because tularemia cases remain extremely rare in the French Alps, where the patient lived, this diagnosis was not considered at the time of the first medical consultation.

Because the rabbit meat was not consumed by the patient, we suspected she became infected at the time she skinned these animals. *F. tularensis* resists low temperatures, including freezing (*2*,*6*). However, the animals had no overt disease at the time they were killed, so low bacterial inoculum may explain why the tests performed on rabbit meat had negative results.

Diagnosis of tularemia is often made by serologic tests (*3*), although these are negative during the first 2 weeks following the onset of symptoms (*1*). In the case described here, the same high antibody titers were obtained in 2 serum samples taken 2 weeks apart, which indicates that the peak secretion of specific antibodies was achieved. Culture of *F. tularensis* from clinical samples remains poorly sensitive, but PCR-based testing of pharyngeal swab specimens or lymph node suppurations or biopsy specimens enables rapid diagnosis of oropharyngeal tularemia and identification of the *F. tularensis* subspecies involved (*1*,*4*,*5*,*7*,*8*).

A few tularemia cases occurring in pregnant women have been reported (*9*). Severe illness or death caused by infection with *F. tularensis* could be a risk for a pregnant woman or her fetus; the role of *F. tularensis* as an agent of abortion and intrauterine death is well recognized in sheep (*10*) but not in pregnant women. A major difficulty in this instance was the choice of the antimicrobial drug regimen, because first-line antibiotics currently recommended for treatment of tularemia, including the aminoglycoside gentamicin, fluoroquinolones, and tetracyclines (*1*,*2*,*7*,*8*), may be toxic for pregnant women or fetuses. No treatment recommendation for tularemia during pregnancy is available (*9*).

This patient recovered after removal of suppurated lymph nodes and treatment with azithromycin. The pregnancy outcome was favorable, and the patient and the infant were healthy at 12-month follow up. Macrolides are usually not recommended for treatment of tularemia patients (*2*,*7*,*8*), especially because *F. tularensis* subsp. *holarctica* biovar 2 strains, mainly found in Eastern Europe and Asia, are naturally resistant to macrolides (*11*–*14*). The ketolides (e.g., telithromycin) are highly active against *F. tularensis* in vitro (*11*,*15*), but their use in pregnant women is currently discouraged. This case emphasizes the usefulness of azithromycin as a first-line treatment for tularemia in pregnant women in areas where infections caused by biovar 2 strains of *F. tularensis* subsp. *holarctica* do not occur.

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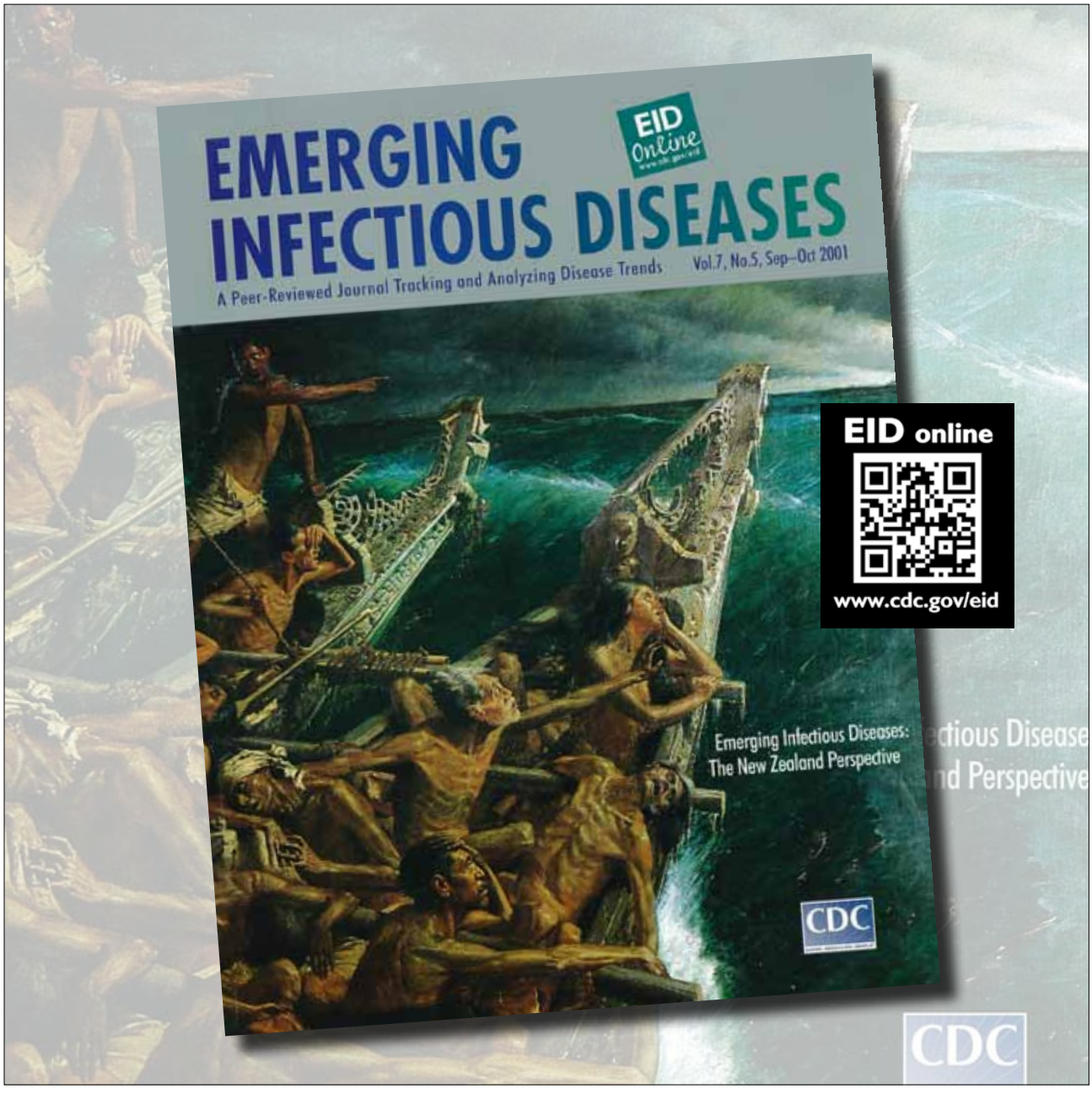

Ciprofloxacin-Resistant *Salmonella enterica* Serovar Kentucky in Canada

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We report emergence of ciprofloxacin-resistant *Salmonella enterica* serovar Kentucky in Canada during 2003–2009. All isolates had similar macrorestriction patterns and were multilocus sequence type ST198, which has been observed in Europe and Africa. Ciprofloxacin-resistant *S. enterica* serovar Kentucky represents 66% of all ciprofloxacin-resistant nontyphoidal *Salmonella* sp. isolates observed in Canada since 2003.

Infections with *Salmonella* spp. are a major health concern for humans and animals on a global scale. Although most cases of salmonellosis result in uncomplicated diarrhea, elderly and immunocompromised persons can be at risk for more severe invasive infections, which can be life-threatening and may require antimicrobial drug therapy (*1*). The drugs of choice for treating these invasive infections are fluoroquinolones (for adults) or cephalosporins.

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One of the main drivers of antimicrobial drug resistance in *Salmonella* spp. is use of antimicrobial drugs in food-producing animals. For example, high rates of cephalosporin resistance in *Salmonella enterica* serovar Heidelberg isolated from poultry, retail chicken meat, and humans were observed in Quebec, Canada in 2003. After a voluntary withdrawal of cephalosporins was instituted by the Quebec broiler industry in 2005, rates of ceftiofur resistance dramatically decreased in animals and humans (*2*).

As with cephalosporin resistance, ciprofloxacin resistance in *Salmonella* spp. is a growing concern. Recently, *S. enterica* serovar Kentucky isolates have been described in Europe and Africa that were ciprofloxacin resistant (*3*). In addition, these isolates were resistant to multiple classes of antimicrobial drugs, which further complicates treatment options for invasive disease. No *S. enterica* serovar Kentucky isolates submitted to the National Antimicrobial Resistance Monitoring System in the United States were ciprofloxacin resistant (*3*). The purpose of this study was to describe the epidemiology and characterize isolates of ciprofloxacin-resistant *S. enterica* serovar Kentucky identified in Canada.

The Study

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS), established in 2003, monitors antimicrobial drug use and resistance in selected species of enteric bacteria from humans, animals, and animal-derived food sources across Canada (www.phac-aspc.gc.ca/ cipars-picra/surv-eng.php). Human *Salmonella* isolates were submitted by all provincial public health laboratories in Canada to the National Microbiology Laboratory for further characterization. Antimicrobial drug susceptibility testing was performed by using broth microdilution (Sensititer Automated Microbiology System; Trek Diagnostic Systems Ltd., Westlake, OH, USA) and breakpoints established by the Clinical Laboratory Standards Institute (*4*).

A total of 76 *S. enterica* serovar Kentucky isolates were submitted to the CIPARS program during 2003–2009, and 23 (30%) isolates showed ciprofloxacin resistance (MIC ≥4 mg/L) during the study (Figure 1). Thirty-five (46%) isolates were susceptible to all antimicrobial drugs tested. Ciprofloxacin-resistant isolates were identified from human case-patients in British Columbia (n = 2), Alberta (n = 2), Saskatchewan (n = 1), Ontario (n = 12), Quebec (n = 5), and Prince Edward Island (n = 1). Age information was available for 54 of 76 case-patients infected with *S. enterica* serovar Kentucky during the study period.

Of these isolates, 11 (14.5%) were resistant to ciprofloxacin. Ciprofloxacin resistance was observed among case-patients 18–69 years of age, and 5 of 11 were 18–29 years of age. Case-patients 18–29 years of age were 8 times

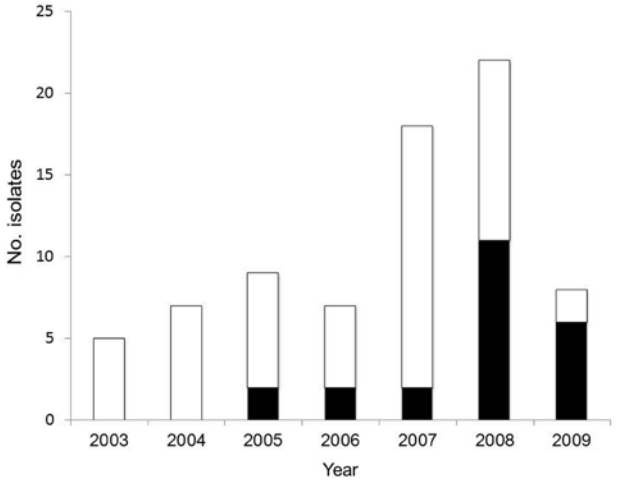

Figure 1. *Salmonella enterica* serovar Kentucky isolates identified in Canada, 2003–2009. Black bars indicate ciprofloxacin-resistant isolates, and white bars indicate non–ciprofloxacin-resistant isolates.

more likely to have a ciprofloxacin-resistant strain than case-patients 50–69 years of age (odds ratio [OR] 8.3, 95% CI 1.034–67.198, p = 0.046). Of 21 ciprofloxacin-resistant isolates from case-patients who reported site of isolation, 20 were identified from feces and 1 from urine. There were no differences in site of isolation between ciprofloxacin-resistant and ciprofloxacin-susceptible *S. enterica* serovar Kentucky isolates. Although the total number of isolates associated with human infections was rare, of the 21,426 nontyphoidal *Salmonella* spp. submitted for susceptibility testing as part of the human component of the CIPARS program since 2003, *S.* enterica serovar Kentucky had a significantly higher rate of ciprofloxacin resistance than all other nontyphoidal *Salmonella* isolates and comprised 66% (23/35; p<0.0001) of all ciprofloxacin-resistant isolates identified during that period.

In Canada, ciprofloxacin-resistant *S. enterica* serovar Kentucky was first identified in 2005, when 22% (2/9) of isolates submitted for drug susceptibility testing were resistant to this drug. A significant increase (OR 10.5, 95% CI 1.115–9.913, p = 0.04) in the number of isolates resistant to ciprofloxacin was observed in 2009 compared with results in 2005. The largest number occurred during 2008–2009, when ciprofloxacin-resistant isolates comprised 57% (17/30) of all *S. enterica* serovar Kentucky isolates identified (Figure 1). The number of cases reported in Canada is comparable with that reported in Denmark over a similar period (*3*).

We typed all isolates by using pulsed-field gel electrophoresis as described and restriction enzyme *Xba*I (*5*). A dendrogram depicting the results was generated with BioNumerics version 3.5 (Applied Maths, Sint-Martens-Latem, Belgium) and is shown in Figure 2. All ciprofloxacin-resistant isolates clustered with a percentage similarity >80%;

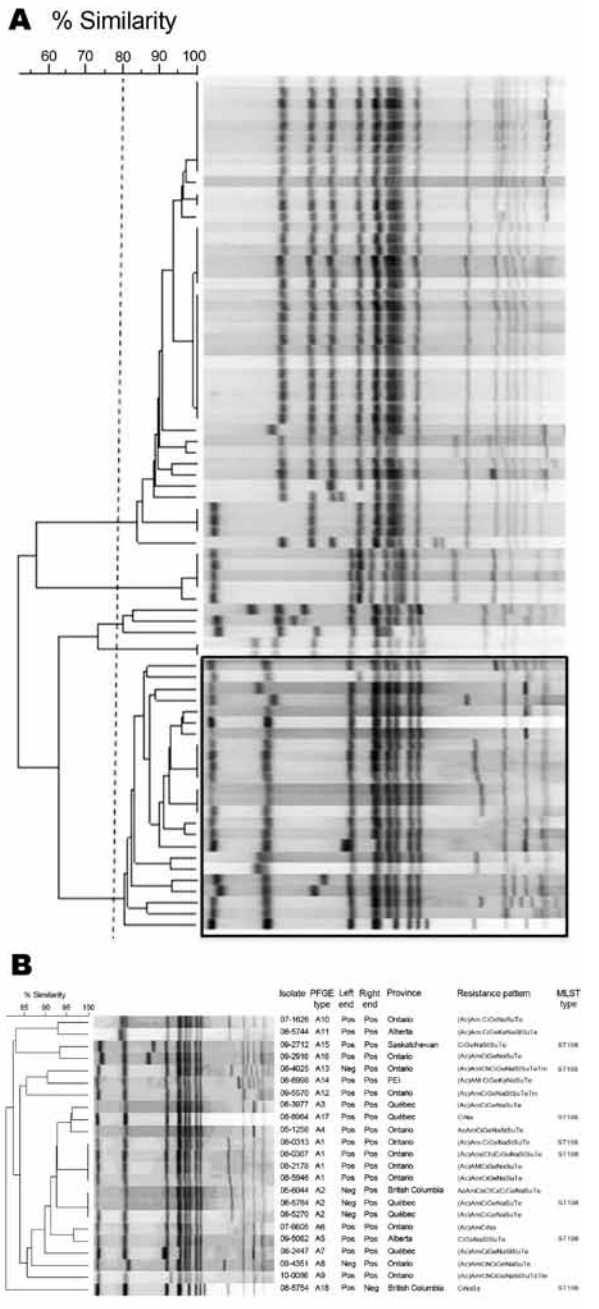

Figure 2. Dendrograms of macrorestriction fragments of all (A) and ciprofloxacin-resistant (B) *Salmonella enterica* serovar Kentucky isolates identified in Canada, 2003–2009. The dotted vertical line in panel A indicates a cutoff value of 80% similarity, and the box indicates ciprofloxacin-resistant isolates. Left end and Right end in panel B indicate PCR results for presence (Pos) or absence (Neg) of left and right junctions of *Salmonella* genomic island 1. PFGE, pulsed-field gel electrophoresis; MLST, multilocus sequence typing; Ac, amoxicillin clavulanate; Am, ampicillin; Ch, chloramphenicol; Ci, ciprofloxacin; Ge, gentamicin; Na, nalidixic acid; St, streptomycin; Su, sulfisoxazole; Te, tetracycline; ST, sequence type; Tm, trimethoprim; PEI, Prince Edward Island. Letters in parentheses indicate drugs that had intermediate MICs.

only 1 ciprofloxacin-susceptible isolate was found in this cluster (Figure 2, panel A).

Multilocus sequence typing (MLST) was performed on a subset of 8 isolates on the basis of differences in pulsed-field gel electrophoresis patterns and variations in antimicrobial drug resistance. Data were submitted to the MLST database website (http://mlst.ucc.ie/mlst/dbs/Senterica) to determine MLST types (*6*). All isolates tested were sequence type (ST) 198 (Figure 2, panel B). This sequence type and similar antimicrobial drug resistance patterns have been recently reported in France, England and Wales, Denmark, Belgium, and Africa (*3,7,8*).

Many ST198 multidrug-resistant isolates observed in Europe and Africa contained *Salmonella* genomic island 1 (SGI1) variants, particularly, SGI1-K, SGI1-Q, and SGI1-P. To determine whether ciprofloxacin-resistant isolates from Canada harbored similar SGI1 variants, we used PCR to detect the chromosomal left and right junctions of SGI1 as described (*9,10*). The right junction was found in 22 of 23 isolates, and the left junction was found in 18 of 23 isolates (Figure 2, panel B). Further studies are needed to identify specific SGI variants in the isolates.

Analysis of *S. enterica* serovar Kentucky isolates obtained during 2003–2009 from animal and retail meat samples as part of CIPARS did not identify any ciprofloxacin-resistant isolates (www.phac-aspc.gc.ca/cipars-picra/index-eng.php). This finding suggests that human infections in Canada were not acquired from domestically produced food. Many ciprofloxacin-resistant *S. enterica* serovar Kentucky human infections identified in Europe have been linked to travel to countries in Africa (*3*). Of 23 case-patients in Canada, we obtained travel history for 11. Travel history was defined as previous travel out of Canada within the past 7 days. Four case-patients had traveled to Morocco (1 also had traveled to Spain and Portugal), 3 had traveled to Egypt, 1 had traveled to Libya, and 3 had traveled to Africa (no country reported).

Conclusions

Resistance to ciprofloxacin in *Salmonella* spp. is a growing concern because it limits the ability to treat invasive disease. In this study, we described the characteristics of ciprofloxacin-resistant *S. enterica* serovar Kentucky isolates in Canada. Similar drug-resistance patterns and genetic backgrounds of *S. enterica* serovar Kentucky have been observed in Europe and linked to travel to countries in Africa (*3*). That most isolates had multidrug resistance phenotypes is of particular concern. Further studies are required to determine risk factors for acquisition of these infections in Canada.

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Novel Poxvirus in Big Brown Bats, Northwestern United States

Ginny L. Emerson, Robert Nordhausen, Michael M. Garner, John R. Huckabee, Steven Johnson, Ron D. Wohrle, Whitni B. Davidson, Kimberly Wilkins, Yu Li, Jeffrey B. Doty, Nadia F. Gallardo-Romero, Maureen G. Metcalfe, Kevin L. Karem, Inger K. Damon, and Darin S. Carroll

A wildlife hospital and rehabilitation center in northwestern United States received several big brown bats with necrosuppurative osteomyelitis in multiple joints. Wing and joint tissues were positive by PCR for poxvirus. Thin-section electron microscopy showed poxvirus particles within A-type inclusions. Phylogenetic comparison supports establishment of a new genus of *Poxviridae*.

Bat species worldwide have been implicated as reservoirs for several emerging viruses, such as lyssaviruses, henipahviruses, severe acute respiratory syndrome–associated coronaviruses, and filoviruses. Bats have several physiologic, cellular, and natural history characteristics that may make them particularly suited to their role as reservoir hosts (*1,2*).

Chordopoxviridae is a subfamily of *Poxviridae* that contains large double-stranded DNA viruses that replicate in the cellular cytoplasm and are known to infect a wide range of vertebrates. Many of these viruses cause zoonotic disease in humans. Although poxviruses are known to have incorporated host genes into their genomes to subvert the host immune system (*3*), bats and poxviruses may also serve as facilitators in the horizontal transfer of transposable elements to other species (*4–6*). We report the isolation and characterization of a viable poxvirus from bats.

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The Study

During 2009–2011, six (5 male and 1 sex unknown) adult big brown bats (*Eptesicus fuscus*) were brought to a wildlife hospital and rehabilitation center (PAWS Wildlife Center, Lynnwood, WA, USA) during late spring or summer because they could not fly. All but 1 of the bats had ≥1 visibly swollen and occasionally contused joints involving the long bones of the legs and wings; 1 had contusions of the oral commissures.

All bats received care that included antimicrobial drugs and nutritional and fluid support. However, minimal or no clinical improvement was observed, and the bats showed progressive joint swelling and increased lethargy. All bats were eventually euthanized. In all instances, gross lesions were limited to the joints.

Bat tissues were sent to a facility that specializes in the pathologic analysis of nondomestic species (Northwest ZooPath, Monroe, WA, USA) for further investigation. Histologic examination showed severe fibrino-suppurative and necrotizing tenosynovitis and osteoarthritis that involved the long bones and occasionally facial flat bones and joints with occasional localized vasculitis. No bacterial or fungal agents were seen by light microscopy of specimens stained with hematoxylin and eosin, Giemsa, Warthin-Starry, Brown and Brenn, or Gomori methenamine silver stains or in a Wright-Giemsa–stained cytologic preparation of a joint aspirate. Cultures for aerobic and anaerobic bacteria, and cultures of the joint from 1 bat for mycoplasma showed negative results.

Thin-section electron microscopy of synovial tissue extracted from a wax histoblock showed poxvirus particles in inflammatory cells (Figure 1, panel A). A 906E transmission electron microscope (Carl Zeiss, Peabody, MA, USA) at an accelerating voltage of 80 kV was used for initial imaging. Digital images were captured by using a 2K ´ 2K camera (Advanced Microscopy Techniques, Danvers, MA, USA). The state veterinarian and the Centers for Disease Control and Prevention (Atlanta, GA, USA) were subsequently consulted.

Material was taken from the wing and joint of an affected bat for real-time PCR testing and cell culture isolation. Wing and joint material was positive by real-time PCR for a poxvirus with low genomic G + C content (*7*). The elbow joint of 1 bat was then processed for poxvirus growth in cell culture. The specimen was emulsified in 500 mL of sterile phosphate-buffered saline by using a tissue grinder. Viral nucleic acid was extracted by using EZ1 Advanced XL (QIAGEN, Valencia, CA, USA). Ten microliters of homogenate was added to 1 mL of RPMI 1640 medium supplemented with 2% fetal bovine serum, L-glutamine, and penicillin/streptomycin. Growth medium from a T25 flask containing green monkey kidney epithelial cells (BSC40) was removed and the virus mixture added.

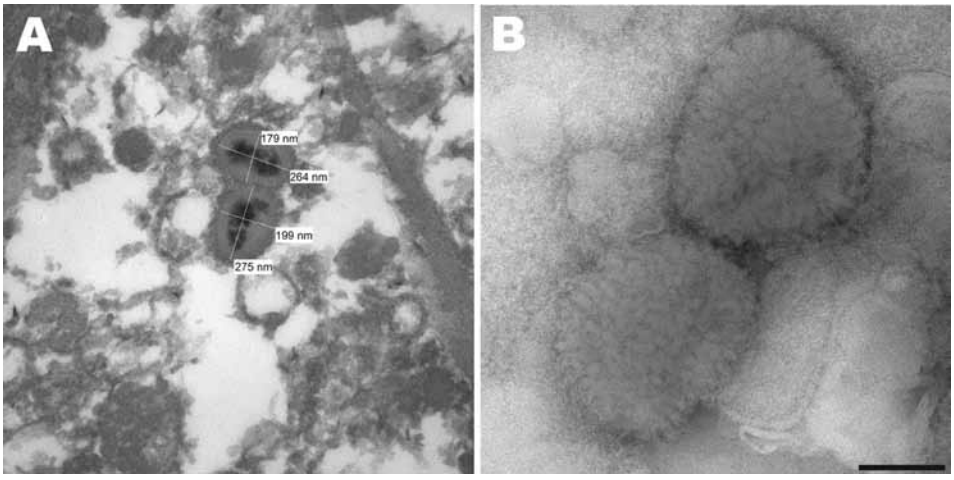

Figure 1. A) Electron micrograph of poxvirus particles in synovium of a big brown bat, northwestern United States. B) Negative staining of poxvirus particles in cell culture supernatant. Scale bar = 100 nm.

The flask was incubated for 1 hour at 56°C, after which 6 mL of RPMI 1640 medium was added to each flask. Cells and supernatant were harvested after 95% of the monolayer was infected.

Negative stain electron microscopy was performed by using cell culture supernatant. Two microliters of supernatant was pipetted onto a 300-mesh formvar-carbon–coated nickel grid. After a 10-min incubation, supernatant was blotted and the grid was rinsed. A negative stain composed of 5% ammonium molybdate, pH 6.9, and 0.1% trehalose (wt/vol) was briefly applied to the grid and blotted. The grid contents were visualized by using a Tecnai BioTwin electron microscope (FEI Company, Hillsboro, OR, USA) operating at 120 kV. Digital images were captured by using a 2K ´ 2K camera (Advanced Microscopy Techniques). Poxvirus particles were identified in cell culture supernatant (Figure 1, panel B).

Genome sequencing produced data that were used to construct a phylogenetic tree (Figure 2). Virus DNA sequence data were collected by using the Illumina platform (www.illumina.com/technology/sequencing_technology.ilmn). DNA sequences from 7 open reading frames (A7L, A10L, A24R, D1R, D5R, H4L, and J6R, according to reference sequence vaccinia virus Copenhagen) were extracted on the basis of sequence similarity. Data were deposited in GenBank under accession nos. KC181855–KC181861. Open reading frames were translated into amino acid sequences and aligned by using the ClustalW alignment option in Geneious version 6.0.5 (www.geneious.com). The tree search was conducted by using MrBayes in Geneious 6.0.5 under default settings and Amsacta moorei (red hairy caterpillar) entomopoxvirus (Moyer) was used as the outgroup with a burn in of 10%.

Conclusions

Historically, osteomyelitis with arthritis has been reported in smallpox patients (osteomyelitis variolosa) and in smallpox vaccine recipients (vaccinia osteomyelitis), but did not occur frequently (*8*). In such cases, variola virus particles were detected in joint fluid (*9*), and vaccinia virus was isolated from a bone biopsy specimen of an affected limb (*10,11*). It is unclear whether the manifestation of arthritis in bats is a normal or rare result of the infection, or a new development in the evolution of the virus. Likewise,

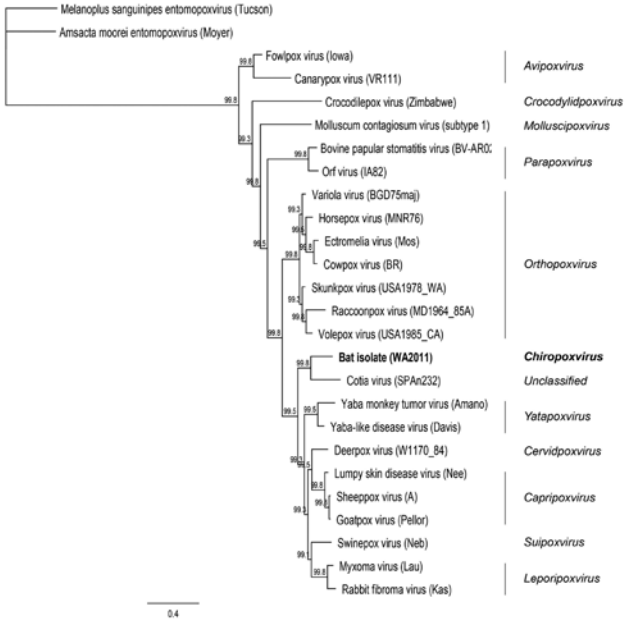

Figure 2. Maximum clade credibility tree generated by MrBayes in Geneious version 6.0.5 (www.geneious.com/) using amino acid sequences from 7 open reading frames (final chain length 240,000 at an average SD of split frequencies of zero) for poxviruses. Clade credibility values are indicated at each node. The virus isolated in this study is shown in **boldface**. Amsacta moorei (red hairy caterpiller) entomopoxvirus (Moyer) was used as the outgroup. Scale bar indicates amino acid substitutions per site.

the frequency of poxvirus infection in big brown bats is impossible to estimate at this stage. Because the public is generally cautioned against handling downed bats because of possible rabies infection, underestimation of prevalence is likely. Infectious disease surveys of bats might have missed the infection up to this point because no obvious lesions are apparent on the skin, and swollen joints might have been classified as being arthritis without suspicion of infectious disease involvement.

Results of at least 3 investigations that involved detection of viral DNA detection in bat guano have been published; 2 involved bats from North America and 1 involved bats in China (*12–14*). No evidence of poxviruses was found in the animals investigated in those studies. The zoonotic potential or host range of the virus described herein is not known, but at a minimum, the virus could pose a newly emergent threat to bat populations. Likewise, it is not clear if the infection seen in bats is a result of spillover or possibly an introduction of the virus into a new area. The isolate does not group with any of the 8 characterized genera of *Chordopoxvirus*; its nearest neighbor is Cotia virus, an as yet unclassified chordopoxvirus first isolated from sentinel suckling mice in a state reserve in Cotia County, São Paulo State, Brazil, in 1961 (*15*).

Although the 2 viruses are nearest neighbors, levels of shared nucleotide and amino acid identity between them suggest they should likely be considered separate genera (Table, Appendix, wwwnc.cdc.gov/EID/article/19/6/12-1713-T1.htm). We propose that the bat-derived isolate be distinguished as part of a new lineage with the suggested genus designation *Chiropoxvirus*. Further efforts should be undertaken to determine whether Cotia virus should be included in this genus. The bat-derived virus requires a new species designation for which we propose *Eptesipox virus* because of its isolation from an *Eptesicus fuscus* bat specimen.

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We thank Heather Bair-Brake for initially contacting the poxvirus staff at the Centers for Disease Control and Prevention regarding the events.

Dr Emerson is a microbiologist at the Centers for Disease Control and Prevention. Her research interests include the evolution, ecology, and epidemiology of viral zoonoses.

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Fatal Influenza A(H1N1)pdm09 Encephalopathy in Immunocompetent Man

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We report an immunocompetent patient who had fatal encephalopathy after mild influenza. He rapidly died after unusual symptoms related to intracerebral thrombosis and hemorrhage. A brain biopsy specimen was positive for influenza A(H1N1)pdm09 virus RNA, but a lung biopsy specimen and cerebrospinal spinal fluid samples were negative.

Influenza-related neurologic complications are rare, especially in immunocompetent adults. The clinical signs and severity of this pathology are variable. We report a life-threatening specific complication of influenza A(H1N1) pdm09 infection that was responsible for lethal central venous thrombosis.

The Study

A previously healthy 26-year-old man from northern Africa was admitted to our emergency department in Lyon, France, in November 2009, during the peak of influenza A(H1N1)pdm09 infection in France (*1*), because of cephalalgia, confusion, and lethargy. A Glasgow Coma Score was 12. He had no history of influenza vaccination. Initial symptoms (fever, cough, and myalgia) began a week before admission. Several members of his family had similar symptoms. There were no risk factors indicative of a complicated disease. Body temperature at admission was 36.8°C, and he had no respiratory distress or signs of shock. Results of a chest radiograph were normal.

During the first hours after admission, the patient lost consciousness (Glasgow coma score 3), which was associated with a seizure. His pupils were anisocoric and nonreactive to light. Intubation was then required to protect the airways. A cranial computed tomographic (CT) scan showed thrombosis of the superior sagittal sinus associated with 3 cerebral hematomas (left frontal and bilateral parieto-occipital) and diffuse cerebral edema with signs of increased intracranial pressure (Figure).

Biologic results showed an increased neutrophil count (14.5×10^9 cells/L), thrombocytopenia (25×10^9 platelets/L), and an inflammatory syndrome (C-reactive protein level 49.7 mg/L). There was no renal dysfunction and no increases in levels of serum lactate or abnormalities in levels of cardiac, hepatic, and pancreatic enzymes. Toxicology screening showed no alcohol or drugs present. Results of thrombophilia screening (standard blood coagulation tests and tests for antibodies against thrombin III and phospholipid) were negative.

Real-time PCR for nasopharyngeal swab specimens rapidly confirmed influenza A(H1N1)pdm09 infection. Test results for cerebrospinal fluid (CSF) (312,000 erythrocytes/mm^3, 1,000 leukocytes/mm^3, glucose level 0.84 mmol/L, and protein level 2.7 g/dL) were not informative because of massive hemorrhaging. Results of real-time PCR for CSF were negative for influenza A(H1N1)pdm09 virus, herpes simplex virus (HSV1 and HSV2), and enterovirus. Results of serologic analyses for infectious agents often associated with encephalopathy (cytomegalovirus, Epstein-Barr virus, HSV, rubella virus, enterovirus, and *Mycoplasma pneumoniae*) were negative. The patient was also negative for HIV. Surgery was not considered because the neurologic condition was irreversible. Two electroencephalographic records showed no cerebral activity, confirming this poor prognosis. The patient died 72 hours after admission.

An autopsy was performed. Macroscopic examination showed a congested and edematous brain. Thrombosis of the superior sagittal sinus was caused by a platelet–fibrin thrombus. Acute subarachnoid hemorrhage was found with multiple intraparenchymal infarcts involving the frontal and parietal lobes. Cerebral tonsillar and bilateral uncal herniations were noted. Inflammatory infiltrates were scarce, and few perivascular lymphocytes were found. Immunohistochemical analysis showed no macrophagic infiltration, suggesting recent (<3 days) infarcts. A brain biopsy specimen was positive by real-time PCR for influenza A(H1N1)pdm09 virus RNA, but a lung biopsy specimen was negative by real-time PCR and culture.

Conclusions

We describe fatal encephalitis in the form of central venous thrombosis associated with influenza A(H1N1)pdm09 virus infection in an immunocompetent man. Influenza-associated encephalopathy (IAE) is a rare complication

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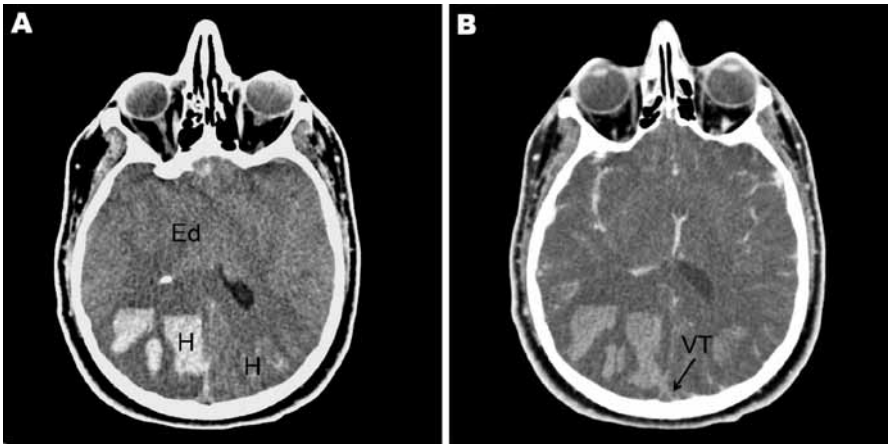

Figure. A) Noncontrast cranial computed tomographic (CT) scan of a 26-year-old immunocompetent man with influenza, showing diffuse cerebral edema (Ed) and bilateral parieto-occipital hematoma (H). B) Cranial CT scan with contrast injection, showing diffuse cerebral edema (Ed) and cord sign (arrow) related to a venous thrombosis (VT) of the superior sagittal sinus.

of a common disease and is more frequently described in children (1–4 cases/100,000 person-years) (*2,3*). In children, IAE related to seasonal influenza results in variable relapse and a mortality rate as high as 30% (*3*). In adults, seasonal IAE is infrequent and poorly characterized (*4*). Symptoms in the patient were typical of neurologic disorders of IAE, including disorientation, meningismus, agitation, seizures, and coma (*2,4*). The incidence of neurologic complications from influenza A(H1N1)pdm09 has not been determined, and it is not clear whether this pandemic was associated with increased neurologic complications compared with those of seasonal influenza (*5*).

Several series of neurologic complications, especially those involving children, have been published and occasionally reported poor prognosis (*5–7*). To our knowledge, only a few reports have described cases in adults (*8–12*). A 20-year-old man had refractory seizures in association with malignant edema and survived with severe neurologic sequelae (*8*). A 22 year-old woman showed development of persistent Parkinsonian features and hypothalamic dysfunction manifestations after IAE (*9*). As in our patient, these 2 patients had no respiratory distress. A 40-year-old patient had prolonged hypoxemia secondary to the acute respiratory distress syndrome (ARDS) associated with acute hemorrhagic leukoencephalitis, which was responsible for severe disability (*10*). Two patients had fatal cerebral edema and transtentorial brain herniation syndrome associated with ARDS and renal failure (*12*).

The pathogenesis of IAE remains unclear (*2*). As demonstrated in the case reported, influenza virus is rarely detected in CSF and pleiocytosis is often absent, suggesting that direct invasion by influenza A virus is unlikely to be the cause of encephalopathy (*2*). Hematogenous spreading is unlikely because viremia is rare in humans, and influenza virus–associated neurotropism has not been demonstrated. In addition, influenza virus viremias are often associated with ARDS caused by massive virus replication in the lungs during infection, but our patient had no pulmonary infection.

Pathogenesis might be related to a hyperactivated cytokine response in the context of a systemic inflammatory response syndrome. In patients with influenza encephalopathy, levels of proinflammatory cytokines and soluble cytokines receptors are increased in serum and CSF (*13*). Symptoms may be caused by cytokines, which could cause direct neurotoxic effects, cerebral metabolic changes, or breakdown of the blood–brain barrier (endothelial injury) (*14*). However, lack of benefit from use of steroids or intravenous immunoglobulin for influenza-associated encephalopathy does not support this potential mechanism (*4*).

Neuroimaging findings by CT or magnetic resonance imaging (MRI) for this pathogenic process usually include focal or diffuse cerebral edema, necrosis (especially in children), demyelinization or hemorrhagic injury (*2*). Patients exhibiting neuroradiographic abnormalities have more severe sequelae or higher mortality rates than patients with normal CT or MRI results (*4*). Influenza A(H1N1) pdm09 virus infections might be associated with increased abnormalities detected by MRI compared with those associated with seasonal influenza (*6*). To our knowledge, there has been no report of IAE related to cerebral venous thrombosis, including influenza A(H1N1)pdm09 infections.

Histologic abnormalities of the brain are often absent in patients who die with clinical signs of IAE (*15*). Influenza virus antigens are generally not detected in the brain (*8*). We found evidence of direct viral neuroinvasion and positive results by real-time PCR for a brain biopsy specimen for influenza A(H1N1)pdm 09 virus RNA, which indicates microbiologically documented encephalitis associated with influenza A(H1N1)pdm09 infection.

In summary, IAE is a rare complication of a common disease that was also diagnosed during the influenza A(H1N1) 2009 virus pandemic. Cases in adults usually remain mild, but our results show that clinicians should be alert to potential neurologic complications of influenza, even without respiratory symptoms. The severity of neurologic sequelae warrants investigation of these sporadic cases.

Increased knowledge of host–virus interaction in the brain and necropsy studies of cases with cerebral involvement could provide better understanding of this interaction.

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Recombinant Vaccine–derived Polioviruses in Healthy Children, Madagascar

To the Editor: Poliomyelitis outbreaks caused by pathogenic vaccine-derived polioviruses (VDPVs) are primarily a result of low polio vaccine coverage. Low coverage enables interhuman circulation of polioviruses (PVs) from the oral polio vaccine (OPV), and it enables genetic drift of the viruses and their subsequent reversion to neurovirulent phenotypes (*1*). Polio outbreaks associated with type 2 or 3 VDPVs (VDPV2s or VDPV3s) were reported in 2001–2002 and 2005 in Toliara Province in southern Madagascar (*2*,*3*). These VDPVs were found in patients with acute flaccid paralysis (AFP) and in healthy children who were contacts of the patients with AFP (*2*,*4*). The genomes of these VDPVs belong to several independent, complex mosaic recombinant lineages composed of sequences derived from vaccine polioviruses and other co-circulating species C human enteroviruses (human EV-C) (*4*,*5*). The 2001–2002 and 2005 outbreaks in Toliara Province were stopped after rapid and efficient OPV vaccination campaigns.

No polio cases have been detected in Madagascar since 2005. However, OPV coverage fluctuates in Toliara Province. In June 2011, to determine if VDPVs were circulating in the province, we collected fecal samples from 616 healthy residents <5 years of age (Madagascar Ethics Committee agreement 011-MSANP/CE). Sample extracts were used to inoculate human RD and HEp-2c cells and mouse L cells expressing the human poliovirus cellular receptor CD155 (L20B cells) (*6*). Of the 616 samples, 238 induced cytopathogenic effects in human cells, of which 20 also induced cytopathogenic effects in L20B cells; the latter samples were confirmed by reverse transcription PCR molecular testing (*7*) to contain PV strains. All PV isolates originated from children who had not received OPV in the 30 days before samples were collected.

We sequenced the genomic regions encoding the capsid viral protein (VP1) of the 20 isolates confirmed to contain PV strains: 3 type 2 PV isolates showed >0.5% nt sequence divergence from the type 2 OPV strain (Sabin 2) and were thus identified as potentially pathogenic VDPV2s. Two of these VDPV2s carried numerous mutations in the VP1 region (903 bp): isolates MAD-2593-11 (30 nt mutations) and MAD-2642-11 (33 nt mutations). The third

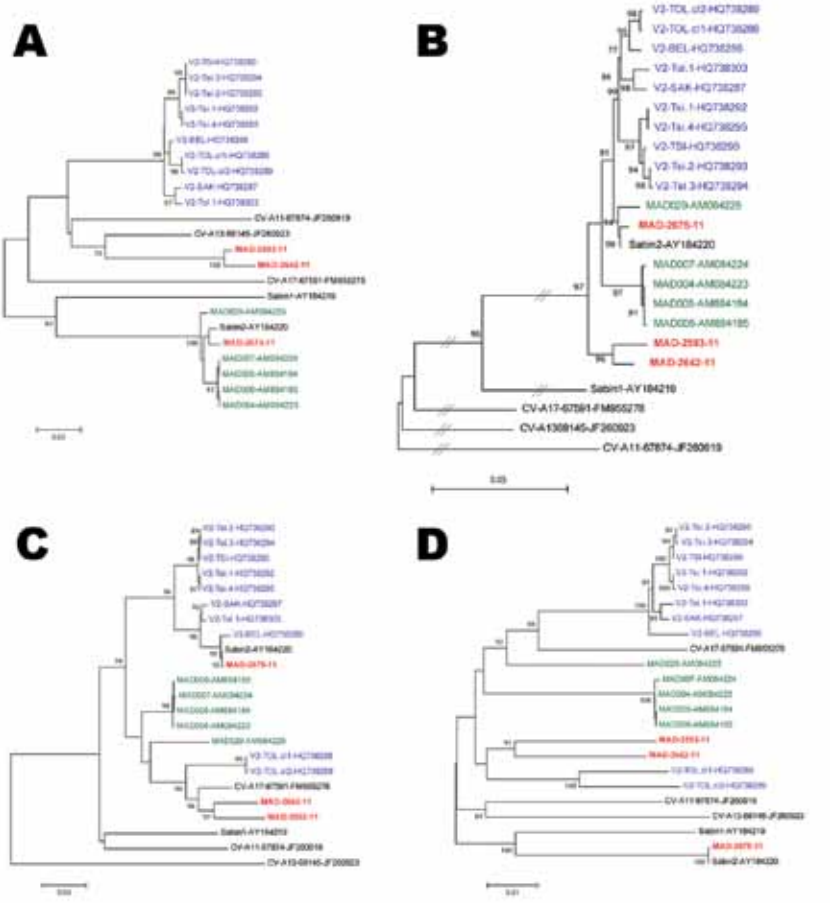

Figure. Phylogenetic trees showing genetic relationships between sequences of vaccine-derived poliovirus (VDPV) isolates. The trees are based on nucleotide sequence alignments of various subgenomic regions. Multiple sequence alignments were performed with CLC Main Workbench 5.7.2 software (CLC bio, Aarhus, Denmark). Phylograms were constructed with MEGA4 (http://megasoftware.net/mega4/mega.html), using the Jukes-Cantor algorithm for genetic distance determination and the neighbor-joining method. The robustness of the resulting trees was assessed with 1,000 bootstrap replications. A) Tree built from 712-bp fragments of the 5′–untranslated region (nt 36–747, with reference to Sabin 2 nucleotide numbering). B) Tree built from 2,637-bp fragments of the P1 region (nt 748–3,384). C) Tree built from 1,725-bp fragments of the P2 region (nt 3,385–5,109). D) Tree built from 2,259-bp fragments of the P3 region (nt 5,110–7,368). Names (isolate name-accession no.) are VDPV isolates recovered in Madagascar in 2011, 2005, and 2001–2002, respectively. Sequences of other isolates (CV-A11, 13, and 17) from Madagascar were also used. The percentage of bootstrap replicates is indicated at nodes if >70%. The length of branches is proportional to the number of nucleotide differences (percent divergence). Scale bars indicate genetic distances. A color version of this figure is available online (wwwnc.cdc.gov/EID/article/1916/13-0080–F1.htm).

VDPV2, MAD-2675–11, carried 6 nt mutations. The nucleotide differences between Sabin 2 and MAD-2593–11 (3.3%) and MAD-2642–11 (3.6%) suggest that these VDPV2s had been multiplying or circulating for ≈3.0 years, and the differences between Sabin 2 and MAD-2675–11 suggest it had been multiplying or circulating for ≈0.5 year. Almost complete genomic sequencing, from nt 36 to nt 7,420 (Sabin 2 numbering), was subsequently performed (EMBL accession nos. HF913426– HF913428).

Isolate MAD-2675–11 was shown to be a mutated Sabin 2 PV, but isolates MAD-2593–11 and MAD-2642–11 displayed mosaic recombinant genomes composed of sequences derived from Sabin 2 and other human EV-Cs. Both recombinants had the 5′–untranslated region (UTR), the nonstructural P2–P3 regions, and the 3′-UTR derived from non-PV human EV-C. The 2 recombination sites were similarly located in each of these recombinant genomes (approximately at nt 760 and nt 3,368) at the 2 extremities of the structural genomic P1 region. Although these recombinants appeared to be related, they had different non-PV human EV-C sequences in the P2–P3 region and the 3′-UTR (6.0%–20.0% nt sequence difference). The phylogenetic relationship of these isolates to the previous Madagascar VDPV2s was assessed: the 2011 isolates originated from 2 novel, independent events (Figure). The 2011 VDPVs had lost the major attenuating determinants in the 5′-UTR (guanine to adenine at nt 481) and VP1 region (isoleucine to threonine at codon 143) regions (*8*). This finding strongly suggests that the 2011 VDPVs had regained a degree of neurovirulence.

Although there is currently no evidence of AFP cases linked to these new VDPVs, their detection in 3 children and their genetic characteristics strongly suggest insufficient OPV coverage in Toliara Province. We could obtain proof of vaccination (vaccination card) for only 27% of the participants. To prevent a third poliomyelitis outbreak, the Ministry of Health of Madagascar organized house-to-house OPV vaccination campaigns within Toliara Province in October and December 2011 and in January 2012. No polio cases have been reported in the province since then, suggesting that VDPV circulation was limited or stopped by these campaigns.

Massive OPV immunization campaigns worldwide have greatly decreased the frequency of poliomyelitis caused by wild-type PVs. However, after the disease has been eliminated in developing countries, low polio vaccine coverage frequently enables the emergence of VDPVs or the reintroduction of wild-type PV from disease-endemic countries; both scenarios threaten the success of the poliomyelitis eradication program (*9*,*10*). More than 640 polio cases caused by circulating VDPVs have been reported in 21 developing countries. We have shown that even in the absence of polio cases, potentially pathogenic circulating VDPVs can be detected in fecal samples collected from children living in areas with fluctuating and often low polio vaccine coverage. Thus, such sampling can help determine whether vaccine campaigns should be implemented in areas where polio could reemerge. In Madagascar, vaccination appears to have cleared emerging VDPVs and to have prevented polio cases in children.

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Hepatitis E Outbreak, Dadaab Refugee Camp, Kenya, 2012

To the Editor: Hepatitis E virus (HEV) is transmitted through the fecal-oral route and is a common cause of viral hepatitis in developing countries. HEV outbreaks have been documented among forcibly displaced persons living in camps in East Africa, but for >10 years, no cases were documented among Somali refugees (*1*,*2*). On August 15, 2012, the US Centers for Disease Control and Prevention (CDC) in Nairobi, Kenya, was notified of a cluster of acute jaundice syndrome (AJS) cases in refugee camps in Dadaab, Kenya. On September 5, a CDC epidemiologist assisted the United Nations High Commissioner for Refugees (UNHCR) and its partners in assessing AJS case-patients in the camp, enhancing surveillance, and improving medical management of case-patients. We present the epidemiologic and laboratory findings for the AJS cases (defined as acute onset of scleral icterus not due to another underlying condition) identified during this outbreak.

Dadaab refugee camp is located in eastern Kenya near the border with Somalia. It has existed since 1991 and is the largest refugee camp in the world. Dadaab is composed of 5 smaller camps: Dagahaley, Hagadera, Ifo, Ifo II, and Kambioos. As of December 2012, a total of 460,000 refugees, mainly Somalians, were living in the camps; >25% were recent arrivals displaced by the mid-2011 famine in the Horn of Africa (*3*). Overcrowding and poor sanitation have led to outbreaks of enteric diseases, including cholera and shigellosis (*4*); in September 2012, an outbreak of cholera occurred simultaneously with the AJS outbreak.

During July 2–November 30, 2012, a total of 339 AJS cases were reported from the camps and 2 nearby villages: 232 (68.4%) from Ifo II, 57 (16.8%) from Kambioos, 26 (7.7%) from Ifo, 12 (3.5%) from Dagahaley, 10 (3.0%) from Hagadera, and 1 each (0.6%) from the nearby Kenyan villages of Biyamadow and Darkanley. The epidemic curve of the outbreak is shown in the Figure.

Of the 339 AJS case-patients, 184 (54.3%) were female. The overall median age was 23.5 years (range 1 month–91 years). The median age among female and male residents was 24 years and 20 years, respectively. Among the 134 women of reproductive age (15–49 years), 72 (53.7%) reported being pregnant; the median gestational age was 17.4 weeks (range 8.7-35.3 weeks). Death was reported for 10 of the 339 case-patients (case-fatality ratio 2.9%), 9 of whom were postpartum mothers (case-fatality ratio 12.5%) and 1 a 2-year-old child.

Serum samples were obtained from 170 (50.1%) AJS case-patients for testing at the Kenya Medical Research Institute/CDC laboratories in Nairobi, Kenya. Of the 170 samples, 148 were tested for hepatitis E virus (HEV) IgM by using an ELISA

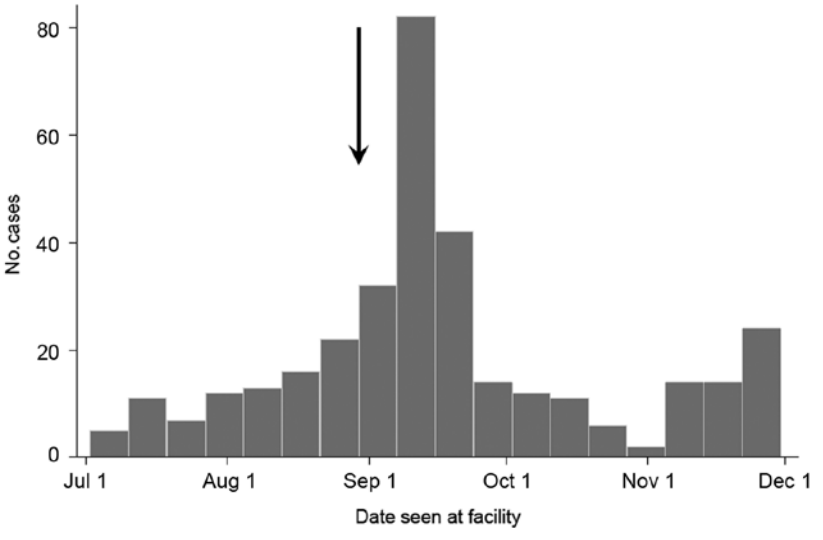

Figure. Cases of acute jaundice syndrome, Dadaab, Kenya, July–November 2012. The arrow indicates the point at which outbreak control measures (e.g., construction of new latrines and hygiene messaging) were initiated by health authorities.

(Diagnostic Systems, Saronno, Italy), and 93 were tested for HEV RNA by using the GeneAmp Gold RNA PCR Reagent Kit (Applied Biosystems, Foster City, CA, USA). Of the 170 samples tested, 131 (77.1%) were positive for HEV IgM, HEV RNA, or both: 120 (81.1%) of 148 tested for HEV IgM and 48 (51.6%) of 93 tested for HEV RNA were positive. In response to the outbreak, UNHCR and partners initiated control measures, including training of health care workers, increasing community awareness, improving hygiene promotion activities, and hastening latrine construction.

The outbreak also affected refugee resettlement to the United States and other countries. At the onset of the outbreak, ≈100 Dadaab refugees per month were scheduled for US resettlement. The incubation period for HEV is 15–60 days (*5*); thus, there was concern that refugees could become ill in transit or within weeks of US resettlement. Acute HEV infection, including progression to fulminant hepatitis, had been reported among travelers returning from regions where the disease is endemic (*6*). As a precaution, the International Organization for Migration and CDC conducted heightened AJS surveillance during pre-departure and arrival health screenings. As of February 2013, no cases of AJS were reported among refugees from Dadaab who resettled in the United States.

Dadaab has faced grave insecurity: aid workers were abducted from the camp in late 2011, and Dadaab has experienced numerous blasts from explosive devices (*7*). Thus, UNHCR and CDC have been limited in their capacity to collect data and conduct a thorough outbreak investigation to identify risk factors. An earlier study in the Shebelle region of Somalia suggested an increased incidence of HEV during the rainy season and elevated risk for infection in villages dependent on river water (*8*). Further evaluation is needed to identify the risk factors for HEV transmission and HEV-associated deaths in this region, including the role of person-to-person transmission. UNHCR and CDC investigations of HEV outbreaks in refugee camps in southern Sudan may provide data to answer these questions.

HEV is believed to have infected humans for centuries (*9*); however, the reemergence of the disease in refugee camps is a major concern because of the difficulty in implementing effective preventive measures under camp conditions. Point-of-care tests will be useful for rapidly detecting outbreaks and could potentially save lives. The progress made in developing effective vaccines is encouraging (*10*). Once available, HEV vaccination should be prioritized in this population, especially for pregnant women.

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Wild Poliovirus Importation, Central African Republic[1]

To the Editor: Since the Global Polio Eradication Initiative was launched in 1988, indigenous transmission of wild poliovirus (WPV) has been interrupted in all countries except Afghanistan, Pakistan, and Nigeria (*1*). However, during 2003–2011, outbreaks resulting from importation of WPV occurred in 29 previously polio-free countries in Africa, including Central African Republic (CAR) (*1–3*). In 2011, 350 WPV cases were reported from 12 countries in Africa, a 47% decrease from the 657 cases reported by 12 countries in Africa in 2010 (*1*).

In CAR, the last case of poliomyelitis caused by indigenous transmission of wild poliovirus was reported in 2000, but importation of WPV type 1 has been reported (*4*). We describe the importation of WPV1 and WPV3 into CAR during successive events in 2008, 2009, and 2011.

To investigate importation of WPV into CAR, we conducted a study using fecal samples collected from patients in CAR who had acute flaccid paralysis (AFP) during 2008–2011. The samples were analyzed for virus isolation, typing, and intratypic differentiation at the Regional Reference Laboratory for Polio, Institut Pasteur de Bangui, using World Health Organization (WHO) standard procedures (*5*). Isolated WPV strains were sent to the Centers for Disease Control and Prevention (Atlanta, Georgia, USA) or the National Institute for Communicable Diseases (Johannesburg, South Africa) for sequencing according to WHO guidelines (*6–8*). Cases were classified as laboratory confirmed or polio-compatible according to WHO recommendations; a polio-compatible case was defined as AFP for which stool samples were not adequate or a situation in which the patient was lost to follow up or had residual paralysis 60 days after testing.

Of 141 AFP cases from 2008, three, from Bangui, Ouham, and Ouaka districts, were laboratory confirmed as WPV1; this cluster was designated B2D1B (Figure). Sequencing results showed that the virus in this cluster belonged to the South Asia A (Indian) genotype, which was circulating in Angola and Democratic Republic of Congo at that time (Figure).

Of 163 AFP cases from 2009, 14 in Ouham-Pende district were laboratory confirmed as WPV3; this cluster was designated D2B2B1. Sequencing results showed that the virus in this cluster belonged to West Africa B genotype, which was circulating in Nigeria and southern Chad at that time (Figure).

Of 142 AFP cases from 2011, four in Ouham district were laboratory confirmed as WPV1; this cluster was designated I6C2B4C1A2. Sequencing results showed that the virus in this cluster belonged to West Africa B genotype, which was circulating in south Chad and Nigeria at the time (Figure).

The importation of wild poliovirus strains into CAR appeared to follow 3 different routes. In 2008, WPV1 originated from Democratic Republic of Congo and was first detected in the capital, Bangui, which is located in

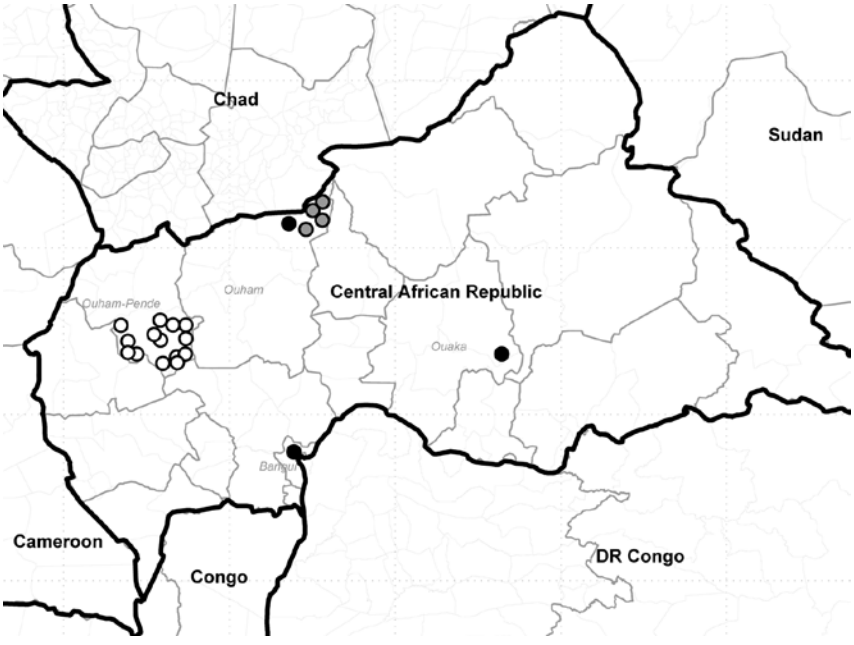

Figure. Clusters of polio cases caused by wild poliovirus importations, Central African Republic, 2008–2011. Each circle represents 1 case of acute flaccid paralysis confirmed as polio. Black circles, cluster B2D1B, 2008 poliovirus (PV) type 1 SOAS importation from Democratic Republic of Congo (DR Congo); white circles, cluster D2B2B1, 2009 PV3 WEAF-B importation from Nigeria and southern Chad; gray circles, cluster I6C2B4C1A2, 2011 PV1 WEAF-B importation from southern Chad.

[1]Data from this report were presented to the Global Polio Laboratory Network, Geneva, Switzerland, and at the First International Conference of the African Society of Laboratory Medicine, 2012 Dec 1–7, Cape Town, South Africa.

the southern part of the country. Two more cases were detected in 2 other districts, in the north (Ouham) and in the middle (Ouaka) of the country. During that year, 2 AFP cases were classified as polio-compatible; these cases originated from Haute Kotto and Ouham districts. In 2008, routine coverage of oral polio vaccine (OPV) was 45%, 33%, and 57% for Bangui and Sanitary Regions 3 and 4, respectively. (Sanitary regions are equivalent to provinces and have several districts under their jurisdiction; Bangui is considered a Sanitary Region containing 8 districts.) To interrupt wild poliovirus circulation, health authorities implemented 4 rounds of national immunization days, 2 using monovalent OPV (mOPV) type 1 and 2 using trivalent OPV; 1 local immunization day using mOPV1 was also instituted.

In 2009, WPV3 was imported from southern Chad to the Ouham-Pende district in CAR. This insecure district is difficult to access, but routine OPV coverage was reported as 61% for 2009, compared with the country's official OPV coverage of 55%. The apparently higher coverage in areas of insecurity is likely the result of inaccurate target population estimates. Five additional AFP cases were classified as polio-compatible; these occurred in Ouham (1), Ouham Pende (2), Mambere Kadei (1), and Mbomou (1) districts. To interrupt wild poliovirus circulation, 8 supplementary immunization activities were organized.

The 2011 polio outbreak occurred in the district of Ouham and was caused by WPV1 poliovirus imported from southern Chad (Figure). Fourteen additional cases of AFP were classified as polio-compatible; these occurred in Ouham-Pende (6), Ouham (1), Ombela M'Poko (1), Kemo (1), Ouaka (1), Haute Kotto (2), and Mbomou (2) districts. Routine OPV coverage for this insecure and difficult-to-access region was 55% for 2010 and 58% for 2011.

CAR is one of the countries with the highest predicted risk for WPV circulation after importation (*9*). The 3 WPV importation events we report demonstrate that current immunization levels in this country are insufficient to guard against polio. High, sustained levels of routine OPV coverage in every district, supplemented by high-quality supplementary immunization activities, will help prevent future outbreaks. Surveillance standards must also be maintained to ensure the rapid detection of WPV importation, thus enabling timely response and containment.

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Human Gyrovirus in Healthy Blood Donors, France

To the Editor: Gyroviruses (GyVs) are naked, single-stranded DNA viruses that were described in chickens in 1979 (*1*). Chicken anemia virus (CAV), initially the sole member of the genus *Gyrovirus* (family *Circoviridae*), possesses a genome of ≈2.3 kb, containing 3 major partially overlapping open reading frames, viral proteins [VP] 1–3, and a short untranslated region (*1*). For >30 years, this virus, which was responsible for severe anemia and increased death rates in young chickens, was considered to have an extremely low genetic diversity and to be specific to this animal host. In 2011, however, sequence-independent molecular protocols enabled the characterization of highly divergent, GyV-related sequences in human and chicken biological samples. Human GyV 1 (HGyV1), avian GyV 2, and GyV3 sequences were identified from human skin, chicken blood, and human feces, respectively (*2*–*4*). These genomes harbor a genetic organization similar to CAV, despite a high genetic divergence (49%–65%).

One study described the detection of GyVs in HIV-positive patients and kidney transplant recipients (0.7% and 6%, respectively) (*5*), but these viruses had not been identified in blood samples from healthy persons. We investigated the presence of HGyV DNA in 352 blood samples from healthy blood donors in France (mean age 39 years; 185 men; M:F ratio 1:1.11).

Plasma samples were prepared as described (*6*), and 1-mL aliquots were used for nucleic acids extraction (MagNA pure LC; Roche Diagnostics, Meylan, France). HGyV DNA was detected by using 2 systems in separate real-time TaqMan amplification assays (StepOne Plus; Applied Biosystems, Courtaboeuf, France). The first detection assay (VP1 gene) was described previously (HGyV-rtFP/HGyV-rtRP primers, HGyV-rtP probe, 72 nt) (*5*). The second assay was designed following the analysis of available HGyV sequences (Figure): sense primer HGyVsPBs 5′-GCTAAGACTGTRACATGGC-3′, reverse primer HGyVsPBr 5′-CTCCGGGAATAGCGTCTTC-3′, probe HGyVsPBp 5′-FAM-TGGCACTGGAGACACAGACTGCG-TAMRA-3′. This assay targets the VP2 gene of the viral genome, with an expected length of 118–115 bp, depending on the reference sequence considered.

Amplification reactions were performed by using 10% of extracted material with the TaqMan Fast Universal PCR Kit (Applied Biosystems) in a final volume of 20 µL. Cycling conditions for both assays were 95°C for 20 s, followed by 50 cycles of 95°C for 1 s and 60°C for 20 s. The sensitivity of TaqMan assays was estimated to be 10 copies of HGyV DNA by using dilutions of a synthetic template. Each amplification product was subjected to additional agarose gel electrophoresis to help eliminate potential false-negative real-time PCR results.

Among the 352 plasma samples tested, 3 (0.85%) resulted in a positive signal by using our in-house real-time detection assay; no positive signal was identified by using the other system tested. When the tests were repeated, identical results were obtained. No additional amplicons were identifiable after agarose gel analysis. HGyV DNA titers in the 3 positive samples were low (<500 copies/mL plasma). Positive blood samples originated from 1 woman (age 44) and 2 men (ages 29 and 39). No biological or serologic marker evaluated for routine blood donor screening in France was associated with these donations.

Partial HGyV sequences obtained were cloned and sequenced (7 clones each). All sequences characterized clustered with HGyV1 sequences, exhibiting either 100% nucleotide identity or 1 point mutation (corresponding to a nonsynonymous substitution, ala →thr) (Figure). No intragenetic diversity was identified.

Our results demonstrate that recently discovered HGyVs are detectable in blood of healthy persons. The low prevalence (0.85%) suggests, however, that the virus is infrequently found in the general population in France. A study from Italy of HGyVs in blood from healthy donors did not detect such viruses (*5*). This result may be linked to the small number of samples tested (n = 50) or to the use of a nonoptimized detection system designed on the basis of HGyV1 sequences only; moreover, the possibility that the VP1 and VP2 amplified

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9B1         acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
9F2         acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
13F1        acatggcAAGACTACGAAGAAGACGACCTCACGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
HGyV1-915   acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
HGyV1-CL33  acatggcAAGACTACGAAGAAGACGACCTCGCGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
AGV2        acatggcAAGATTACGACGACGACGACCTCGTGGACGCTTTGGCTTCTACCACAGAGGACGATGGCACTGGAGACACAGACTGCGACgaagacg
GyV3        acatggcTAGA---CGATTTCGCCGGCACAGAGGAAAGTTCGGATACTACAGACGGGGAAGATGGCACTGGAGACACAGACTGCGACgaagacg
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Figure. Alignment of partial sequences of human gyroviruses (HGyVs) from healthy blood donors, France. Point mutation corresponding to a nonsynonymous substitution is in **boldface** (13F1 isolate). Reference sequences and GenBank accession nos.: HGyV1-915, FR823283; HGyV1-CL33, JQ308212; avian gyrovirus (AGV) 2, JQ690763; gyrovirus (GyV) 3, JQ308210). Bar above sequences indicates location of the HGyVsPBp probe; lowercase letters indicate 5′/3′ ends of HGyVsPBs/HGyVsPBr real-time primers; asterisks (*) indicate conserved positions.

regions would be conserved differently must be considered.

It is probable that subsequent sequences of HGyVs remain to be identified in human blood. A recent study reported the characterization of a highly divergent GyV sequence (GyV4) in human fecal samples and chicken meat (*7*); as with avian GyV2 and GyV3, further research is needed to determine whether this variant replicates in the human body or is solely ingested in food and passively excreted. A better knowledge of the genetic diversity of these newly discovered viruses will enable development of improved molecular detection systems and their subsequent use in epidemiologic studies involving diverse human cohorts.

The potential clinical importance of HGyVs remains to be clarified. Although infection with CAV in birds is frequently associated with clinical signs and disease, the presence of HGyVs in immunocompromised or immunocompetent humans does not appear to be correlated with visible symptoms. Further studies of the natural history and distribution of HGyVs in human hosts are needed.

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Vibrio cholerae O1 Isolate with Novel Genetic Background, Thailand–Myanmar

To the Editor: *Vibrio cholerae* O1, a causative agent of cholera, was classified into 2 biotypes, classical and El Tor (*1*). However, accumulating evidence suggests that atypical El Tor *V. cholerae*, which possesses traits of both classical and El Tor biotypes, has replaced the seventh pandemic prototypic El Tor *V. cholerae* worldwide in recent years. Cholera outbreaks in Thailand during 2007–2010 were caused by atypical El Tor isolates carrying the classical type cholera toxin gene (*2*). Epidemiologic surveys in a Thailand–Myanmar border area during 2008–2012 yielded more than 500 isolates of *V. cholerae* O1. We identified an isolate that possessed the typical El Tor type cholera toxin gene (genotype 3) and designated it MS6 (later assigned strain number DMST28216). It does not belong to either the seventh pandemic prototypic biotype identified in 1961 or the group of atypical El Tor strains found during 1991–present (*3*).

MS6 was isolated from stool samples from a 26-year-old woman (migrant worker) from Myanmar who had been admitted to Mae Sot General Hospital in Tak Province, Thailand, for 3 days with vomiting, watery diarrhea, nausea, fever, and headache. The illness was considered mild to moderate. Acute gastroenteritis was diagnosed on the basis of the symptoms and laboratory results. The key virulence factors of *V. cholerae* O1 include cholera toxin (CTX), which is responsible for profuse watery diarrhea, and a pilus colonization factor known as toxin-coregulated pilus (TCP). The virulence-related genes (*ctxAB* and *tcpA*) and the phage repressor gene (*rstR*) of MS6 had identical sequences to those of the seventh pandemic prototypic El Tor *V. cholerae* O1 N16961 strain. The isolate was found to be positive for enteric bacteria in the Voges-Proskauer test and resistant to polymyxin B (50 units). We further investigated 2 gene clusters, *Vibrio* seventh pandemic island I (VSP-I) and II (VSP-II), associated with the seventh pandemic strains and absent in classical and pre–seventh pandemic strains (*4*–*7*). The common genes on the VSP-I island in N16961, including VC0175, VC0178, VC0180, VC0181, and VC0183, were detected by PCR (*8*) in MS6 but were lacking in VSP-II; 26.9 kb of VSP-II was originally found in N16961. Moreover, PCR analysis showed that the isolate did not

possess the VC2346 gene, a specific marker of the seventh pandemic clone (*5*,*9*). However, we found a VC2346 homolog with 83.9% sequence identity with VC2346 at the nucleotide level and 97% at the amino acid level in the 624-bp region. The coding region of the homolog is considered to be shorter than VC2346 (684 bp) because it contains the stop codon, TAG. This homolog was identified in environmental, classical, or pre–seventh pandemic strains of *V. cholerae* O1 (*5*), including MS6, and in 2740–80 (US Gulf Coast, 1980), 3569–08 (US Gulf Coast, 2008), BX33026 (environmental water in Australia, 1986), RC27 (classical, human isolate in Indonesia, 1991), O395 (classical, human isolate in India, 1965), MAK757 and M66–2 (pre–seventh pandemic, human isolate in Indonesia, 1937), and NCTC 8457 (pre–seventh pandemic, human isolate in Saudi Arabia, 1910), excluding seventh pandemic strains.

This conservation of the homologue of VC2346 in strains isolated over the course of a century and the geographic distribution of the strains suggest a notable biologic function and a specific marker. In addition, we determined the sequences of 15 housekeeping genes which exhibited sequence variations in toxigenic *V. cholerae* (*10*). The results indicated that by comparison, MS6 is closely related to the US Gulf clones (Figure). However, 2 genes, *malP* and *pepN*, of MS6 are remotely related to them and are novel sequence types, based on results of a BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search. Antimicrobial susceptibility testing by using the disk diffusion method revealed that MS6 was susceptible to chloramphenicol, ciprofloxacin, gentamicin, sulfamethoxazole/trimethoprim, tetracycline, streptomycin, furazolidone, doxycycline, and norfloxacin, and had intermediate susceptibility to ampicillin and erythromycin, suggesting that MS6 had not been exposed to several antimicrobial drugs. The *V. cholerae* SXT element, which usually shows code drug-resistance markers, integrates into a specific site of the *prfC* gene. In MS6, the complete *prfC* gene was detected. The accession numbers of DDBJ for nucleotide sequences determined in this study are AB699244-AB699265. MS6 possesses unique properties in terms of the ribotype, pulsed-field gel electrophoresis pattern, and multiple-locus variable-number tandem-repeat analysis profile, compared with other *V. cholerae* O1 isolates (*2*).

This case was probably an episode of sporadic cholera from indigenous *V. cholerae* O1, such as US Gulf Coast and Australian clones, which are mainly associated with environmental sources. We have been unable to isolate another MS6-like clone, which could have escaped detection because of low prevalence or might exist in a dormant state in a rural area. Nevertheless, the transmission route and its pathogenicity must be of concern for public health.

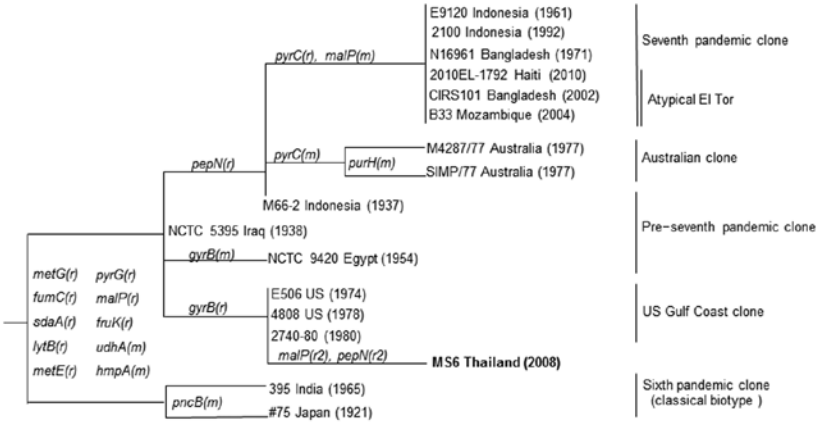

Figure. Relationships among MS6, *Vibrio cholerae* O1 strain, isolated in Thailand in 2008, and other *V. cholerae* O1 strains based on 15 housekeeping genes referenced in Salim et al. (*10*). **Boldface** indicates the MS6 strain. The mutational (m) and recombinational (r) changes with gene names are marked on the branches ($r \neq r2$). Numbers in parentheses represent the year of isolation. DNA gyrase subunit B gene (g*yrB*) of MS6 is 22 nt differences from that of the seventh pandemic clone. Two genes of MS6, *malP* and *pepN*, exhibit novel sequence types based on results of a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

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Crimean-Congo Hemorrhagic Fever Asia-2 Genotype, Pakistan

To the Editor: Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic disease caused by a member of the virus family *Bunyaviridae*, genus *Nairovirus*. This virus (CCHFV) has caused illness throughout Asia, Europe, Africa, and the Middle East (*1*). CCHFVs are clustered among 7 genotypes (Asia-1, Asia-2, Euro-1, Euro-2, Africa-1, Africa-2, and Africa-3) on the basis of genetic variation in the small segment (*2*). These genotypes are well conserved among their regions of origin; however, >1 genotype is prevalent in many countries (*2*). In Pakistan, the first CCHF case was reported in 1976; multiple sporadic cases and outbreaks have occurred in subsequent years (*3*).

To determine which genotypes were present in Pakistan, we performed molecular analysis of archived serum samples collected during 2008 in Fatima Jinnah General and Chest Hospital, Quetta, Baluchistan, in southwestern of Pakistan. Because of limited diagnostic facilities for CCHFV in this country, samples collected during 1976–2002 were occasionally sent to laboratories in countries such as South Africa and the United States, where genetic analysis showed that all viruses tested from that location belonged to the Asia-1 genotype (*4*). Data beyond this period are not available; however, because of improved molecular diagnostic facilities at the Department of Virology, National Institute of Health, Pakistan, blood samples collected from patients with suspected cases attending in-country hospitals are now examined by the institute for confirmation. Our findings substantiate the presence of Asia-1 and Asia-2 genotypes in Baluchistan.

Thirteen IgM-positive samples collected during 2008 and stored at –70°C were available for study. The samples were processed for amplification of 260 bp of the small segment by using reverse transcription PCR with a previously described protocol (*5*). The mean age of patients with serology-confirmed CCHF was 31.3 (range 18–40) years; male-to-female IgM positivity ratio was 1:2. Common symptoms were fever, headache, and nosebleeds. Platelet counts ranged from 16,000 to 43,000/μL of blood.

Of the 13 samples, viral RNA was detected in 2 (CCHF-65–2008PAK and CCHF-43–2008PAK); the amplicons were subjected to bidirectional sequencing by using the BigDye Terminator v3.1 cycle sequencing kit (Applied BioSystems, Foster City, CA, USA). Sequences were analyzed with Sequencher (GeneCodes Corp., Ann Arbor, MI, USA) and MEGA v4.0 (http://megasoftware.net/). The 2 viruses were phylogenetically clustered into Asia-1 and Asia-2 genotypes, with 7% nucleotide divergence, although both samples were collected during September–October, 2008.

The closest nucleotide identity (99%–100%) for CCHF-65–2008PAK was found with the previously reported Asia-1 strains from Pakistan, Afghanistan, and Iran; CCHF-43–2008PAK had 96%–97% similarity to viruses from Dubai and Tajikistan (Figure). The sequences reported from United Arab Emirates, Pakistan, Afghanistan, Iran, and Iraq belong to the Asia-1 genotype; the Asia-2 genotype sequences were mostly from China and Central Asian countries such as Uzbekistan, Tajikistan, and Kazakhstan (*6*). All viruses detected intermittently in Pakistan during 1976–2002 were of the Asia-1 genotype (*4*). However, the analysis of the 2 samples reported here enhances our knowledge of CCHFV genetic diversity in Pakistan.

The closest phylogenetic positioning of CCHF-43–2008PAK with

Asia-2 strain Dubai-616 (GenBank accession no. JN108025) indicates that the probable route of CCHFV transmission was through animal trade between the United Arab Emirates and Pakistan. This finding supports the proposition that animals imported from Pakistan were the probable source of a 1979 outbreak in the United Arab Emirates (*7*). However, we cannot determine the direct source of the Asia-2 genotype in Pakistan, nor confirm the transmission link between the 2 countries. We attribute this to a lack of consistent, contemporary viral genetic information of CCHFV strains in Pakistan and the United Arab Emirates. This lack of data necessitates intensive surveillance and epidemiologic investigations in animal and human populations because geographic factors alone do not provide comprehensive information about the diversity of CCHFV strains circulating in Asia (*6*).

Figure. Phylogenetic analysis of partial small gene fragment (220 bp) obtained from Crimean-Congo hemorrhagic fever virus strains analyzed in this study (black circles). Reference strains belong to different genogroups as retrieved from GenBank. Diamonds indicate virus sequences previously reported from Pakistan. Evolutionary tree and distances (scale bar indicates number of base substitutions per site) were generated with the maximum composite likelihood method with Kimura-2 parameter distances by using MEGA 4.0 (http://megasoftware.net/). Numbers next to branches indicate the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates). The GenBank accession numbers, country, year of sample collection, and respective genotype information have been provided where available.

The presence of geographically distant, but genetically similar, strains suggests that the viruses are dispersed either through animal trade or migratory birds (*8*). No clear evidence of CCHFV infection in migratory birds has been found, but they may play a major role in translocation of infected ticks to distant areas (*9*). Birds are known to be parasitized by these vectors of CCHFV in eastern Europe and Asia and disseminate the virus by transporting infected immature ticks between continents (*4*). It is therefore highly advisable to develop an active surveillance system with appropriate laboratory facilities to conduct the seroepidemiologic surveys and screening of household animals and vectors for CCHFV to rule out potential risks.

Our study was limited by a low number of samples, resulting in availability of only a short fragment of the small gene for analysis. However, similar partial small gene sequencing has been used in previous studies (*2*) and has supported the classification of CCHFV strains correctly into 7 genotypes.

In conclusion, because tick control is not feasible, surveillance activities and laboratory facilities should be improved. Health care workers should also be aware of proper patient management and standard prophylactic and preventive measures, particularly in areas where CCHFV is endemic, such as Baluchistan, where many deaths associated with nosocomial transmission have been reported (*10*).

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Shewanella haliotis Associated with Severe Soft Tissue Infection, Thailand, 2012

To the Editor: Marine bacteria of the family Shewanellaceae, genus *Shewanella*, are gram-negative, motile bacilli that grow aerobically or anaerobically and produce hydrogen sulfide (*1*). Organisms belonging to a *Shewanella* species were first isolated in 1931 by Derby and Hammer from dairy products and classified as *Achromobacter putrefaciens* (*2*). Members of *Shewanella* species usually are found in marine environments in warm climates or during summer in temperate climates (*3*). In humans, most *Shewanella* species infections occur in skin and soft tissues (*4*). One species (*S. algae*) and possibly a second (*S. putrefaciens*) have been isolated from human samples on multiple occasions (*5*). A third species, *S. haliotis*, was implicated in human infections during 2010 (*6*) and *S. xiamenensis* was reported as the fourth infectious species among humans during 2011 (*7*). *S. haliotis* is a novel bacterial species that was isolated from the gut microflora of abalones (*Haliotis discus hannai*) in 2007 (*8*). We report the second description, to our knowledge, of S. haliotis involved in human disease.

In September 2012, a 52-year-old woman, living in Bangkok, Thailand, was hospitalized after experiencing drowsiness for 2 hours. She had a low-grade fever, chills, and swelling, erythema, and tenderness in her left leg. During the previous week, she had handled fresh seafood in a market and had eaten cooked mackerel. She denied having eaten uncooked food or wading into flooded areas or the sea. She had undergone orthotopic liver transplantation 6 months previously to excise hepatocellular carcinoma related to Child-Pugh class C hepatitis C cirrhosis; since that procedure, she had been under treatment with immunosuppressive drugs. She also had diabetes, hypertension, and nephrotic syndrome. Physical examination revealed that in addition to above-named symptoms, multiple blisters were noted (Figure, panel A). Her oral temperature was 37.8°C, blood pressure 80/40 mm Hg, pulse was 110 bpm, and respiratory rate was 24 breaths/minute. A complete blood count showed a leukocyte count of 2,250 cells/μL (91.2% neutrophils). Despite adequate rehydration, monitored by central venous pressure, the patient required norepinephrine to stabilize her vital signs. The clinical diagnosis of her condition was septic shock with suspected necrotizing fasciitis.

After tissue and blood samples were collected and submitted for microbiological analysis, shock resuscitation and an emergency fasciotomy (Figure, panel B) were performed, and antimicrobial drug treatment with meropenem and vancomycin was started. Surgeons did not confirm the suspected necrotizing fasciitis. Two sets of blood cultures and fluid culture sampled from the left leg identified *S. algae* by conventional biochemical methods. The MICs of antimicrobial drugs were determined by Etest (bioMérieux, Solna, Sweden). This strain was susceptible to ciprofloxacin (0.25

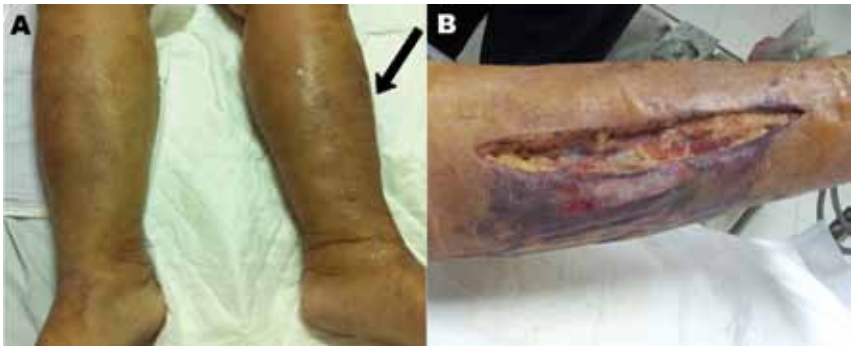

Figure. *Shewanella haliotis* severe soft tissue infection of woman in Thailand, 2012. The patient sought treatment for painful erythematous swelling of the left leg. A) Arrow indicates affected area. B) Postsurgical fasciotomy wound with necrotic tissue.

mg/L), piperacillin-tazobactam (1.0 mg/L), ceftriaxone (1.0 mg/L), and meropenem (0.38 mg/L). The patient had fever for the first 2 days of hospitalization. After 2 weeks of treatment, the antimicrobial drug was switched to oral ciprofloxacin; treatment was continued after dressing and debridement of the fasciotomy wound.

The organism produced yellowish-brown mucoid colonies on sheep blood agar and chocolate agar after 18 hours of incubation at 35°C under CO2 atmosphere. MacConkey agar showed non–lactose-fermenting colonies that were oxidase-positive, motile, and produced hydrogen sulfide on triple sugar iron agar. Growth at 42°C with 6.5% NaCl suggested that this organism was S. algae. Because phylogenetically related *Shewanella* species may be misidentified by routine biochemical tests, the strain was confirmed by using 16S rRNA gene sequencing.

Molecular characterization of 16S rRNA gene sequencing was performed by using PCR with *Shewanella* species consensus primers (online Technical Appendix Table, wwwnc.cdc.gov/EID/article/19/6/12-1607-Techapp.pdf) and direct sequencing from PCR product (JX968803). Phylogenetic analysis of the 16S rRNA gene sequence showed clustering with *S. haliotis* (NR_044134T) and 99.9% similarity and 1 base difference (online Technical Appendix Figure). By using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis, JX968803 showed the closest match (99.9%; 1 base difference) with Alteromonadaceae bacterium PH39 (AF513471).

The strain was confirmed as *S. haliotis* by using additional biochemical tests and API 20 NE System (bioMérieux, Durham, NC, USA). It was positive for ornithine decarboxylase, gelatinase, reduction of nitrates to nitrites, tolerance to 6% NaCl, and assimilation of caprate and malate, but negative for citrate utilization, arginine dihydrolase, lysine decarboxylase, urease, indole production, assimilation of mannose, glucose, arabinose, mannitol, maltose, adipate, and acidification of glucose. This strain was resistant to polymyxin B (300 μg/disc).

More than 50 species of Shewanella have been reported. The route of *Shewanella* infection is associated with direct contact with the organism through seawater or ingestion of raw seafood (*9*). Japan reported 1 case of *S. haliotis* infection in an elderly patient in whom *Vibrio vulnificus* infection was initially suspected (*6*), and various clinical manifestations of *S. algae* infection have been reported (*5*). Community- and hospital-acquired infection with *Shewanella* species from contaminated medical devices have also been reported (*10*). *S. haliotis* and *S. algae* are closely related organisms; discriminating between them on the basis of biochemical tests is difficult. Molecular characterization of 16S rRNA gene sequencing can be used to differentiate the 2 species. In summary, this case suggests that immune-compromised persons in tropical climates could be susceptible to *S. haliotis* soft tissue infection in the absence of typical exposures.

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Murine Typhus in Humans, Yucatan, Mexico

To the Editor: *Rickettsia typhi* is the causal agent of murine typhus, a febrile illness affecting humans worldwide (*1*). In Mexico, recent studies demonstrated a 14% prevalence of antibodies against typhus group rickettsiae in healthy adult blood donors in Mexico City, and a recent nonfatal case of endemic typhus was reported in Yucatan (*2*,*3*).

In May 2011, a 42-year-old woman and her 12-year-old son sought care at the clinical service of the Autonomous University of Yucatan. They had malaise, headache, fever (39°C), fibromyalgia, sore throat, and fatigue and an erythematous rash on the chest that after 6 days spread to the abdomen and extremities.

Dengue fever was diagnosed, and the patients were treated empirically with acyclovir, methanesulfonamide, N-(4-nitro-2-phenoxyphenyl) and clarithromycin. Dengue could not be confirmed by laboratory testing.

Murine typhus was diagnosed on the basis of PCR amplification and immunofluorescent assay for antibodies to *R. typhi*. *Rickettsia* species was determined by sequencing of rickettsial genes. Three serum samples were collected from the woman (8, 12, and 16 days after illness onset) and 1 from the boy (8 days) in 3.8% sodium citrate as anticoagulant, and DNA was extracted immediately by QIAamp DNA Blood Mini Kit (QIAGEN Valencia, CA, USA) in accordance with the manufacturer's instructions. Single-step PCR amplification was performed by using genus-specific primers for the rickettsial 17-kDa protein and citrate synthase (*gltA*) genes as reported (*4*)

Sequences of the citrate synthase and 17-kDa PCR products were compared at the National Center for Biotechnology Information BLAST software (*5*). Three PCR amplicons of both genes were fully sequenced and compared with sequences in GenBank. The 17-kDa and citrate synthase fragment sequences (GenBank accession nos. JX198507 and JX458814) showed 99% and 100% identity, respectively, with *R. typhi* strain Wilmington strain (GenBank accession no. AE017197.1) (Table).

Immunofluorescent assay was performed by using *R. rickettsii* and *R. typhi* antigen fixed on slides. We examined the serum samples for IgG and IgM, assessing reactivity of γ chain–specific and m heavy chain–specific secondary conjugates, respectively, with rickettsial antigens. All 3 samples from the woman and the sample from the boy contained antibodies to *R. typhi* (Table). Both patients were treated with 100 mg of oral doxycycline 2×/day for 7 days (boy), and 10 days (woman); symptoms improved in 72 hours for the child. The woman's symptoms resolved completely in 5 days.

Typhus has been endemic in Mexico since before the conquest period (*6*). Socioeconomic aspects play a major role in zoonotic diseases, such as rickettsioses, especially in their distribution in urban and suburban areas because of factors such as marginalized communities, animal breeding, education levels, poverty, and social exclusion from health systems.

Overcrowding resulting from migration from rural areas to large urban centers contributes to increased zoonoses in urban areas. Also contributing is the ecologic imbalance of flora and fauna associated with deteriorating sanitary conditions in areas where mammals involved in the cycle of *R. typhi*, such as rodents and opossum, may live in the same habitat as humans and colonize backyards, waste deposit area, and areas around the neighborhoods where they can find food. The concurrence and presence of mammals, vectors, and humans may contribute to maintaining transmission of endemic typhus in a reduced area, with the possibility to cause outbreaks

Table. Ig detection by immunofluorescent assay and molecular results by PCR and sequence identity of the amplicons of *gltA* and 17-kDa genes of *Rickettsia* spp. from 2 patients with murine typhus, Yucatan, Mexico

Patient age, y/sex	Days after illness onset	Immunofluorescence assay				PCR/sequence test		*Rickettsia* species*
		R. typhi		*R. rickettsii*				
		IgM	IgG	IgM	IgG	17 kDa	*gltA*	
45/F	8	256	128	Neg	128	Pos	Pos	*R. typhi*
	12	128	128	Neg	64	Pos	Pos	*R. typhi*
	16	64	128	Neg	64	Neg	Neg	ND
12/M	8	128	128	Neg	64	Pos	Pos	*R. typhi*

*Identified species showed 99% identity with *R. typhi* Wilmington strain 17-kDa antigen gene (GenBank accession no. AE017197.1) and 100% identity with *R. typhi* Wilmington strain *gltA* gene (GenBank accession no. AE017197.1). ND, species not determined.

(*7*,*8*). Housing conditions and culture, such as courtyards with vegetation and presence of pets, in several suburban areas of Yucatán encourage close contact between humans and possible reservoirs for several infectious diseases, such as mice, rats, opossums, dogs and cats, and their ectoparasites (*9*).

Because housing and cultural conditions are similar in all countries of Latin America, endemic typhus probably is transmitted in the same way: by coexistence with domestic animals and close contact with wild reservoirs. Epidemiologic control should be closely linked to education aimed at encouraging villagers to interrupt the cycle of transmission and thus prevent the disease.

We have confirmed the presence of murine typhus in Yucatan. The finding of human cases demonstrates the need to consider *R. typhi* infection in the differential diagnosis of febrile illnesses considered endemic in Yucatan, such as dengue fever and leptospirosis (*10*). Early and accurate diagnosis should enable physicians to treat this disease appropriately and early in the clinical course to prevent increased illness and death.

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Flaviviruses in Game Birds, Southern Spain, 2011–2012

To the Editor: Certain arthropod-borne epornitic flaviviruses, namely, West Nile virus (WNV) and Usutu virus (USUV), have spread recently in parts of Europe (*1*,*2*). In southern Spain, the emergence of a third virus of this type, known as Bagaza virus (BAGV), is of concern (*3*). Because of the outbreaks in 2010 in Cádiz (southern Spain) of WNV infection, which affected birds, horses, and humans, and of BAGV infection, which affected game birds (partridges and pheasants), and the reported presence of USUV in mosquitoes in this area (*4*), a surveillance program was implemented in partridges and pheasants during the next hunting season (October 2011–February 2012) to assess the possible circulation of these 3 flaviviruses in the area.

Serum samples and brain tissue from 159 hunted-harvested wild red-legged partridges (*Alectoris rufa*) and 13 common pheasants (*Phasianus colchicus*) were collected on 12 hunting properties from Cádiz (online Technical Appendix Figure, wwwnc.cdc.gov/EID/article/19/6/13-0122-Techapp1.pdf]). All sampled birds were reared and shot in the wild. The age of the partridges was determined according to plumage characteristics.

Presence of antibodies against WNV was tested with a commercial epitope-blocking ELISA (Ingezym West Nile Compac, INGENASA, Madrid, Spain) (*5*). Virus-neutralization titers against WNV (strain

Table. Results of serologic studies in red-legged partridges and common pheasants, southern Spain, 2011–2012*

Species, no.	Age	ELISA, WNV	VNT titers†			Interpretation‡
			WNV	BAGV	USUV	
Partridges (*Alectoris rufa*), n = 159						
6	Juvenile	+	**10–20**	≤5	≤5	WNV
7	Adult	+	**10–20**	≤5	≤5	WNV
1	Adult	+	**160**	**20**	**20**	WNV
4	Not determined	+	**10–80**	≤5	≤5	WNV
1	Not determined	+	**80**	≤5	**10**	WNV
2	Juvenile	+	**10**	**40**	≤5	BAGV
1	Juvenile	+	**20**	**80**	**10**	BAGV
1	Juvenile	–	≤5	**160**	**10**	BAGV
2	Adult	+	**20, 40**	**80, 320**	**10**	BAGV
1	Adult	+	≤5	**20**	≤5	BAGV
1	Adult	+	≤10‡	**40**	≤10‡	BAGV
1	Adult	–	≤5	**40**	≤5	BAGV
1	Adult	+	≤5	≤5	**40**	USUV
2	Juvenile	+	**10, 20**	**20, 40**	≤5	Flavivirus
3	Adult	+	≤5	≤5	≤5	Flavivirus
2	Adult	+	**20, 80**	**80, 160**	**40**	Flavivirus
1	Adult	+	**20**	≤5	**10**	Flavivirus
1	Adult	+	**160**	≤20‡	**80**	Flavivirus
1	Adult	+	**40**	**40**	≤10‡	Flavivirus
1	Adult	–	≤10‡	**20**	≤10‡	Flavivirus
3	Not determined	+	≤5	≤5	≤5	Flavivirus
1	Not determined	+	≤5	**10**	≤5	Flavivirus
Pheasants (*Phasianus colchicus*), n = 13						
1	Adult	+	**80**	**20**	**10**	WNV
2	Not determined	+	**10, 20**	≤5	≤5	WNV
2	Adult	+	**10, 80**	**80, 640**	**20**	BAGV
1	Adult	–	≤5	**20**	≤5	BAGV
3	Adult	+	**20–40**	**20–160**	**40–160**	Flavivirus
1	Adult	+	**10**	≤5	≤5	Flavivirus
1	Adult	–	≤5	**10**	≤5	Flavivirus
1	Adult	Not determined	≤20‡	≤20‡	**40**	Flavivirus
Positive, no. (%)						
Partridges		40 (25)	31 (19)	17 (11)	11 (7)	
Pheasants		9 (69)	9 (69)	8 (62)	7 (54)	
Total		49 (29)	40 (23)	25 (15)	18 (10)	

*WNV, West Nile virus; VNT, virus neutralization test; BAGV, Bagaza virus; USUV, Usutu virus; +, positive; –, negative. **Boldface** indicates VNT-positive serum.

†Serum samples were titrated from 5 to 1,280 dilutions and neutralization titers of ≥10 were considered positive.

‡Differentiation was based on comparison of VNT titers obtained in parallel against the 3 flaviviruses: the neutralizing immune response observed was considered specific when VNT titer for a given virus was ≥4-fold higher than titers obtained for the other viruses; samples showing VNT titer differences <4-fold between the viruses examined were considered positive for flavivirus but not conclusive for any specific virus.

‡VNT at the indicated (or lower) dilution(s) could not be determined because of cytotoxic effect caused by the sample.

Eg-101), BAGV (strain Spain/2010), and USUV (strain SAAR1776) were determined by micro virus neutralization test (VNT) as described (*6*).

Viral genome in brain tissue samples was examined by heminested pan-flaviviral reverse transcription PCR (*7*). All 172 tissue homogenates examined were negative by this test.

Overall seroprevalence for WNV by epitope-blocking ELISA was 29%. Prevalence of neutralizing antibodies measured by VNT was 23% for WNV, 15% for BAGV, and 10% for USUV. Seroprevalence rates were higher for pheasants than for partridges for WNV (Fisher exact test, $p = 0.0003$), BAGV ($p<0.0001$), and USUV ($p<0.0001$) (Table). The significance of this result is uncertain, given that just 2 hunting areas were sampled for pheasants.

Neutralizing antibodies to >1 flavivirus were detected in 15 of the 45 VNT-positive partridges and in 6 of the 12 VNT-positive pheasants (Table). Specificity, as determined by neutralizing antibodies titer comparisons (*8*), showed virus-specific neutralizing antibodies to WNV, BAGV, and USUV in 19 partridges, 9 partridges, and 1 partridge, respectively, in 3 pheasants to WNV and in another 3 pheasants to BAGV (0 to USUV). Serum from 9 partridges and 6 pheasants remained inconclusive (neutralizing antibodies titer differences <4-fold [*8*]). WNV-reacting antibodies by ELISA were shown in 11 of 12 hunting properties (online Technical Appendix Figure). In all locations but 1, ELISA-positive results were confirmed by VNT for NT-Abs to WNV, BAGV, or USUV. Of them, neutralizing antibodies to only WNV were detected in 2 locations, whereas neutralizing antibodies to at least 2 (WNV/USUV or WNV/BAGV) of the 3 flaviviruses were detected in 8 locations. Within these locations, flavivirus-specific NT-Ab responses were differentiated in several samples: neutralizing antibodies to either WNV or BAGV were detected in samples from 6 locations, whereas samples with neutralizing antibodies to either WNV or USUV were detected in 1 location.

Analysis of VNT results in juvenile partridges showed specific neutralizing antibodies to WNV (13%) or BAGV (9%); 4% of these samples were positive for flavivirus but inconclusive for any of the flaviviruses tested (Table). Overall, these results indicated recent circulation of 3 different epornitic flaviviruses—WNV, USUV, and BAGV—in resident game birds in Cádiz, the southernmost province in Spain. A high proportion of birds showed neutralizing antibodies to >1 flavivirus. Some are likely to be attributable to cross-neutralization, although co-infection cannot be ruled out because the results showed co-circulation of >1 flavivirus in game birds in most locations studied. Furthermore, the presence of specific neutralizing antibodies in juvenile partridges indicated that WNV and BAGV circulated in the area 1 year after the outbreaks of 2010. For USUV, specific neutralizing antibodies were detected only in an adult partridge, indicating infection acquired during the previous years. Nevertheless, this finding does not rule out recent co-circulation of USUV together with the other 2 viruses in the same game bird populations, considering that USUV has been repeatedly detected in mosquitoes in nearby areas (*4*).

Evidence of infection by at least WNV and BAGV in 2 consecutive seasons strongly supports the premise that these viruses overwintered in the area. Capability of WNV to overwinter in southern Europe was shown in Italy during 2008–2009 (*9*) and in Spain during 2007–2008 (*10*). Overwintering of BAGV after its introduction into Spain could indicate a risk for its expansion in areas with similar climates (Mediterranean basin). The risk for dissemination of WNV, BAGV, or USUV infections not only to game birds, but also to other wildlife, domestic animals, and humans, calls for improvements in surveillance programs, particularly those that monitor susceptible hosts, such as game birds.

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Books, Other Media

Reviews (250-500) words of new books or other media on emerging disease issues are welcome. Name, publisher, number of pages, and other pertinent details should be included.

Absence of Rift Valley Fever Virus in Wild Small Mammals, Madagascar

To the Editor: Rift Valley fever virus (RVFV) is a mosquito-borne zoonotic virus in the family *Bunyaviridae*, genus *Phlebovirus*, which affects mainly domestic ruminants and humans on continental Africa, Madagascar, and the Arabian Peninsula (*1*). RVFV is transmitted between ruminants mainly by bites of mosquitoes of several genera (*1*). Infection can lead to mild symptoms or can cause abortion in pregnant animals and high mortality rates among newborns. Humans are mostly infected by aerosol transmission when handling infected tissues (aborted fetuses or meat), which results in dengue-like illness. Some cases in humans can be in a severe form (hemorrhagic fever and meningoencephalitis), which can be fatal. Outbreaks in southern and eastern Africa are associated with periods of heavy rainfall (*1*). In eastern Africa, RVFV is believed to be maintained during interepizootic periods through vertical transmission in *Aedes* spp. mosquitoes (*1*). It has been suspected that wild mammals, especially rodents, play a role in the maintenance of RVFV during interepizootic periods (*2*). However, evidence of a wild mammal reservoir in the epidemiologic cycle of RVFV has yet to be demonstrated (*2*).

In Madagascar, the first RVFV isolate was obtained from mosquitoes captured in the Périnet Forest (Andasibe, Moramanga District) in 1979, outside an epizootic period (*3*). Two epizootic episodes occurred, during 1990–91 and 2008–09 (*4*). After the most recent episode, domestic ruminants were shown to be involved in RVFV circulation during interepizootic periods (*5*,*6*); together with the potential vertical transmission in *Aedes* spp. mosquitoes in Madagascar, they might play a role in the maintenance of RVFV. However, genetic evidence indicates that RVFV outbreaks in Madagascar are not associated with emergence from enzootic cycles but that they are associated with recurrent virus introductions from mainland east Africa (*7*). Although these mechanisms for RVFV epidemiology on Madagascar are documented, the possibility of a wild mammal reservoir cannot be excluded. We therefore explored the role of wild terrestrial small mammals in the maintenance of RVFV in Madagascar, especially the nonnative, abundant, and ubiquitous black rats (*Rattus rattus*) (*8*), as has been suggested in rural Egypt (*9*,*10*).

For this study, 1,610 blood samples were obtained from different species of wild terrestrial small mammals in Madagascar (Figure). Permits to capture and collect animals were obtained from national authorities. Animals were sampled from October 2008 through March 2010 at a site in the Anjozorobe-Angavo (Anjozorobe District) forest corridor (18°18′′41.9′ S, 48°00′′57.6′ E), where RVFV was first detected in humans and cattle in February 2008 (*4*) and within 100 km from where the first RVFV was isolated in 1979 (*3*). We collected 378 serum samples from 11 native Tenrecidae (Afrosoricida) tenrecs, 114 samples from 6 native Nesomyidae (Rodentia) rodents, and 471 samples from introduced *R. Rattus* (Muridae, Rodentia) rats (online Technical Appendix, wwwnc.cdc.gov/EID/article/19/6/12-1074-Techapp1.pdf). In addition, during 2008, we obtained serum samples from 647 *R. rattus* or *R. norvegicus* rats living near humans in areas where RVFV was reportedly circulating during 2008 and 2009: the districts of Ankazobe, Antsiranana, Betafo, Ihosy, Marovoay, and Moramanga (*4*,*5*) (Figure).

Serum samples were tested for IgG against RVFV by ELISA, as described (*4*), by using peroxidase-labeled recombinant protein A/G (Pierce, Rockford, IL, USA) or

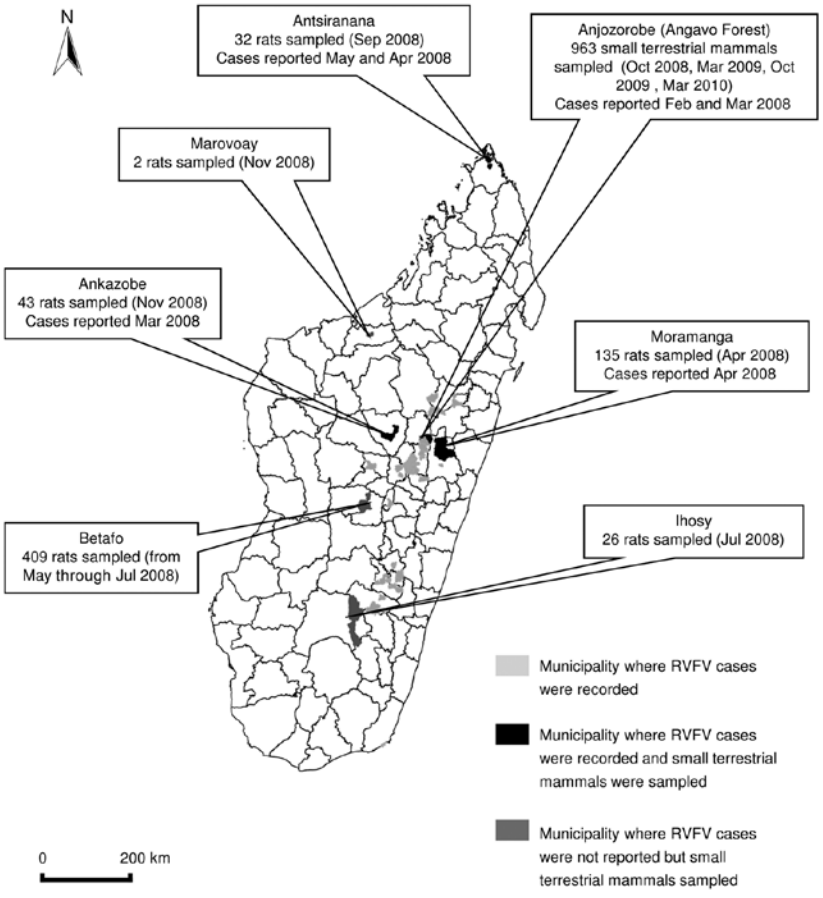

Figure. Collection sites of wild terrestrial small mammals on Madagascar and the number of mammals tested for Rift Valley fever virus (RVFV). At certain localities, the genus and species of sampled rats were *Rattus rattus* or *R. norvegicus*.

anti-mouse or rat IgG (H+L) according to the ability to recognize the immunoglobulin of species endemic to Madagascar (data not shown). The results were negative for all samples tested. Liver and spleen samples from 947 animals caught in the Anjozorobe-Angavo forest corridor were also tested. Approximately 50–100 mg of liver and spleen from each individual was mixed and homogenized at a dilution of 1:10 in culture medium containing 40% fetal bovine serum. After centrifugation, supernatants were collected and pooled by species (maximum 5 individuals/pool). RNA was extracted from pooled supernatants by using TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturers' instructions. Detection of RVFV RNA was attempted by using real-time reverse transcription PCR (*4*). The results were negative for the 220 monospecific pools tested.

Serologic and virologic results from rodent and tenrec samples collected during and after the epizootic 2008–2009 periods were negative for RVFV; 72.8% had been collected in municipalities where RVFV cases were reported. This finding does not indicate a role of native Rodentia and Afrosoricida mammals in the epidemiology of RVFV in Madagascar, nor does it indicate evidence of infection of *Rattus* spp. rats, as suggested in Egypt (*9*,*10*). The absence of infection in *Rattus* spp. rats during a period of intense RVFV circulation does not support its potential role during the outbreak and, a fortiori, in the maintenance of RVFV during interepizootic periods. Among wild terrestrial mammals in Madagascar, animals of the orders Carnivora and Primata are not considered as candidates for the maintenance of RVFV; however, bats (order Chiroptera) and introduced bushpigs (order Artiodactyla , family Suidae, genus *Potamochoerus*) could be candidates, and their role in RVFV maintenance should be investigated (*2*). At present, no evidence is available for the maintenance of RVFV in wild terrestrial small mammals (native and introduced) in Madagascar.

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Colostrum Replacer and Bovine Leukemia Virus Seropositivity in Calves

To the Editor: Bovine leukemia virus (BLV), a deltaretrovirus in the family *Retroviridae*, is the causative agent of enzootic bovine leukosis (EBL). Although EBL is still endemic to the Americas and eastern Europe, most countries in western Europe are EBL free in accordance with European Union legislation; for example, Great Britain has held EBL-free status since 1999. EBL is notifiable to the World Organisation for Animal Health (*1*), with disease incursion affecting international trade. Infection with BLV is life-long and persistent; the presence of antibodies or integrated proviral DNA are indicators of virus exposure. Two tests prescribed for international trade are the agar gel immunodiffusion test (AGIDT) and ELISA (*1*); these tests are used widely for diagnosis (*2*).

We report 5 cases that occurred in the United Kingdom during 2009, in which calves became seropositive for BLV after consuming colostrum replacer. In Dumfriesshire, Scotland, routine serologic screening for BLV detected seropositivity in 2 calves, which were artificial insemination bull candidates. In Newport, Wales, a BLV-seropositive calf was identified during pre-export testing. And in Yorkshire, England, 2 more BLV-seropositive calves, also artificial insemination bull candidates, were identified. All calves were home bred, and there was no evidence (as documented by serologic testing) or history of EBL within the herd. The farms were considered to have low risk for disease incursion because the introduction of new animals was limited.

Further inquiry revealed that the calves had each been exclusively fed a colostrum replacer from North America, where BLV is endemic. Antibodies to BLV might have been present in the colostrum replacer and thus passively acquired by the calves, resulting in seropositivity.

The hypothesis was tested by monthly blood sampling and ELISA analysis for antibodies against BLV (Institute Pourquier, Montpellier, France). Although the batch of colostrum replacer that had been fed to the calves from Dumfriesshire was not available for investigation, another colostrum sample was obtained from the same manufacturer for analysis. The reconstituted colostrum replacer was tested by AGIDT (IDEXX, Bern, Switzerland) at the following dilutions: neat (manufacturer's guidelines), 1:2, 1:4, and 1:8. In addition, 2 commercial ELISA tests (Institute Pourquier and IDEXX) were used over a series of dilutions to 1:125. All serologic tests were conducted according to manufacturer's recommendations. To examine the samples for proviral DNA, we conducted PCRs to amplify a 385-bp fragment of the envelope gene (*3*).

At the various dilutions of colostrum replacer, all serologic tests gave clearly positive reactions. Proviral PCR of the colostrum replacer also returned positive results, which were confirmed by sequencing. The resultant envelope sequence (GenBank accession no. HF545344) was aligned with 23 other sequences obtained from GenBank, which encompassed all known BLV genotypes. Phylogenetic analysis

Table. Bovine leukemia virus seropositivity of calves fed colostrum replacer, UK, 2009*

Location	Time from initial sampling, mo				
	0	1	2	3	4
Dumfriesshire, Scotland					
Calf 1	+++	++	+	IC	–
Calf 2	+++	+	+	–	–
Newport, Wales					
Calf 1	++	++	NS	NS	–
Yorkshire, England					
Calf 1	++	+	+	NS	–
Calf 2	++	+	+	NS	–

*Tested by ELISA (Institute Pourquier, Montpellier, France). +++, strong positive, sample to positive (S/P) ratio ≥200; ++, positive, S/P ratio ≥100; +: weak positive, S/P ratio 60–99; –, negative, S/P ratio <60; IC, inconclusive, S/P ratio 60–70; NS, not sampled.

was conducted as described (*4*) and revealed clustering within genotype 1, which is consistent with BLV of North American origin (*4*). The hypothesis that colostrum intake had caused the seropositivity was supported by the declining antibody titers found in serial blood sampling of all 5 calves (Table).

The same brand of colostrum replacer was used on all 3 farms. For the farms in Wales and England, it was possible to sample the batch of colostrum powder being used; aliquots from each farm were BLV positive by AGIDT and ELISA.

Reactions to passively acquired antibodies would be expected to decrease and become undetectable. After exposure to virus and subsequent infection, antibody titers would not wane to undetectable levels. Our results (Table) provide evidence that the serologic reactions reported here resulted from ingestion of the colostrum replacer rather than BLV infection. The policy and international trade implications of such cases for Great Britain have been discussed (*5*). To maintain the disease-free status of the country, it was necessary to follow up with these cases, which inconvenienced farmers because of movement restrictions and, consequently, financial loss.

The cases described were all linked by the brand of colostrum used; however, our additional investigations found that other brands also tested BLV positive by AGIDT and thus could cause an effect similar to that described here. These data would therefore be useful to any organization involved in BLV serologic surveillance. As a result of this investigation, in March 2010 the European Union banned the import of calf colostrum from herds that are not EBL free.

Although PCR confirmed the presence of BLV proviral DNA in the colostrum, detection of such does not mean that the colostrum contained viable virus. Retroviruses, including BLV, are heat labile; thus, it is unlikely that viable BLV would survive the spray-drying production process. Whether other agents can survive warrants further investigation.

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Novel Respiratory Syncytial Virus A Genotype, Germany, 2011–2012

To the Editor: Respiratory syncytial virus (RSV) is a major cause of severe respiratory disease in infants and elderly persons. RSV strains have been divided into 2 major antigenic groups (A and B), which are further divided into several genotypes. The main genetic and antigenic differences between genotypes are found within the 2 hypervariable regions of the attachment (G) glycoprotein. In 1999, a novel RSV B genotype, which contained a 60-nt duplication in the second hypervariable region of the G protein, was discovered in Buenos Aires, Argentina, and named BA (*1*). Since then, genotype BA has almost completely replaced other RSV B strains worldwide and has diversified into several subgenotypes (*2*).

In February 2012, as part of routine RSV surveillance, we identified a novel RSV A genotype with a 72-nt duplication in the second hypervariable region of G, thus representing the first RSV A genotype with nucleotide duplications in the G gene. Shortly thereafter, circulation of this genotype was reported in Ontario, Canada, in 2010–11 and 2011–12, and the genotype was named ON1 (*3*). To investigate the frequency of genotype ON1 in Germany, we extended the molecular analysis of RSV strains from the previous 2 RSV seasons. The study was approved by the ethics committee of the medical faculty at the University of Würzburg, Germany.

From July 2010 through June 2011 and from July 2011 through June 2012, we identified 271 and 181 RSV-positive patients, respectively. Patients were identified from respiratory specimens sent by hospitals in Bavaria, Germany, for routine testing of respiratory viruses at the Institute of Virology and Immunobiology at the University of Würzburg. The mean age of all patients was 1.2 years (median 8.2 years; range 0.03–81.4 years), and 259 were male. Of the RSV-positive samples, 183 (67.5%) from season 2010–11 and 171 (94.5%) from season 2011–12 were analyzed by sequencing a fragment of ≈500 nt that encompassed the complete second hypervariable region of the G gene (*4*). Alignment with reference sequences and phylogenetic analyses were conducted by using MEGA 5.0 (*5*).

Molecular analysis of RSV-positive samples revealed that RSV A and B cocirculated during both seasons (98 A and 85 B during 2010–11; 99 A and 73 B during 2011–12). In accordance with previous reports (*2*), all RSV B strains from both seasons were identified as genotype BA. The novel RSV A genotype ON1 was not detected during 2010–11. However, 10 (10.1%) of 99 RSV A strains were assigned to genotype ON1 during 2011–12. All other RSV A strains of both seasons belonged to genotype GA2. An amino acid alignment of ON1 sequences is shown in the Figure. The duplication regions of 2 of the 10 ON1 strains contained 2-aa and 3-aa exchanges compared with the ON1 reference sequence (which has no exchanges) (*3*). Of note, an ON1 sequence from Japan with similar, partially even identical mutations was retrieved from GenBank (Figure). All mutations observed so far did not affect potential O-glycosylation sites.

Of the 99 patients with RSV A infection diagnosed during 2011–12, a total of 91 were hospitalized children. Genotype ON1 was identified in 7 (25.0%) of 28 children in intensive care units (ICUs) and in 2 (3.2%) of 63 children in other wards ($p = 0.003$, Fisher exact test). Children admitted to an ICU were younger (median 0.2 years) than those not in an

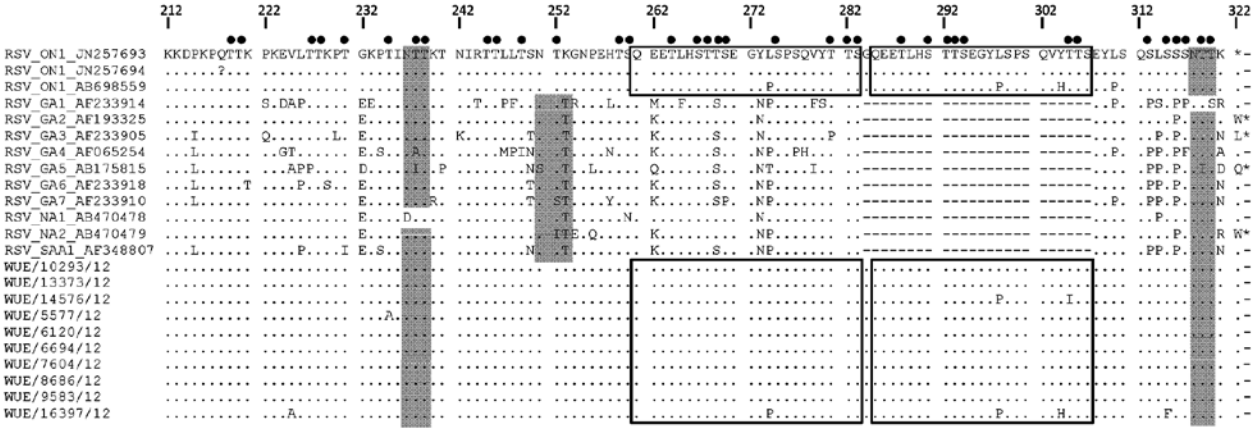

Figure. Amino acid sequence alignment of the second hypervariable region of the respiratory syncytial virus (RSV) G gene. RSV ON1 sequences and RSV A reference sequences in the upper part of the alignment are designated by GenBank numbers. ON1 sequences in the lower part were obtained in this study and are available at GenBank under accession nos. JX912355–JX912364. Black boxes indicate the duplicated region; black circles indicate potential O-glycosylation sites; and gray shading indicates potential N-glycosylation sites. Dots indicate nucleotide identities, dashes indicate adjustment of nucleotide insertions, and asterisks indicate stop codons. WUE, Würzburg.

ICU (median age 1.2 years; p<0.001, Mann-Whitney U test). An exploratory logistic regression analysis on ICU admittance, adjusted for age, confirmed a strong association between RSV genotype ON1 and ICU admittance (adjusted odds ratio 8.4; 95% CI 1.5%–46.6%; p = 0.015). However, this significant difference should be interpreted with caution for 2 reasons: 1) samples from patients in wards other than an ICU originated mainly in the Würzburg area, whereas samples from patients in ICUs were received from pediatric hospitals in various regions of Bavaria; 2) clinical information on patients not in ICUs was not available for assessment of whether the difference persisted when taking into account other risk factors for severe RSV disease.

In summary, the novel RSV A genotype ON1 containing a 72-nt duplication in the G gene was not found during 2010–11, but it constituted already 10.1% of all RSV A strains in a patient cohort from Bavaria, Germany, in the next season, 2011–12. In the context of the primary report of ON1 in Ontario, Canada (*3*), and the GenBank entry from Japan, our data suggest worldwide emergence of ON1. The almost complete worldwide replacement of circulating RSV B genotypes with the BA strain containing a comparable 60-nt duplication, which began in 1999, suggests that these duplications provide a selective advantage (*2*). Thus, molecular analysis of circulating RSV strains should be continued to determine whether ON1 has the potential to replace other RSV A strains in the years to come as did RSV B genotype BA during the past decade.

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Travel-related *Neisseria meningitidis* Serogroup W135 Infection, France

To the Editor: A multinational outbreak of infection with *Neisseria meningitidis* serogroup W135 belonging to the sequence type (ST) 11 clonal complex started in the year 2000 among pilgrims to Mecca, Saudi Arabia, and their contacts and continued in 2001 in countries of sub-Saharan Africa (primarily Burkina Faso) (*1*). Thereafter, infection caused by these isolates decreased (*2*), but quadrivalent meningococcal vaccine (against serogroups A, C, Y, and W135) was recommended for pilgrims and travelers to countries in the meningitis belt of Africa, which spans sub-Saharan Africa from Ethiopia to Senegal. After 2001, infections caused by serogroup A predominated in the meningitis belt, but isolates of serogroup X also emerged (*3*); isolates of serogroup W135/ST11 increased again in Niger in 2010 (*4*).

During January 1–March 11, 2012, >4,000 suspected cases of meningococcal disease caused mainly by serogroup W135 were reported in countries of the African meningitis belt, including Benin, Burkina Faso, Mali, and Côte d'Ivoire (*5*). We present extensive bacteriologic and molecular characterization of *N. meningitidis* W135 isolates from 6 patients with meningococcal disease reported in France since January 2012; we also present typing data from 8 cases of meningitis in Côte d'Ivoire. None of the patients had received meningococcal vaccine.

The cases in France were neither epidemiologically nor geographically linked; 4 were in residents of the Paris region. All cases were linked to recent travel to sub-Saharan Africa by the patient or patient contacts; 4 patients reported recent travel to Benin, Sene-

Table. Characteristics of serogroup W135 *Neisseria meningitidis* isolates from patients in France and Côte d'Ivoire, 2012*

Patient no.	Location†	Date of illness onset	Travel history	Date of return from travel	Patient age/sex	Test site	*N. meningitidis* isolate test results						
							Sero	ST	CC	PorA VR1	PorA VR2	FetA	*penA*
1	France/ Paris region	Jan 14	Benin	2011 Dec 28	1 y/M	CSF	2a	11	11	5	2	F1-1	1
2	France/ Loire	Feb 15	Senegal	Feb 12	62 y/F	Blood	2a	11	11	5	2	F1-1	1
3	France/ Rhône-Alpes	Feb 19	Senegal	Feb 19	53 y/F	Blood	2a	11	11	5	2	F1-1	1
4	France/ Paris region	Feb 27	Mali	Feb 23	4 y/M	AF	2a	11	11	5	2	F1-1	1
5	France/ Paris region	Mar 3	Mali (family)	UNK	5 y/F	CSF	2a	11	11	5	2	F1-1	1
6	France/ Paris region	Mar 6	Senegal (father)	Feb 26	4 mo/F	CSF	2a	11	11	5	2	F1-1	1
7	Côte d'Ivoire/ Kouto	Feb 3	UNK	NA	12 y/M	CSF	2a	11	11	5	2	F1-1	1
8	Côte d'Ivoire/ Korhogo	Feb 3	UNK	NA	18 y/M	CSF	2a	11	11	5	2	F1-1	1
9	Côte d'Ivoire/ Kouto	Feb 23	UNK	NA	7 y/F	CSF	2a	11	11	5	2	F1-1	1
10	Côte d'Ivoire/ Kouto	Feb 24	UNK	NA	65 y/F	CSF	2a	11	11	5	2	F1-1	1
11	Côte d'Ivoire/ Kouto	Feb 24	UNK	NA	1 y/F	CSF	2a	11	11	5	2	F1-1	1
12	Côte d'Ivoire/ Tengrela	Feb 24	UNK	NA	19 y/F	CSF	2a	11	11	5	2	F1-1	1
13	Côte d'Ivoire/ Tengrela	Feb 24	UNK	NA	3 y/M	CSF	2a	11	11	5	2	F1-1	1
14	Côte d'Ivoire/ Tengrela	Feb 24	UNK	NA	5 y/M	CSF	2a	11	11	5	2	F1-1	1

*All patients had meningitis except patients 2 and 3, who had bronchopneumonia and septicemia, and patient 4, who had arthritis. Sero, serotype; ST, sequence type, CC, clonal complex; VR, variable region; UNK, unknown; CSF, cerebrospinal fluid; AF, articular fluid; NA, not applicable.
†Country/region where case was reported.

gal, or Mali (Table). The 2 other cases were in a 4-month-old infant whose father had returned from Senegal 2 weeks before the onset of the disease and in a 5-year-old child who had several family members who visited Mali regularly, although no recent travel was documented. The delay between the return to France and the onset of the disease was <5 days except for 1 patient (17 days).

N. meningitidis isolates were recovered from blood, cerebrospinal fluid, or articular fluid from all 6 patients in France (Table). Two patients had septicemia after bronchopneumonia, but no respiratory samples were available. One patient had arthritis that was also described in his sister, but no samples were available from the sister. Extrameningeal forms of illness caused by W135/ST11 isolates have been described (*6*). For the patients in Côte d'Ivoire, bacteria were isolated from cerebrospinal fluid during weeks 5–8 in 2012; the patients lived in 3 districts of the country (Kouto, Korhogo, and Tengrela). Mean age was 20.9 years (range 0.33–62) for the patients in France and 16.25 years (range 1–65) for those in Côte d'Ivoire.

Molecular typing was performed by multilocus sequence typing (MLST) using the PubMLST database (http://pubmlst.org/neisseria); typing included the 7 usual genes of MLST, PorA variable regions 1 and 2, and *penA* and *fetA* genes. Results were obtained by using cultured bacteria for all but 1 case in France. All isolates from France and Côte d'Ivoire shared the same tested markers. Eight other cases of infection with serogroup W135 were found in France during the same period, but the patients had no travel history, and all isolates showed different markers (M.-K. Taha, unpub. data).

Serogroup W135 strains are widely distributed worldwide; the emergence of these strains during the 2000s corresponded to a clonal expansion of 1 clone within the ST11 complex (*7*). The subsequent decline of W135/ST11

strains was associated with increased isolate diversification (*8*), which suggests a selective restriction of the dominant circulating strain (*2*). In France, the W135/ST11 strain was rare after 2005; no cases were culture confirmed in 2010, and the 2 cases that were confirmed in 2011 showed the FetA2-19 marker. However, isolates from Africa during 2000–2011 frequently showed the FetA1 marker; in sub-Saharan Africa, the decline of W135/ST11 isolates was also associated with isolates showing diversified FetA markers (M.-K. Taha, unpub. data). The reemergence in 2012 of W135/ST11 strains that had the FetA1-1 marker suggests an antigenic shift that may have involved membrane proteins other than FetA or other surface structures, such as the lipooligosasccharide. Such antigenic shifts were associated with increased incidence of serogroup C and serogroup Y meningococcal disease in the United States (*9*). Antigenic shift could be a marker of changes in virulence and transmission of meningococcal isolates. Extensive molecular typing of meningococcal isolates is more likely to detect antigenic shifts and escape variants that may undergo clonal expansion and therefore should be employed in outbreak investigations. Enhanced surveillance was setup in France to identify imported W135 cases.

Our findings indicate that travelers to the meningitis belt of sub-Saharan Africa may be at risk for infection with *N. meningitidis* of serogroup W135. A vaccination campaign using the meningococcal A conjugate vaccine is ongoing in this region (*10*), but a conjugate bivalent vaccine that includes W135 should also be considered. Vaccination of travelers to this region with quadrivalent meningococcal vaccine should be recommended.

Information regarding the patients and their contacts were provided by the clinicians, the French Institute for Public Health Surveillance (wwwinvs.sante.fr), and the Regional Health Agencies of Pays-de-Loire, Rhône-Alpes and Ile-de-France. In Côte d'Ivoire, the work was supported by the Agence Médecine Préventive and the mobile laboratory; patient information was provided by clinicians at health districts and the National Institute of Public Health.

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Clostridium difficile Infection Associated with Pig Farms

To the Editor: *Clostridium difficile* of PCR ribotype 078 causes enteric disease in humans and pigs (*1*,*2*); a recent pan-European study revealed that this type was the third most frequently found type of *C. difficile* (*1*). The finding of identical *C. difficile* PCR ribotype 078 isolates in piglets with diarrhea and in humans with *C. difficile* infection (CDI) led to the suggestion that interspecies transmission might occur (*3*,*4*). Because *C. difficile* can be detected in the immediate environment of pig farms, we investigated intestinal colonization with *C. difficile* in pigs and in pig farmers, their relatives, and their employees in the Netherlands.

Persons living on 32 pig farms were enrolled as part of a longitudinal intervention study of several zoonotic agents. Pig farmers were partly recruited through the Dutch Farmer's Association or by veterinarians, who informed potential participants about the aims of the study. Inclusion criteria for participants were that they should work and/or live on the farm; the farms were either closed farms or multipliers (farms at which piglets are bred and then sold to other farms, where they are raised until ready for slaughter). The number of persons willing to submit a fecal sample per farm ranged from 1 to 10 (mean 4, median 5). Veterinarians who normally provided veterinary services to each farm collected fresh fecal samples from the floors of 10 animal wards per farm. No a priori knowledge of *C. difficile* colonization status of the pigs on the farms was available. Fecal samples from humans and from animal wards were cultured for the presence of toxinogenic *C. difficile* by using previously described methods (*1*,*3*,*4*).

Of the 128 persons who enrolled in the study, 48 had daily contact with pigs, 22 had weekly contact with pigs, and 36 had contact with pigs varying from monthly to less than yearly; no contact information was available for 22 participants. A total of 12 (25%) of 48 persons who had daily contact with pigs had fecal samples positive for *C. difficile* colonization; for persons who had weekly contact with pigs, 3 (14%) of 22 had positive samples. Daily to weekly contact with pigs versus monthly to less than yearly contact was significantly associated with an intestinal presence of *C. difficile* ($p = 0.003$). *C. difficile* was also found in fecal samples from 3 persons for whom no contact information was available. The *C. difficile* carriage rate among those with daily to weekly contact with pigs (15/70, 21%) was higher than the carriage rate of <5% reported for nonhospitalized adults with CDI (*5*).

A total of 18 *C. difficile*–positive human samples were detected at 16 of 32 pig farms investigated. At 2 of these farms, only 1 person submitted a sample, but at the other 14 farms, the number of participants ranged from 2 to 9 (mean 4, median 3). *C. difficile* was found in pig manure at all farms; 10%–80% of the wards were positive per farm.

Corresponding *C. difficile* PCR ribotypes were cultured from samples from pigs and humans; type 078 was found in humans and pigs on 15 farms and type 045 in a farmer and his pigs on 1 farm. Multilocus variable number tandem repeat analysis (MLVA) and antimicrobial drug susceptibility testing (E-test) were performed on human isolates from 15 farms and 1 porcine isolate per farm. One human isolate could not be typed because the isolate was lost during laboratory activities.

MLVA results showed that, at 2 farms, the human and porcine isolates were not genetically related, whereas at the other 13 farms, human and porcine isolates were genetically related, including 100% identical MLVA results for type 078 human and porcine isolates at 3 farms. Isolates were considered genetically related when the summed tandem repeat differences were <10 (*3*,*4*,*6*).

Antimicrobial drug susceptibility testing demonstrated similar susceptibility levels among isolates. For human and porcine isolates from 9 of 15 farms, MIC variability of <1 μg/L was found for imipenem, cotrimoxazole, erythromycin, clindamycin, tetracycline, and moxifloxacin. For the remaining 6 farms, drug susceptibility patterns for human isolates differed from pig isolates for 1 drug only: for 1 farm, MICs of erythromycin were 256 μg/L for human isolates and 0.38 μg/L for pig isolates; for 3 farms, MICs of erythromycin were 256 μg/L for pig isolates and 0.25 μg/L for human isolates; and for 2 farms, MICs of imipenem were 32 μg/L for pig isolates and 1.5 or 2 μg/L for human isolates.

In summary, the high *C. difficile* carriage rate among persons who had direct contact with pigs and the fact that these *C. difficile* isolates were genotypically and phenotypically similar to the pig isolates from the same farms indicates that transmission occurs either by direct contact or through the environment. Prospective studies are needed to determine the relationship between *C. difficile* carriage and development of CDI in this population.

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Prolonged Incubation Period for *Cryptococcus gattii* Infection in Cat, Alaska, USA

To the Editor: We report a case of *Cryptococcus gattii* infection in a 12-year-old neutered male cat in Alaska. The cat traveled to Anchorage, Alaska (61°N) from San Diego County, California, with its owner in August 2003. Although *C. gattii* has not been detected in Alaska (where only extremely limited sampling has occurred) or above 49°N (i.e., Vancouver Island, British Columbia), the case suggests that the incubation period for *C. gattii* could be >8 years.

In September 2010, the cat was brought to a veterinary clinic in Anchorage because of facial pruritis and excoriation. The cat did not respond to treatment with methylprednisolone acetate and was referred to a veterinary dermatologist in October. At that time, alopecia, thick scaling, and excoriations were observed on ear margins, sides of the head, and between the eyes. The hair coat was sparse, and there was minimal scaling near the tail and lower legs. All foot pads were excessively cross-hatched and scaly. Cytologic analysis of the skin on the head and pinna showed neutrophils and cocci overgrowth. Skin scrapings were negative for mites. Cytologic analysis of the ears did not identify yeast bodies, parasites, or bacteria. A long-acting antimicrobial drug for treatment of skin infections (cefovecin) was given.

Biopsy specimens were obtained from the head, ears, and paws, and analysis of these samples supported a diagnosis of mural folliculitis and mild plasma cell pododermatitis. Chest radiograph findings and results of routine blood analysis were not unusual. Test results for feline leukemia virus and feline immunodeficiency virus were negative. Prednisolone (1.8 mg/kg/d) was given; the cat showed a good response and eventual resolution of scaling. Hair grew back but the steroid dose could not be reduced to <2.5 mg/d because periodic increases were needed when symptoms flared.

In November 2011, the cat was brought back to the veterinary clinic because of worsening of the skin condition even though the owner had increased the prednisolone dose to 7.5 mg/d during the previous 3 weeks. The cat had also started shaking its head frequently and had a unilateral right nasal discharge. Major nasal discharge had not been a symptom previously reported by the owner. Cytologic analysis of the discharge showed large yeast bodies consistent with a *Cryptococcus* sp. interspersed among neutrophils, cocci, and rods.

The cat was treated with fluconazole, and prednisolone was slowly decreased to minimal doses to control the mural folliculitis. After consultation with the Alaska Office of the State Veterinarian and Division of Public Health, a nasal swab specimen was sent to the Centers for Disease Control and Prevention (CDC) (Atlanta, GA, USA) for confirmation and molecular typing. Since 2009, Alaska has participated in the CDC-led Pacific Northwest *C. gattii* working group, which has been interested in enhanced surveillance for *C. gattii* (*1,2*). CDC identified the isolate as *C. gattii* molecular type VGIII; this type is commonly reported in the southern United States, particularly California (*3*).

Given what is known about the potential for dispersal of *C. gattii* (*4*), the owner was extensively interviewed about travel history of the cat and household members, and any travelers to or visitors from California who may have brought items into the house. The cat was rescued as a stray at ≈1 year of age in California, and after traveling to Anchorage had lived as an indoor/outdoor cat without further travel. The family had not transported or received organic materials from California, except for an elongated (≈45 cm) seedpod. All plants and potting soil had been bought locally from national chain vendors. In May 2012, fifteen environmental samples, including soil from the yard, commercial potting soil, and a planter made from the seedpod brought from California, were taken from the home of the cat; all showed negative results for *C. gattii* when tested at CDC.

In humans, the average incubation period for *C. gattii* infection is 6 weeks–13 months (*5–7*). Therefore, case-patients are usually asked to recall potential exposures during the 13 months before symptom onset (*5*). Although most reported cases of *C. gattii* infection appear to be primary infections, infrequent reports of *C. gattii* infections in immunocompetent persons have described symptoms occurring several years after likely exposure, which suggests that *C. gattii* may have a greater capacity to remain dormant than believed (*8–10*). Incubation periods are not well described for animals but are generally reported as 2–11 months (R. Wohrle, pers. comm.). For either animal or human case-patients living in disease-endemic areas, precise incubation periods are likely incalculable because potential exposure to fungi is ongoing.

We suspect that *C. gattii* infection in this cat resulted from a distant exposure in California. Although the cat left California >8 years ago, it recently became immunosuppressed by medication that may have altered host factors, thus enabling latent fungi to clinically manifest. Results of limited environmental sampling in Alaska were negative. However, the molecular subtype supports California as a source, although the possibility that something or someone from California could have acted as a fomite could not be ruled out. Reactivation of a previous infection could also not be ruled out because the cat was a stray for the first year of its life.

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James Barsness (b. 1954) *My Valley* (2003–2005) (Detail) Acrylic, ink on paper mounted on canvas (125.7 cm x 171.5 cm) Copyright courtesy of the artist and George Adams Gallery, New York

More Is More

Polyxeni Potter

"For certain more curious and disenchanted spirits, the pleasure of ugliness comes from an even more mysterious sentiment, which is a thirst for the unknown and a taste for the horrible," wrote Charles Baudelaire. "It is this sentiment, the germ of which all of us carry inside to a greater or lesser degree, that drives certain poets into clinics and anatomy theaters, and women to public executions."

This taste for "ugliness" seems to also drive Jim Barsness' exploits into the absurd and horrific, the landscape of nightmares rife with physical and moral decay. Taken to excess on the artistic canvas, ugliness and the absurd have been characterized as maximalism, a modern movement in literature and the arts that celebrates richness, decoration, sensuality, luxury, and fantasy. A genre that thrives on redundancy and overt accumulation, maximalism emphasizes a creative process or art-making that is also laborious and cumulative. "I use paper mounted on canvas because I like that sense of it being really hard to get the image ground down through all the layers of stuff. It becomes indelible."

"The first time I did anything in art that had any profound consequences was to Draw Winky." That was in sixth grade at a cartoon drawing contest. Sponsored by the Famous Artists Institute in Minneapolis, the contest was advertised on matchbook covers and comic books and invited children to draw a lumberjack or a baby deer. The drawing won Barsness a scholarship at the Institute's correspondence school. His roots in colloquial drawing, doodling, and pop culture remain strong in many of his pieces, where comic strips provide the background for figures, showing his penchant for poking fun at sacred cows in society and in art. "My art partly has to do with my crackpot ideas about life and living." These ideas lay out aspects of human behavior in mocking and irreverent scenes often filled with beastly or hybrid characters. "I try to hold myself to certain big ideas, but if something wants to go in another direction, I tend to let it go that way."

"My most recent work is about finding, or knowing, my place," the place where "we all belong" or fit in, where we know the rules, Barsness says. This all-important "place" is in various locations: the West, Bozeman, Montana, where he was born; Idaho and California, where he grew up and was educated; Athens, Georgia, where he lives and teaches art; Cortona, Italy, where he visits and works. Search for this metaphorical place also featured in Barsness' decision to become an artist instead of following his other inclination, writing or storytelling. "It was a tossup. I wanted to be both." Now "I can't describe my drawings like stories. I start and try, but I can't finish." "I'm too distractible for the kind of complete narrative storytelling of, say, a novelist." "And that's the whole idea because I want people to supply the stories." It is about story evoking rather than story telling.

"I'm interested in how groups of people interact in a continuous environment like a town," Barsness explained in Monster's Progress, a collection of his works. "It's primarily about suppression of our natural interests for the good of the whole. I think it's that moment of suppression that initiates much of our creative energy." In exploring social interactions, Barsness is guided by psychology and mythology to create narratives with many layers and ramifications. He draws from eastern and western artistic traditions and taps multiple sources, from comic strips and fairy tales to Mughal miniature painting, from graffiti and medieval illuminated manuscripts to Tibetan sacred painting, from Pieter Bruegel the Elder and Hieronymus Bosch folk iconography to pop art. The result is humanity in a labyrinth of situations, puzzling, revealing, shocking.

"Ask a toad what beauty is, true beauty…. He will tell you that it consists of his mate, with her two fine round eyes protruding from her small head, her broad flat throat, her yellow belly, and brown back," writes Umberto Eco in his book On Ugliness. The beholder or the situation, he argues, is judge. Situational ugliness is quite common. Along the

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same lines, imagine being in a familiar room, with a nice lamp sitting on the table. Suddenly the lamp floats upwards in midair. Though the room and the lamp are still the same, the situation has become unusual, disturbing. Something is wrong. This notion of the uncanny (the strange, the unsettling or sinister) is how Barsness creates the context for his ideas in *My Valley*, on this month's cover.

"*My Valley* was inspired by my experience of growing up in suburbia, specifically Boise, where the new subdivisions are at the foot of the mountains, built right at the edge of a massive area of what's left of wild America. But as I remember, there was absolutely nothing going on during the day in the suburbs. It was like a ghost town. There seemed to be nothing and nobody around." Barsness captures the nothing and nobody in empty streets, ending in cartoonish cul-de-sacs and cookie cutter houses on their green lawns in the piedmont. This idyllic community, set against a stylized mountain range, casts colorful homes in suburban congeniality. Yet something is amiss. The inhabitants of the foothills are nowhere to be seen. The bland pleasantness of the homes and the monotony of space with its peculiar ambivalence fill the viewer with uneasiness.

Inside the painting's intricate border, there is an eerie quiet. Uniformly shaded shutterless windows obscure the home interiors, but it is clear that no one is there, and this certainty adds a haunted quality to the scene. No children's toys litter lane or lawn, and there are no vehicles. No neighbor looks over the fence nor old person peeks from behind a curtain. No dog or cat or squirrel in sight. An abandoned development or simply a bedroom community, this town is vacant. A seeming lack of substance gives the image its dramatic force.

Conversion of wilderness areas to agricultural or other commercially viable lands (housing, dams, mining) as a result of global socioeconomic and environmental changes in recent years adds a new angle to Barsness' psychological profile of suburbia. Despite the periodic calm and emptiness, much is actually happening in the periphery of those seemingly vacant enclaves in the foothills: expanded demand for natural resources, deforestation, ecosystem disruptions, demographic pressures, increased urbanization, intensified crop and animal production, population movements. Expanding markets and farms bring diverse species together, facilitating exchange of microbes. The uncanny, so effectively captured in Barsness' painting, becomes even more disquieting seen within a global context. Like the artist's more traditional maximalist creations, this community on the edge of the wilderness is part of modern life's complexity and intrigue.

As people move out of metropolitan areas unwittingly encroaching on the wilderness, they are exposed to and come in contact with wildlife. Animals move into human neighborhoods or are brought in through wildlife trade. And this neighborliness back and forth is not without consequences. Humans are exposed to wildlife microbes, which sometimes make the leap from animal to human hosts. The role of bats and civet cats in SARS, wild waterfowl in avian influenza A(H5N1), and infected birds in West Nile virus infection points to community–wildlife interaction as an effective conduit for zoonotic microbes to enter new niches.

In *My Valley* as in many other works, Barsness includes elements for which he offers no explanation. "I like obsessive detail…. I come from the direction that everything is significant. You have to pretend that nothing matters while proceeding as if everything matters." Fine, draft-like lines and arrows throughout the painting outline some subliminal complexity. These linear parameters, dotted and stamped in fine print and discreetly crisscrossing the canvas, invite metaphorical interpretation. Health emergencies in the past 50 years, among them flu pandemics, anthrax attacks, and a SARS outbreak, have prompted planning and emergency response efforts within the global public health community, a seamless underlying safety network against future crises.

In art, the tension generated by expansive inclusion of elements, the absurd and even ugliness and the uncanny, promotes understanding that may otherwise be lost. Likewise in public health, where an endless supply of unseen creatures, as monstrous and horrific as any found in science fiction, the art of Hieronymus Bosch, or Jim Barsness' imagination, await the opportunity to wreak havoc. Extensive, even obsessive, public health planning is required. "More is more" takes on a new meaning as the wild, the repugnant, and the horrific, meet the banal and the fortuitous in nature.

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Reduction of Visceral Leishmaniasis by Insecticide Impregnation of Bed-Nets

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Article Title

Iatrogenic Blood-borne Viral Infections in Refugee Children from War and Transition Zones

CME Questions

1. You are a public health consultant to a clinic for refugee children from central Asia, Southeast Asia, and Sub-Saharan Africa. Based on the review by Dr. Goldwater, which of the following statements about observations regarding and factors contributing to iatrogenically transmitted blood-borne virus (BBV) infection is most likely to appear in your report?

A. There is extensive literature documenting and characterizing iatrogenic transmission of BBV infection
B Several good studies have proven that refugees are at greater risk of BBV infection than others living in conflict zones
C In iatrogenic cases, vertical transmission of BBV was ruled out by the mothers' negative serostatus and no history of surrogate breastfeeding or sexual abuse
D Virus co-infection was not reported in the 4 cases of presumptive iatrogenic BBV infection described by the authors

2. Based on the review by Dr. Goldwater, which of the following statements about the role of contaminated injections and unsafe blood transfusions in contributing to increased prevalence of BBVs in refugee children is most likely correct?

A. According to the World Health Organization (WHO), about 5% of injections given in Sub-Saharan Africa in 2000 were administered unsafely
B Widespread screening of blood transfusions in developing countries has prevented HCV transmission by this route
C. HIV transmission to babies and children through unscreened blood transfusion has not been reported in Kazakhstan
D. Central Asia's geographic position along major drug trafficking routes has led to rapidly increasing intravenous drug use and corruption of the blood supply

3. Based on the review by Dr. Goldwater, which of the following statements about other factors contributing to increased prevalence of BBVs among refugee children from central Asia, Southeast Asia, and Sub-Saharan Africa would most likely be correct?

A. Political collapse and civil war do not play a significant role in fostering iatrogenically transmitted BBV infection
B. HIV positivity in children of HIV-negative mothers is not linked to breastfeeding practices
C Modes of transmission in HIV-infected African children with HIV-uninfected mothers are well documented
D. Wartime rates of rape and sexual violence are much higher than those in peacetime, and prostitution among refugee children also fosters HIV transmission

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title
Foodborne Botulism in Canada, 1985–2005

CME Questions

1. You are an infectious disease consultant to a public health department in Canada regarding the likelihood of botulism outbreaks. Based on the laboratory database study by Dr. Leclair and colleagues, which of the following statements about the incidence of laboratory-confirmed outbreaks of foodborne botulism occurring between 1985 and 2005 in Canada is most likely to appear in your report?

A. There were 91 laboratory-confirmed outbreaks of foodborne botulism, involving 205 cases
B The annual rate of confirmed outbreaks throughout Canada was significantly lower than that reported for 1971–1984
C. About half of the outbreaks occurred in native communities
D. Outbreaks are less likely among the Nunavut than in Canada as a whole

2. Based on the laboratory database study by Dr. Leclair and colleagues, which of the following statements about pathogens and sources of laboratory-confirmed outbreaks of foodborne botulism occurring between 1985 and 2005 in Canada is most likely to appear in your report?

A. *Clostridium botulinum* type B was the most common pathogen
B. Commercial ready-to-eat meat products were the most common source
C. None of the outbreaks involved food served in restaurants
D. Continuous consumer education is needed on the potential risks of botulism from home-prepared foods, use of a pressure canner, and proper food storage temperatures

3. Based on the laboratory database study by Dr. Leclair and colleagues, which of the following statements about outcomes of laboratory-confirmed outbreaks of foodborne botulism occurring between 1985 and 2005 in Canada would most likely be correct?

A. No change in case identification from the period before 1985 resulted in no change in the case fatality rate
B. Of the 205 reported cases, 11 died
C. Of 3 cases in pregnant women, 1 resulted in stillbirth
D. Antitoxin administration was not associated with reduction in hospital length of stay in type E cases

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.