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EMERGING INFECTIOUS DISEASES

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On the Cover

Rembrandt van Rijn (1606–1669)
The Rat Catcher (1632)
Etching (13 cm × 14 cm)

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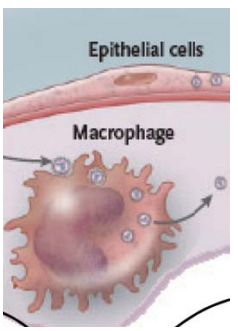

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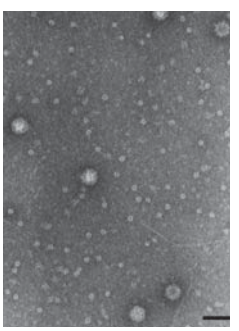

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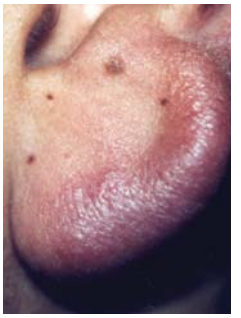

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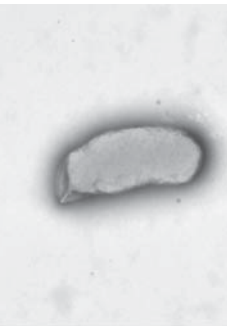

Letters

About the Cover

Global Poverty and Human Development

Emerging Infectious Diseases, along with more than 200 other science journals, is dedicating its October 2007 issue to the theme Global Poverty and Human Development. Emerging infections linked to global poverty are examined in the following articles:

Global Public Health Security; Preparedness for Highly Pathogenic Avian Influenza Pandemic in Africa; Plague Reappearance in Algeria after 50 Years, 2003; HIV and Tuberculosis in Ho Chi Minh City, Vietnam, 1997–2002; Epidemiology of Schistosomiasis in the People's Republic of China, 2004; Dengue Fever Seroprevalence and Risk Factors, Texas–Mexico Border, 2004; Cost-effectiveness of Algorithims for Confirmation Test of Human African Trypanosomiasis; Public Transportation and Pulmonary Tuberculosis, Lima, Peru; Prevalence of *Plasmodium falciparum* Infection in Rainy Season, Artobonite Valley, Haiti, 2006; Evaluating Tuberculosis Case Detection in Eritrea; West Nile Virus Infection among the Homeless, Houston, Texas, USA; *Schistosoma haematobium* and *S. mansoni* among Children, Southern Sudan; Influenza A and B Infection in Children in Urban Slum, Bangladesh; Identification of Rickettsiae, Uganda and Djibouti; and Skin and Soft Tissue Infections and Vascular Disease in Drug Users, England.

Global Public Health Security

Guénaël Rodier,* Allison L. Greenspan,†
James M. Hughes,† and David L. Heymann*

"When the world is collectively at risk, defense becomes a shared responsibility of all nations."
——Dr. Margaret Chan, Director General, World Health Organization; World Health Day 2007

The framework of the newly revised International Health Regulations is a key driver in the effort to strengthen global public health security. Unanimously agreed upon by the World Health Assembly on May 23, 2005, the regulations are the result of experience gained and lessons learned during the past 30 years. This global legal framework includes a commitment from the World Health Organization (WHO) and from each WHO member state to improve capacity for disease prevention, detection, and response. It provides standards for addressing national public health threats that have the potential to become global emergencies. Its success will rely on the capacity and performance of national public health systems, anchored by strong national public health institutes (NPHIs). The new International Association of National Public Health Institutes aims to strengthen and invigorate existing NPHIs, to create new NPHIs where none exist, and to provide funded grants to support NPHI development priorities.

In the wake of the 2003 outbreak of severe acute respiratory syndrome (SARS), preparedness for public health emergencies was propelled into worldwide consciousness. The appearance and rapid international spread of SARS demonstrated to all—including global leaders, ministers of health, prime ministers, and heads of state—how an infectious disease can rapidly cross borders and deliver health threats and economic blows on an unimaginable scale (*1*,*2*). Since then, the entrenchment of highly pathogenic avian influenza virus (H5N1) in poultry flocks of Asian countries, and the spread of the virus across Europe and into Africa, has put the world on high alert for an influenza pandemic and affirmed the urgency of strengthening public health systems and capacity worldwide (*3*,*4*).

Compounding the challenges of threats to public health security from new and reemerging infectious diseases and the concerns about intentional dissemination of chemical or biological substances are the challenges of ensuring individual health security. These latter challenges include the unfinished agenda of broadening access to the drugs, vaccines, and other interventions needed to control endemic diseases such as malaria, acute lower respiratory tract infections, diarrheal diseases, measles, and tuberculosis, as well as to address the ongoing problems of HIV/AIDS, neglected tropical diseases, humanitarian emergencies, and global environmental changes.

The scale, range, and complexity of these modern challenges to health security call for new approaches of comparable dimension and strength. Protecting the world

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from transnational health threats demands a global public health perspective and investment in global public health infrastructure. The theme of this year's World Health Day and the World Health Report 2007 is "Global public health security—the need to reduce the vulnerability of people around the world to new, acute, or rapidly spreading risks to health, particularly those that cross international borders" (*5*). With a call to all nations to "invest in health, and build a safer future," the World Health Organization (WHO) emphasizes the need for collaboration among nations to increase our collective capacity and infrastructure to respond to potential international health emergencies and other public health risks. As recent events have shown, global public health security is a complex, costly, and information-intense undertaking that requires strong national public health leadership and infrastructure, cross-border collaboration, capacity to identify problems rapidly and design real-time evidence-based solutions, well-trained and well-equipped workforces, well-functioning laboratories and service-delivery systems, capacity to sustain interventions, and ability to respond to unexpected events (*5*,*6*). Investment in these elements will strengthen not only global public health security but also the infrastructure needed to help broaden access to healthcare services and improve individual health outcomes, which would help break the cycles of poverty and political instability and thus contribute to national economic development and achievement of the Millennium Development Goals (*7*).

A key driver in the effort to strengthen global public health security is the framework of the newly revised International Health Regulations (IHR [2005]) (*8*), the legally binding global agreement designed to build and strengthen national alert and response systems. Unanimously agreed upon by the World Health Assembly on May 23, 2005, the regulations are the result of experience gained and lessons learned about global public health security over the past 30 years. This global legal framework constitutes a "major development in the use of international law for public health purposes" (*9*). It includes a commitment from WHO and from each of its 193 member states to improve capacity for disease prevention, detection, and response and provides ground rules to address national public health threats that have the potential to become global emergencies. The adoption of the new regulations ended a 10-year process of revision, stimulated by the pneumonic plague outbreak in India in 1994 (*10*) and the Ebola hemorrhagic fever outbreak in the former Zaire in 1995 (*11*). The revised regulations have now entered into force for all WHO member states.

New Times, New Requirements

The revised regulations reflect a growing understanding that the best way to prevent the global spread of diseases is to detect and contain them while they are still local. WHO member states have obligations to rapidly assess and alert the global community about potential disease threats as well as to prevent and control the spread of disease inside and beyond their borders. Compared with the previous regulations, adopted in 1969 (*12*), IHR (2005) expands the scope of internationally reportable diseases and events, provides criteria for identifying novel epidemic events, and specifies conditions for involvement of the international community in outbreak responses. The revision includes the following 5 substantive changes.

Expanded Scope

The previous regulations applied to only 3 infectious diseases: cholera, plague, and yellow fever. IHR (2005) reflects shifting concepts about disease control, shaped by recent and impending disease threats and the experiences of the past 2 decades in detecting and responding to disease outbreaks. The emergence and reemergence of a cascade of infectious diseases fueled by globalization and international travel (*13*), the threat of biological terrorism, and novel environmental threats (*14*) have spotlighted the need for heightened vigilance and increased capacity to recognize and manage public health risks and emergencies. The appearance and rapid international spread of SARS and the pandemic potential of circulating avian influenza (H5N1) strains—with their combined health and economic effects—confirmed the inapplicability of the 1969 IHR to most emerging and reemerging infectious diseases.

The revised regulations replace the previous disease-specific framework with one built on timely notification of all events that might constitute a public health emergency of international concern, taking into account the context in which an event occurs (*15*). The advantage of this approach is its applicability to existing threats as well as to those that are new and unforeseen. The regulations also recognize the existence of threats to public health outside the infectious disease context, such as those associated with natural disasters, industrial or chemical accidents, and other environmental changes, which might cross international borders.

Decision Instrument and Notification

Expanding the scope of the IHR beyond reporting of 3 diseases to reporting of any public health emergency of international concern required an algorithm to assist in identification of such events. The resulting decision instrument (see [*8*], Annex 2, p. 43) identifies a limited set of criteria for use by member states for fulfilling the obligation to determine whether an event occurring within their territory might constitute a public health emergency of international concern and therefore require formal notification to WHO within 24 hours of assessment. Essentially, the events that must be reported are those that fulfill at least 2 of the following criteria:

- Is the public health impact of the event serious?
- Is the event unusual or unexpected?
- Is there a significant risk of international spread?
- Is there a significant risk of international trade or travel restriction?

To facilitate the use of the decision instrument, which requires some judgment to answer each of the questions, Annex 2 of the Regulations provides specific examples of events that might constitute a public health emergency of international concern. In addition to this broad scope for notification, IHR (2005) includes a list of diseases for which a single case must be reported to WHO immediately, regardless of the context in which the disease occurs. This list includes smallpox, poliomyelitis due to wild-type poliovirus, human influenza caused by a new subtype, or SARS. In addition, an event involving certain other diseases (e.g., cholera, pneumonic plague, yellow fever, viral hemorrhagic fevers) calls for a careful evaluation using the decision instrument to determine whether notification is indicated. The need for recognition of specific diseases requires adequate diagnostic laboratory capacity.

After an event is reported, only the Director General of WHO can determine whether the event formally constitutes a public health emergency of international concern. However, the Director General shall first consult with the affected state party and hear the view of the emergency committee. This committee, composed of experts from the newly established IHR roster of experts, is specifically set up to review a reported event and provide advice to the Director General on whether an event constitutes a public health emergency of international concern and whether a temporary recommendation must be issued. On request, WHO will be able to provide technical support to affected countries, including the mobilization of the Global Outbreak Alert and Response Network.

Focal and Contact Points

A third innovation under IHR (2005) is the requirement for member states to designate "national IHR focal points" as the operational link for notification and reporting to WHO and for WHO to name corresponding "IHR contact points." Effective communication between these 2 organizational entities will be central to the rapid management of a possible public health emergency of international concern. IHR focal points, or their designees, are required by IHR (2005) to be accessible at all times.

National Core Surveillance and Response Capacities

Experiences during the past several years have shown that public health emergencies expose the weaknesses and vulnerabilities of national and subnational public health infrastructure. The fourth change calls for member states to develop, strengthen, and maintain core capacities to 1) detect, assess, notify, and report disease events, and 2) respond promptly and effectively to public health risks and public health emergencies of international concern. State parties are required to complete a capacity assessment within 2 years of the revised IHR entering into force and, after this assessment, to develop public health infrastructure and human resources that ensure full compliance within 5 years of the IHR entering into force. This assessment must lead to the development of national action plans to meet the core capacity requirements that Annex 1 of the Regulations specifies for different levels (i.e., local community or primary, intermediate, and national public health response) as well as designated airports, ports, and ground crossings. For these national points of entry, IHR (2005) also introduces special provisions for travelers, including the obligation to treat them with respect for their dignity, human rights, and fundamental freedom.

WHO Support

WHO is required to assist all member states in fulfilling the new obligations. On request, WHO will collaborate with countries to evaluate their public health capacities and facilitate technical cooperation, logistical support, and mobilization of financial resources for strengthening capacity in surveillance and response. Countries will build on existing national or regional strategies such as the Asia Pacific Strategy for Emerging Diseases in WHO's Southeast Asia and Western Pacific Regions (*16*) and the Integrated Disease Surveillance and Response strategy in the African Region (*17*). In many countries, national action plans can also build on the influenza pandemic preparedness plans developed with WHO's guidance. Specific WHO guidelines and initiatives, particularly in the areas of external quality assessment for laboratories, data gathering and analysis at the health district level, and the central and coordination functions of national public health institutes, are being developed. WHO's Lyon Office for National Epidemic Preparedness and Response is specifically dedicated to supporting countries in meeting the core national capacity requirements of IHR (2005).

Under IHR (2005), new powers for WHO include an information-gathering responsibility that is not limited solely to official state notifications or consultations but covers all available scientific evidence and other relevant information. WHO can consult nonofficial reports and require countries to collaborate with a request for verification. WHO is also empowered to recommend and coordinate measures that will help contain the international spread of disease, including public health actions at ports, airports, and land borders, and on means of transportation that involve international travel.

Critical Role of National Public Health Institutes

Because weak national public health capabilities undermine efforts to strengthen global public health security, IHR (2005) imposes substantial responsibilities on countries to improve public health capacity and infrastructure. However, despite the broad new goals included in IHR (2005), improvements in global public health security will depend on what member states are actually able to do. Success will rely on the capacity and performance of national public health systems (*15*), anchored by strong national public health institutes (NPHIs). Low-resource countries, which are particularly vulnerable to emerging threats, will be particularly challenged by the IHR (2005) requirements and the need to ensure an appropriate and coordinated public health response to health emergencies.

Many countries have been well served by centralizing their public health expertise and activities within 1 institution or network of institutions that provides leadership and coordination for public health (*18;* unpub. data). Examples include the US Centers for Disease Control and Prevention, the National Public Health Institute of Finland, and the Chinese Center for Disease Control and Prevention. These NPHIs are usually governmental or quasi-governmental agencies with a central focus and organizational structure that allow coordination of national public health service delivery and ensure a country's ability to detect, investigate, and respond to public health emergencies. The core functions of an NPHI have been defined (unpub. data) and include evaluation and analysis of health status; public health surveillance, problem investigation, and control of risks and threats to public health; and public health research.

Given the scope and range of their activities, NPHIs are a vital asset to health development and security and will have a critically important role in implementing IHR (2005), whether as national focal points or as operational partners in fulfilling the requirements of the regulations. Unfortunately, however, many countries still either have no NPHIs or have institutes with severely limited capacities and capabilities relative to the need. Even in countries with strong NPHIs, unpredictable and rapidly evolving health threats can quickly overwhelm capacity and inhibit a timely and complete response.

A new organization, the International Association of National Public Health Institutes (IANPHI, www.ianphi.org), was created to address these gaps through the enhancement and proliferation of NPHIs throughout the world. Founded in 2006 by 39 NPHI directors who recognized the importance of strong national public health capacity and the mutual benefits of shared information, experience, and expertise, IANPHI aims to be a catalyst for sustained improvements in public health capacity and infrastructure globally. With the partnership of WHO and funding first from the Rockefeller Foundation and now from the Bill and Melinda Gates Foundation, the organization focuses on strengthening public health capacity in low-resource countries by strengthening NPHIs and on providing tools and a context that will support all NPHIs. IANPHI is also a professional association for NPHI directors; it fosters leadership development and advocacy for public health and collaborates with WHO.

Since early 2006, the founding members have continued to expand the network and put their shared vision into practice. IANPHI is managed by an executive board and a secretariat located both in Finland and in the United States. With nearly 50 current members and an ambitious agenda for collaboration, service, and growth, IANPHI is committed to a vision of a robust and fully integrated global network of NPHIs equipped to address critical public health challenges. Its mission is to strengthen and reinvigorate existing NPHIs, create new NPHIs where none exist, and provide funded grants to support NPHI capacity development priorities.

IANPHI achieves its service mission through a 3-part approach of advocacy, technical assistance, and linkages. IANPHI advocates for NPHI development and proliferation through partnerships with key global health organizations, such as WHO. Through these partnerships, IANPHI ensures that NPHIs are considered in major global public health initiatives and that public health and the work of NPHIs are included in efforts to strengthen health systems.

Assistance to NPHIs in low-resource countries is provided through 3 grant programs. A short-term technical assistance program helps countries quickly resolve priority gaps in NPHI capability and infrastructure. A medium-term capacity-building program helps NPHIs address high-priority needs for up to 3 years. IANPHI's long-term grant program, the most intensive of the assistance efforts, will help create NPHIs in low-resource countries that currently lack a central public health focus. With funding from a $20 million grant from the Bill and Melinda Gates Foundation, the organization is committed to implementing 60 NPHI development projects by 2011.

As of June 2007, IANPHI had awarded technical assistance grants to public health institutes in 5 nations. The new awards include 3 short-term grants to NPHIs in Thailand, Uganda, and Iran to support training and infrastructure development. A medium-term grant to the Nigerian Institute of Medical Research will support sustainable improvements in disease surveillance, outbreak investigation, and emergency preparedness and strengthen linkages with other groups working to advance public health in the country; special focus will be on public health laboratory capacity building and integration of surveillance, epidemiology, and laboratory programs. Colombia's Institutos Nacional de Salud was awarded a medium-term grant to establish a pilot chronic disease study site to generate, collect, and dissemi-

nate chronic disease data by using multiple mechanisms. The activities are designed to yield a sustainable network of surveillance and research sites to guide national-level public health decision making.

The cornerstone of the IANPHI approach is a peer-assistance model for NPHI strengthening and enhancement, with an emphasis on countries without NPHIs or with NPHIs in their early stages. Experts from IANPHI member institutes provide technical assistance and project support targeted at critical NPHI needs. Teams are guided by the Framework for the Creation and Development of NPHIs (www.ianphi.org/?action=arkisto&RYHMA=47&ID=&valittu=8), a product of IANPHI in partnership with WHO. The Framework provides a working definition of an NPHI and suggests a process for creating or enhancing an institute. By defining the critical characteristics of an NPHI, IANPHI hopes to bring specificity to the organization's vision, align efforts to assist low-resource countries in building NPHIs, and provide benchmarks and resources to help any country assess and improve the functioning of its NPHI. To that end, IANPHI has also developed an NPHI toolkit (www.sph.emory.edu/IANPHI), which provides access to a variety of Web-based information resources for countries, NPHIs, and IANPHI peer-assistance teams to use as they work to assess, develop, and improve NPHIs and build public health capacity around the world.

Through strategies to define and develop core public health functions and to share expertise, IANPHI helps NPHIs sharpen their focus and raise standards of performance. IANPHI also links NPHIs through annual meetings, regional events, leadership development activities, research seed grants, and communication outlets including a website, newsletter, and listserv. By fostering an international community of public health leadership, IANPHI helps NPHIs gain the benefits of shared information, experience, and expertise to address public health threats and opportunities. Through its grant program and other activities, IANPHI aims to help national governments develop organizational infrastructures to devise and implement comprehensive public health priorities, meet global public health goals, develop workforce capacity, effectively absorb donor funds, address emerging threats, and improve the health of their populations (*18*).

Conclusions

In today's global environment, every country confronts similar challenges in keeping its population healthy and preventing the cross-border spread of disease. SARS demonstrated this dramatically in 2003, and the ongoing challenges posed by avian influenza have focused attention on the need for global pandemic influenza preparedness. Polio has reemerged in countries that had virtually eradicated it, while HIV/AIDS and other diseases continue to threaten the stability of communities around the world. Recent examples of emerging and reemerging diseases of global significance are the resurgence of dengue in tropical and subtropical areas of the world; the spread and establishment of Japanese encephalitis and West Nile viruses in new habitats and environments; and the reoccurrence and spread of chikungunya virus in India, East Africa, and several Indian Ocean islands (*19*,*20*). As life expectancy increases worldwide, issues related to noncommunicable conditions are also becoming increasingly common to all. By working within the collaborative framework provided by IHR (2005), countries can benefit through improved national and international surveillance; improved systems for rapid detection of and response to public health emergencies; standardized rules for evaluation, control, and resolution of urgent events; and mechanisms to increase national and local public health security.

Nonetheless, the success of IHR (2005) and other global public health initiatives such as the Millennium Development Goals depends on strong national public health systems with competent, well-trained staff and well-equipped facilities. By targeting the core of public health systems, especially in low-resource countries that currently lag behind in public health capacity and infrastructure, IANPHI will play a key role in improving the capacity of countries to effectively detect, investigate, and respond to public health emergencies. The result will be better control of endemic diseases such as HIV/AIDS, acute lower respiratory tract infections, diarrheal diseases, measles, tuberculosis, malaria, and the neglected tropical diseases. These efforts will strengthen the practice of public health worldwide, yield global public health benefits of disease control and prevention, and ultimately accelerate social and economic development in the poorest countries of the world and progress toward achieving the Millenium Development Goals.

Dr Rodier is director of International Health Regulations coordination at WHO in Geneva.

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Preparedness for Highly Pathogenic Avian Influenza Pandemic in Africa

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Global concerns about an impending influenza pandemic escalated when highly pathogenic influenza A subtype H5N1 appeared in Nigeria in January 2006. The potential devastation from emergence of a pandemic strain in Africa has led to a sudden shift of public health focus to pandemic preparedness. Preparedness and control activities must work within the already strained capacity of health infrastructure in Africa to respond to immense existing public health problems. Massive attention and resources directed toward influenza could distort priorities and damage critical public health programs. Responses to concerns about pandemic influenza should strengthen human and veterinary surveillance and laboratory capacity to help address a variety of health threats. Experiences in Asia should provide bases for reassessing strategies for Africa and elsewhere. Fowl depopulation strategies will need to be adapted for Africa. Additionally, the role of avian vaccines should be comprehensively evaluated and clearly defined.

In January 2006, chickens in Kano and Kaduna States in northern Nigeria exhibited diarrhea and respiratory distress and died within a few days. The National Veterinary Research Laboratory in Plateau State isolated influenza viruses from these dying chickens. A Food and Agriculture Organization laboratory in Rome confirmed that the isolates were highly pathogenic influenza A subtype H5N1and genetically similar to influenza (H5N1) clade 2 viruses from China, Indonesia, Japan, South Korea, and other countries (*1*). Official reports of the outbreak among poultry in Nigeria were disseminated on February 7, 2006, which initiated a wide range of bilateral and multilateral responses to the arrival of avian influenza in sub-Saharan Africa. Subsequently, the influenza viruses subtype H5N1 isolated in Nigeria were confirmed as belonging to 3 distinct phylogenetic lineages, which suggested independent introduction into Nigeria through different sources (*2*).

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Response to Influenza (H5N1) in Africa

After highly pathogenic influenza A virus subtype H5N1 was first detected in Africa, plans were announced to implement strategies to eradicate the virus. Nigerian authorities formed a national steering committee led by the ministers of health, agriculture, and information. The committee focused on detection of affected farms, depopulation efforts on those farms and in surrounding areas, surveillance for human disease, and dissemination of messages to promote understanding and modification of behaviors that encourage virus spread. Despite the endorsement of control strategies at the national and state levels, local resources were limited and the virus spread widely. By the end of February 2006, 4 Nigerian states and the Federal Capital Territory, all within central and northern parts of the country, had confirmed influenza virus subtype H5N1 outbreaks in poultry, and presence of the virus was suspected in 9 other states. More than 770,000 birds had either died from illness or were culled. Bilateral donor agencies donated >$3 million, and the World Bank offered $50 million credit to add to several million dollars committed by the government of Nigeria. However, efforts to get human, material, and financial resources rapidly to locally affected areas were inadequate, which stifled well-conceived national control efforts. By early June, 14 Nigerian states had confirmed cases of influenza (H5N1) infection in birds, and several hundred thousand additional birds were reported dead, including chickens, geese, and ducks. Niger and Cameroon had also confirmed influenza (H5N1) outbreaks near the Nigerian border.

Despite continued spread of H5N1 subtype among poultry, human cases of avian influenza were not identified in Nigeria until January 2007, when 1 human case of infection with influenza virus subtype H5N1 was confirmed in a woman. However, weaknesses in existing disease surveillance systems limited capacity to detect transmission of avian influenza to humans. To rule out avian influenza, as of May 2007, specimens from 301 patients have been tested in Nigeria.

That an epizootic of this magnitude in poultry would have negative economic and nutritional effects is not surprising. Nigeria's estimated 140–160 million poultry account for 10% of its gross domestic product and a substantial proportion of the protein ingested by its 132 million people (*3*). An assessment conducted by the United Nations Development Program showed that the greatest adverse effect was in impoverished areas like rural and semiurban Nigeria, affecting especially backyard and medium-scale farmers. Egg and chicken sales declined by >80% within 2 weeks after the announcement of the outbreak; 4 months later, sales were still <50% of baseline. Poultry feed sales also dropped by >80%; 80% of workers on affected farms and 45% on unaffected farms lost their jobs (*4*). The outbreak caused an immediate decline in chicken consumption, even in areas where the disease was not reported among poultry.

In March 2006, avian influenza outbreaks were reported among poultry in Egypt; these reports were followed by 38 laboratory-confirmed cases and 15 human deaths. As of May 2007, influenza virus (H5N1) has been detected in fowl in 8 countries neighboring Nigeria and Egypt (Figure). Outbreaks of avian influenza have continued in Nigeria; as of May 2007, 24 of 36 states had documented avian influenza in poultry. In many other countries in East and West Africa, bird die-offs were reported, but related disease (influenza A subtype H5N1) in humans has not been confirmed. Whether the lack of reported human cases in sub-Saharan Africa, when compared with Egypt, is due to differences in surveillance systems or in animal handling practices that would promote transmission to humans is not clear.

Figure. Map of Africa, documenting spread of influenza (H5N1).

Implications of Avian Influenza Emergence in Africa on Pandemic Preparedness

The next influenza pandemic, if it plays out like the 1918 pandemic, would likely cause more deaths proportionately in sub-Saharan Africa than anywhere else in the world (*5*). Poor nutritional status, high rates of concurrent diseases such as HIV/AIDS, and limited access to healthcare could contribute to high mortality rates (*5*). The devastating effect of pandemic influenza would likely exacerbate unaddressed public health problems and require immense humanitarian emergency assistance, which might not be feasible because of limited available global resources and restrictions on movement of persons worldwide.

Sub-Saharan Africa is uniquely characterized by vast geographic areas that are difficult to access; uneven socioeconomic development; nearly transcontinental limitations in epidemiologic, surveillance, and laboratory capacity; and profound infrastructure weaknesses relating to communications and health systems and capacity of government organizations to effectively focus limited resources. An awareness of this situation has brought substantial pressure on nations in Africa to implement or strengthen detection and rapid response capacities. Animal health strategies have focused on improving surveillance in birds, enhancing laboratory capability to detect influenza A, including H5 strains, and supporting teams to do rapid depopulation. On the human side, an early warning system is needed for an emerging influenza pandemic, i.e., surveillance to detect clusters of influenza-like illness in healthcare facilities and communities and to rapidly confirm them as influenza in the field and at qualified laboratories.

Effective preparedness and responses will require fresh, innovative thinking relevant to local circumstances, determination, political will, and national and international resources. The African Regional Office (AFRO) of the World Health Organization (WHO) and its country offices have a clear mandate to address regional impending and ongoing health crises. Partners, including other United Nations agencies, development organizations, and allied nongovernment organizations, should line up with AFRO and national ministries of health and livestock to encourage and catalyze multisectoral government and nongovernment commitment and action.

Balancing Pandemic Influenza Concerns with Ongoing Health Priorities

The potential effects of pandemic influenza on human health and political stability in sub-Saharan Africa cannot be denied. Of paramount concern, however, is that this

potential disaster emerges onto a backdrop of countries struggling to address epidemics of HIV, tuberculosis, and malaria; resurgence of paralytic poliomyelitis; and high childhood mortality rates due to pneumonia and diarrheal diseases. Many of the severe effects of these diseases could be prevented by use of proven public health tools (e.g., antiretroviral drugs; improved detection and treatment of tuberculosis; insecticide-treated bednets; vaccines against *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and rotavirus), but African countries lack the resources and infrastructure to take full advantage of these tools.

In fact, the frenetic global activity around avian influenza's potential threat to human health is diverting critical financial and human resources and focus from the real ongoing, distressing effects of the major infectious disease syndromes. This diversion may be acceptable for a short time if the most pressing challenges for preparedness can be quickly and effectively addressed. However, for many months, ministries of health personnel in countries throughout Africa have been deeply immersed in pandemic preparedness and response planning; they have been pulled away from routine activities, and critical programs have been put on hold. In Nigeria, while senior health officials were struggling to maintain Global Fund resources for HIV and malaria, as well as to improve polio vaccination coverage and stop the export of polio to other nations, considerable immediate pressure was exerted to ramp up influenza surveillance, containment, and preparedness activities after the initial confirmation of influenza virus (H5N1). Similarly, when avian influenza was confirmed in poultry in Juba, Sudan, in September 2006, the newly formed Ministry of Health staff of the government of South Sudan had to balance already demanding commitments to battle HIV, tuberculosis, and an ongoing cholera epidemic with the need to prepare for potential influenza A virus (H5N1) infection of humans. The substantial effort required added more weight to a grossly overstressed public health system.

Tension between existing disease concerns and the potential threat of a pandemic raises several questions: whose concern is avian influenza; and what are appropriate, balanced responses, particularly in countries with severely limited public health resources and overwhelming health problems? A pandemic strain emerging in Africa is a global concern as much as an African concern. Without rapid detection systems and effective control measures, a highly transmittable strain would quickly spread globally. Wealthier nations have recognized this and pledged nearly $2.5 billion to address the problem, although most is not specifically for use in Africa.

In addition, an influx of massive resources from more developed nations, if not well coordinated and balanced in scope, could distort the sense of priorities and damage other critical public health and agricultural programs. How then can a balance be achieved and result in appropriate enhancements to address the threat of pandemic influenza while keeping focus on the existing major health priorities facing Africa? One approach, detailed below, would be to use new resources in a way that is broadly applicable to building public health capacity in recipient nations.

Improving Pandemic Preparedness

The need and resources for rapid detection of potentially pandemic strains of influenza should be leveraged with support for surveillance (Table) for other epidemic-prone and vaccine-preventable diseases; parallel, vertical systems will ultimately not be sustainable in Africa. The drive for improved influenza surveillance can fuel the implementation of Integrated Disease Surveillance and Response (IDSR), promoted by WHO and its partners and endorsed in 1998 by 46 countries in the African region (*6*–*8*). IDSR includes surveillance for vaccine-preventable diseases and epidemic-prone diseases such as meningococcal disease, measles, cholera, typhoid, yellow fever, and viral hemorrhagic fever. If functioning well, IDSR should provide health officials with signals when disease incidence passes thresholds, suggesting an impending epidemic and the need to ramp up disease prevention activities like vaccination, safe water campaigns, or quarantine. Presently, however, IDSR is functional in a few countries, including Eritrea, Ghana, and Kenya. Strengthening IDSR throughout Africa would create an effective early warning system capable of detecting a pandemic strain of influenza or clusters of patients with severe acute respiratory illness, which could signal the beginning of person-to-person transmission. Early detection of a pandemic would facilitate the timely implementation of pharmaceutical and nonpharmaceutical containment measures. In addition, strengthening IDSR would have the dual benefit of enhancing capacity for early detection of outbreaks of more conventional diseases and perhaps currently unrecognized threats.

For IDSR to effectively function as an early warning system for influenza and other communicable diseases, a

Table. Surveillance enhancements needed to address pandemic influenza and other emerging threats, Africa

Method	Needs
Integrated disease surveillance and response	Resources, training, local commitment
Surveillance among health workers, students	Standard operating procedures, resources
Mobile phone messaging	Resources and targeted application of existing technologies
Veterinary surveillance for zoonoses	Political will to encourage stronger linkages between health and animal ministries, resources, effective models to emulate

number of steps should be taken. First, additional resources should be made available for field training of national and local staff and subsequent site assessments. Second, this conventional public healthcare facility–based surveillance system should be expanded to cover private healthcare institutions and include community-level surveillance, through reporting by village chiefs, traditional healers, dispensaries, and primary schools. Finally, existing technology in Africa should be leveraged to make IDSR a user-friendly, more efficient surveillance system. The widespread use of mobile telephones and extensive mobile phone networks throughout Africa could be extremely helpful for reporting unusual health occurrences through inexpensive, potentially automated, text messaging to an identified, widely recognized, and toll-free number. Reports of disease clusters could then be immediately verified and investigated by public health personnel (*9*). This tool could also be used for animal surveillance.

Intersectoral Communication and Collaboration

With similar urgency, enhanced veterinary surveillance and strong, functional networks of communication and collaboration between animal and human health experts would increase potential for prevention of transmission of influenza from animals to humans. Currently, linkages and communication mechanisms between health and veterinary officials are neither explicit nor functional. A major challenge in Nigeria's response to avian influenza (H5N1) was to ensure communication between Ministry of Health and Ministry of Agriculture staffs on the ground so that when poultry outbreaks occurred on farms, the Ministry of Health was alerted to look for human cases.

Laboratory Capacity

Resources for improving infrastructure to address threats of avian influenza must be channeled to improve local laboratory capacity and strengthen laboratory networks. WHO and AFRO have a critical role to promote, select, develop, train, and sustain laboratory capacity for diagnosis of influenza within regional reference laboratories and national influenza centers. AFRO has already designated several existing laboratories in Africa as regional reference laboratories for testing seasonal and avian influenza in humans. With enhanced surveillance, these laboratories should be able to rapidly confirm or rule out influenza as the cause of a cluster of severe respiratory illness, which would allow for rapid epidemic responses. Already, laboratories in several countries in Africa have been upgraded, laboratorians have been trained, equipment (real-time and conventional PCR machines) and appropriate reagents (primers and probes) have been procured, and quality control mechanisms have been put in place so that PCR can be used in a timely manner to reliably document the presence of highly pathogenic influenza A virus subtype H5N1. With additional reagents and training, these laboratories could have the capacity to rapidly detect causes for other outbreaks and assist with surveillance for routine and emerging diseases. As laboratory surveillance is initiated and strengthened, training, leveraging of equipment maintenance and service contracts, and resource sharing across animal and human facilities will be essential.

Epidemiologic Capacity

Being prepared for an influenza pandemic will require having a well-trained corps of epidemiologists. Field epidemiology training programs and Public Health Schools without Walls are in place in several countries in Africa; these programs are successfully training medical doctors (and laboratorians in the case of Kenya's Field Epidemiology and Laboratory Training Program) to become public health experts. To minimize out-migration, graduates should be rewarded through career advancement pathways within national public health systems.

Response and Containment

In a joint effort by WHO and the Centers for Disease Control and Prevention, rapid outbreak response teams are being trained in sub-Saharan African countries to recognize outbreaks, collect information and specimens, and implement quarantine measures, if indicated, while using and distributing personal protective equipment and antiviral drugs. Training rapid response teams in each country is a relatively new concept for Africa, but such teams in Asia have responded to dengue epidemics and, more recently, to avian influenza. If these teams are functional, they will strengthen responses in Africa to a wide array of epidemics, enabling timely implementation of interventions to prevent illness and death.

Should a major health disaster such as an influenza pandemic occur, essentially no capacity exists to appropriately address the healthcare crisis while continuing to deal with other routine health problems, which themselves can at times overwhelmingly stress healthcare systems. Preparation for a pandemic must strengthen hospital capacity to optimally and safely manage severely ill patients with potentially highly contagious illnesses. Although aiming for parity with hospitals in industrialized nations on quality standards for infection control is excessively ambitious, simple, practical approaches to implement basic infection control measures developed by WHO can be used (*10*).

Although data on the topic are limited, public awareness of avian influenza is minimal in Africa, specifically concerning the link between dying birds and human illness and the potential of catastrophic emergence of pandemic influenza. The United Nations Children's Fund has conducted participatory action research, which has shown

that while community outreach campaigns will be needed across the continent, messages must be locally customized because of wide variations in literacy, awareness, experience, and beliefs (*11*). Recent efforts to strengthen information, education, and communication in Africa will improve societal mobilization for behavior modification capacity, which can be called upon to address public health threats (*12*). Ultimately, by developing effective health communication measures, functional surveillance systems with strong epidemiologic and laboratory support, and capable multisectoral rapid response teams, it may be possible to curtail spread of a lethal pandemic strain and buy time to develop and deliver effective human-administered vaccines (*13*,*14*).

A Call for New Strategies

In Asia, the principal control approach has been depopulation of poultry in affected and nearby farms along with attempts to restrict movement of poultry and poultry products from affected areas. Even when aggressively pursued, these efforts were not always timely or comprehensive, and avian influenza became endemic among birds in many parts of Asia and has now spread to Europe and Africa. The diffuse spread of the virus in avian species and some transmission to humans and other mammals has provided numerous opportunities for a pandemic virus to appear; however, a strain easily transmittable from person to person has not yet emerged. Although this provides some comfort for now, a variety of ecologic and health factors could affect the emergence of a devastating pandemic strain.

The massive geographic spread of avian influenza in birds should cause a re-evaluation of bird depopulation as the principal strategy for control. Although depopulation appears to have worked when the virus was localized during 1997 in Hong Kong and 1.5 million birds were culled, widespread presence of the virus in migratory and domestic birds has severely limited the usefulness of this approach. In addition to direct costs, massive culling operations dramatically affect the economic and nutritional status of impoverished people. Compensation programs, critical to success of culling efforts, have not been implemented optimally in most countries where influenza virus (H5N1) epizootics have occurred. Without credible, adequate, and rapidly implemented compensation programs linked to depopulation efforts, farmers are not encouraged to report bird die-offs and may transport birds to unaffected areas, thereby spreading the virus (*15*). Furthermore, depopulation may counter evolutionary pressure. If the birds that survive local outbreaks are the fittest, killing them provides no evolutionary advantage to survival.

Vaccination of poultry may be a useful adjunct to depopulation in a defined area surrounding an affected farm (ring depopulation). Some available vaccines seem to provide immunity to infection without substantially prolonging viral shedding (*16*–*19*). If so, focusing vaccination programs on farms around affected areas may be a less costly, more socially acceptable, and more effective solution than mass culling, with or without compensation. Effective use of influenza vaccines in avian populations could theoretically reduce the risk for emergence of a pandemic strain by decreasing the numbers of circulating avian influenza strains that could mutate or reassort with other circulating influenza viruses (*19*). Additionally, use of emergency vaccination can be effective in conjunction with other measures at the time of poultry outbreaks (*19*). Ultimately, control measures will be implemented by agriculture and livestock ministries, which must address potentially conflicting commercial and public health interests.

Current vaccine supplies for poultry in risk areas may be insufficient. A clear avian vaccination strategy, articulated and implemented by international organizations, would encourage adequate production of effective vaccines for use in currently and prospectively affected areas. Analyses comparing costs and potential cost-effectiveness of depopulation and vaccination strategies (and strategies that use both approaches) would be helpful.

The Path Forward

Activities to address the threat of pandemic influenza should, whenever possible, build upon existing public health systems and enhance available health infrastructure rather than create new systems. In Vietnam, for instance, research organizations like Oxford University and The Wellcome Trust, and partners they support, were able to refocus their efforts onto avian influenza and provide substantial new knowledge relevant for protecting health (*20*,*21*). Likewise in Africa, many nongovernment and religious organizations can build on their existing networks and groundwork to assist governments with surveillance and risk-reduction efforts, as has been the case for initiatives to address HIV/AIDS, provide safe water, and eradicate poliomyelitis (*22*–*26*).

Identifying existing laboratory and disease-reporting mechanisms in individual countries is crucial so they can be augmented to achieve sustainable capacity for surveillance for influenza and other emerging diseases. National disease reporting systems—IDSR and polio surveillance—may provide entry points for such activity but will require strategic investments that in large part need to be defined in the context of national priorities and balanced with global needs. Early detection and improvement of the continent's preparedness for emerging diseases has been brought into focus by the recent adoption of a treaty by the World Health Assembly of new International Health Regulations (IHR [2005]), which now compels countries to have more effective surveillance and reporting systems to improve the

ability for early detection of global disease threats (*27*). More specific to influenza, IHR (2005) compels countries to implement optimal communications, rapid risk assessment, and containment and response measures, should a pandemic strain emerge.

An old expression says, "You can't slide uphill." The difficult work associated with preparedness for pandemic influenza will help bring Africa closer to the top of the hill, making it possible to efficiently respond to this and other threats and, when necessary, slide toward a beneficial outcome.

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Plague Reappearance in Algeria after 50 Years, 2003

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An outbreak of plague occurred in the region of Oran, Algeria, from June to July 2003. Algeria had not reported this disease for >50 years. Eighteen bubonic cases were identified, and *Yersinia pestis* was isolated from 6 patients. Except for the index case-patient, all patients recovered. Targeted chemoprophylaxis, sanitation, and vector control played a crucial role in controlling the outbreak. Epidemiologic and biomolecular findings strongly suggested the existence of a local animal reservoir during this period, but its origin (resurgence or re-importation) could not be determined. This sudden and unexpected reemergence of plague, close to an important commercial seaport, is a textbook illustration of a public health event of international importance. It also demonstrates that the danger of plague reoccurrence is not limited to the currently indexed natural foci.

Plague is primarily a bacterial zoonosis affecting rodents. It is caused by *Yersinia pestis* and is transmitted from animal to animal by fleas. Humans usually become infected through the bite of an infected rodent flea. Bubonic plague, a severe infectious disease which, in the absence of appropriate antimicrobial drug therapy, can evolve to a rapidly fatal septicemia or pneumonia, can develop. A pneumonia form, which enables direct transmission to contacts, can be responsible for highly lethal outbreaks.

Currently, plague natural foci persist in Asia, the Americas, and Africa (where most human cases occur) (*1*). Plague foci have previously existed in the northern part of Africa but gradually disappeared in the last century, for unknown reasons. Libya is the only north African country that has experienced human cases in the past 40 years (*2*). In Algeria, archives report epidemics of plague as far back as the 14th century. These epidemics mainly affected ports, particularly that of Oran in 1556 and 1678 (3,000 deaths). In 1899, after an absence of nearly 100 years, plague reappeared in the port of Philippeville (now Skikda). Three large epidemics were subsequently reported in 1921 (185 cases), 1931 (76 cases), and 1944 (95 cases) as well as 158 sporadic cases. All but 2 cases occurred in ports (*3*,*4*). No natural focus of plague had ever been described in Algeria (*5*). We describe an outbreak of bubonic plague that occurred in 2003 in Algeria, where the last reported human case occurred in Oran in 1946 (*6*).

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Methods

During June 9–18, 2003, several patients with signs of severe infection and painful inflammatory adenopathy were admitted to the University Hospital of Oran. All came from Kehailia (35°29′N, 0°32′E), a village of 1,300 inhabitants 25 km south of Oran. After eliminating all other possible differential diagnoses, clinicians suspected plague. The diagnosis was confirmed on June 18 by results of analysis of a bubo (lymph node) aspirate. A technical crisis committee was set up, and a case definition was adopted (Table). Any patient with a febrile syndrome and adenopathy who resided in the prefecture of Oran was hospitalized.

Clinical samples collected from patients (blood, bubo aspirate, cerebrospinal fluid) were sent to the Microbiology Department, University Hospital, Oran. Several of the initial cases were first diagnosed with the rapid diagnostic test (RDT) for plague developed by the Institut Pasteur (*7*); however, all samples were also examined with standard bacteriologic methods. Direct examination of smears was performed after Wayson and Gram staining. Blood samples were cultured in Castaneda medium for at least 10 days

Table. Plague case definition adopted by technical crisis committee, 2003 plague outbreak, Oran region, Algeria*

Case definition	Criteria
Suspected	Clinical and epidemiologic characteristics compatible with plague; or, observation of suspect microorganisms on direct examination of clinical samples
Probable	Suspected case with anti-F1 antibodies in patient's blood; or, suspected case with a positive RDT without isolation of *Yersinia pestis* or in the absence of other cases reported in a radius of 10 km around the case
Confirmed	Culture positive for *Y. pestis*; or, RDT positive and *Y. pestis* isolated from patients living in a radius of 10 km around the case

*RDT, rapid diagnostic test.

at 28°C and examined daily. Suspected samples were inoculated into brain heart infusion and peptone broth and streaked on blood agar and cefsulodin-irgasan-novobiocin (Merck, Rahway, NJ, USA) plates. All media were incubated at 28°C. Bacterial identification was conducted with API 20 E strips (Analytab Products, Syosset, NY, USA) or individual tests in tubes. The biovar was determined (*8*). Antimicrobial drug susceptibility testing (ampicillin, amoxicillin-clavulanic acid, cefazolin, cefotaxime, gentamicin, amikacin, sulfamethoxazole, doxycycline) was conducted according to the technique of the Clinical and Laboratory Standards Institute (www.clsi.org). The serodiagnosis was determined by the ELISA-F1 technique (*9*). Serum samples from 30 study participants who had not contracted the disease but lived in the same area as the patients were used to determine the positive threshold of the technique. A serum was regarded as negative if its optical density at 490 nm (OD_{490}) was lower than a threshold defined as the mean (M) OD_{490} value of normal sera + 3 standard deviations (SD): $OD_{490} < M + 3$ SD. Sera with OD higher than this threshold were regarded as weak when the ratio $R = OD_{490}/(M + 3SD)$ was <2 and positive if R was ≥2.

Results

On June 9, 2003, a 19-year-old shepherd living in Kehailia was hospitalized with signs of septic shock (patient no. 2) (online Appendix Table, available from www.cdc.gov/EID/content/13/10/1459-appT.htm). He had been treated at home unsuccessfully with cephalosporins for inguinal adenopathy and fever during the previous 8 days. In the same village, 6 similar cases (nos. 3–8) occurred in the following days, until the diagnosis of plague was suspected and confirmed on June 18, first by RDT and then by isolation of a bacterium that had all the characteristics of *Y. pestis* biovar Orientalis and was susceptible to the antimicrobial agents tested. The epidemiologic investigation uncovered the index patient (no. 1), an 11-year-old child from Kehailia who was a cousin of case-patient 2. On June 2, an inguinal adenopathy with fever developed, and patient 2 was transferred to the hospital. He died 3 hours later, without a precise diagnosis.

Following the sanitation measures (reduction of rodent harborage, garbage removal, and vector control) implemented in Kehailia, no new cases of plague were reported in this locality after June 17. On June 19, a woman living in the suburbs of Oran (Hai Oussama) was hospitalized with bubonic plague (patient 9). The investigation showed that she had gone to Kehailia in the preceding days to consult a healer. Five cases of bubonic plague (nos. 10, 11, 14, 15, and 17) subsequently occurred from June 21 to July 16 among persons living in villages around Kehailia.

On June 28, a farmer and his wife (patients 12 and 13) who resided in Ain Temouchent, 50 km west of Kehailia (Figure), were hospitalized in Oran for symptoms suggestive of plague. The patients reported that they had not left their farm during the weeks preceding their illness. On July 1, a child from Beni Saf, on the Mediterranean coast 100 km southwest of Kehailia (Figure), had clinical signs of bubonic plague and a positive RDT result (patient 16). Neither he, nor his parents, had gone to the area of Kehailia or Ain Temouchent during the previous days. The last case (patient 18) occurred on July 22. The patient, a hunter who lived in Oran, had walked in the forest of M'sila, 30 km northwest of Kehailia, a few days before onset of his clinical signs.

Altogether, 18 cases were identified June 4–July 22, 2003: 10 confirmed, 3 probable, and 5 suspected (or 12 confirmed, 2 probable, and 4 suspected, according to the new World Health Organization case definition [*1*]). Most of the patients lived in unsanitary conditions, in close contact with livestock, and in the vicinity of storage areas of grain and fodder. In Kehailia, all the case-patients resided in different dwellings located within a 200-m radius. None of them reported direct contact with rodents. Sixteen of the 18 patients had an inguinal bubo, indicative of a flea bite on the leg. A septicemic form of plague developed in patients 1 and 2. Patient 1 died very soon after hospital admission. Patient 2 was admitted with a severe fever and neurologic syndrome and fell into a deep coma, despite broad-spectrum antimicrobial drug treatment that included vancomycin, cefotaxime, and gentamicin. He recovered from the coma 48 hours after treatment with ciprofloxacin (500 mg 2×/d for 30 days) was completed (F. Razik et al., unpub. data). No case of secondary pulmonary dissemination was observed. Other plague patients were treated with either doxycycline for adults (200 mg/d for 10 days) or cotrimoxazole for children (40 mg/kg/d for 10 days). All recovered without sequelae.

On the whole, 60 bubo aspirates, 143 blood samples, 6 sputum samples, and 2 cerebrospinal fluid samples were analyzed. In 5 samples, smear stains suggested infection

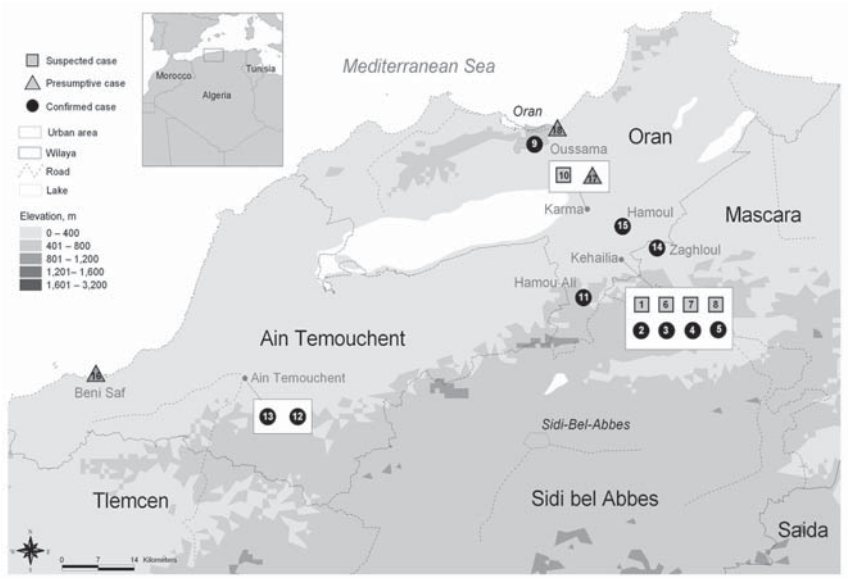

Figure. Geographic distribution of plague cases, Oran region, Algeria, June–July 2003. Boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization (WHO) concerning the legal status of any country, territory, city, or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted lines on maps represent approximate border lines for which there may not yet be full agreement. Data source: Ministry of Health Algeria. Map production: Public Health Mapping and GIS, Communicable Diseases, WHO. Copyright WHO, 2006. Used with permission.

with *Y. pestis* (online Appendix Table). Among the 18 patients, 12 had a positive RDT result, but *Y. pestis* was isolated from only 6 patients: 5 from bubo aspirates and 1 from the blood culture of a patient whose bubo was too small to be punctured (patient 13). Results of ELISA-F1 serologic test conducted on the serum samples from 15 of the 18 patients were strongly positive 3 times and slightly positive 3 times (online Appendix Table).

Discussion

Epidemiologic investigation did not identify any other plague patients before patient 1. It is unlikely that other cases occurred and remained undetected during this period since plague, even in its bubonic form, is a severe infection with high fatality rates.

For the first time, the RDT was used in an epidemic situation outside of Madagascar, where it was developed. The case definition had to take into account this particularity. The bacteriologic diagnosis is a long procedure (at least 4 days) and, in this epidemic context, RDT contributed to the effectiveness of the response. Of the 44 RDTs that were conducted, 12 had positive results; by contrast, culture was positive only for 6. Among the 15 patients for whom a serologic test was conducted (online Appendix Table), a specific antibody response developed only in 6. This absence of specific antibodies can be explained by the fact that serum specimens were taken before the appearance of anti-F1 immunoglobulin G, or by a rapid administration of antimicrobial drugs, which stopped development of an immune response. The 3 clearly seropositive patients were those from whom a positive culture was obtained.

The outbreak occurred in a poor rural settlement, with inadequate sanitation. The residents observed an increase in the population of commensal rodents, which is often associated with the harvesting period, but no unusual rodent mortality was noted during the weeks preceding the outbreak. The appearance during the same week of 2 new cases in Ain Temouchent (50 km west of Kehailia) and then 1 case in Beni Saf (100 km southwest of Kehailia) could not be explained. Nonetheless, the fact that the *Y. pestis* strains isolated in Kehailia and Ain Temouchent had identical pulsotypes (V. Chenal-Francisque et al., unpub. data) argues for a single focus and not for independent foci that emerged simultaneously.

A crisis committee designed and supervised a control strategy based on standardized case management, prophylactic treatment and follow-up of contacts sharing the same dwelling as plague patients, and vector control. Environmental sanitation measures in Kehailia contributed to reduction in the occurrence of new cases in this village. Intra- and peridomestic spraying with permethrin was conducted. Deltamethrin was dusted on the tracks and around the burrows of rodents located in a radius of 10 km around the dwelling of the patients. Uncontrolled killing of rats was prohibited.

No natural focus of plague had ever been described in Algeria. Past cases were always regarded as imported through the ports. The reappearance of human cases in this area can be explained in 2 ways: a recent importation of infected animals or a sudden manifestation of a natural focus that had remained silent for decades. It is noteworthy that Kehailia, the epicenter of the outbreak, is in the vicinity of flour mills built 4 years before the outbreak. These mills are supplied regularly with cereals by trucks arriving from the port of Oran. A part of this traffic was still run by railway a year before the outbreak, and a marshalling yard was installed a few kilometers from Kehailia. In 1919, this mode of importation was responsible for the plague outbreak that

occurred 75 km south of the port of Skikda (*10*). The hypothesis of recent importation of the plague bacillus in Kehailia is therefore tempting but is tempered by the fact that 1) the grain is primarily imported from Europe, which is not affected by plague, and from North America where natural foci exist but have very limited areas of overlap with those regions where cereal grains are grown, 2) no higher mortality rate in the murine population of the port was noted, 3) no human cases occurred in this sector of the city, and 4) a 3IS–restriction fragment length polymorphism (*11*) analysis grouped these strains in a cluster clearly distinct from the strains isolated from Africa and America (V. Chenal-Francisque et al., unpub. data).

The geographic concentration of the cases in 2 foci, both contiguous in the mountainous area of Tessala, suggested the existence of a natural focus in this area. Moreover, *Meriones* are present in Tessala, and these rodents are a well-known potential reservoir of *Y. pestis* (*12*). The outbreak occurred at harvest time, and it is possible that the abrupt reduction in the source of food pushed the wild rodents to approach houses in which grain was stored.

The current challenge in terms of public health is to determine if this animal reservoir has disappeared or if it is well established in the ecosystem. The capture of 3 seropositive small mammals (2 *Mus musculus* and 1 *Aleterix algerius*) in July 2004 (J.L. Soares et al., unpub. data) and the identification of several *Y. pestis* infected fleas in the same area (*13*) favor the second option.

Beyond the local problem, the proximity of a possible natural reservoir of plague to Oran, a large international commercial port, raises the possibility of the risk for an urban outbreak. At the time of the investigation, the sanitation in the city and port were poor and rodents proliferated. These urban rodents could come in contact with infected rodents from rural areas in the uncontrolled dumps at the periphery or through a dry riverbed that penetrates as far as the city center. Because of Oran's population density and the commercial activities of its seaport, a plague outbreak would have international implications.

This outbreak is a textbook illustration of the unexpected and sudden reemergence of an infectious disease epidemic that is potentially highly lethal. It also demonstrates that the danger of a plague outbreak is not limited to the currently indexed natural foci.

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"So many scientists think that once they figure it out, that's all they have to do, and writing it up is just a chore. I never saw it that way; part of the art of any kind of total scholarship is to say it well."

—Stephen Jay Gould

HIV and Tuberculosis in Ho Chi Minh City, Vietnam, 1997–2002

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In Ho Chi Minh City, Vietnam, reporting rates for tuberculosis (TB) are rising in an emerging HIV epidemic. To describe the HIV epidemic among TB patients and quantify its impact on rates of reported TB, we performed a repeated cross-sectional survey from 1997 through 2002 in a randomly selected sample of inner city TB patients. We assessed effect by adjusting TB case reporting rates by the fraction of TB cases attributable to HIV infection. HIV prevalence in TB patients rose exponentially from 1.5% to 9.0% during the study period. Young (<35 years), single, male patients were mostly affected; injection drug use was a potent risk factor. After correction for HIV infection, the trend in TB reporting rates changed from a 1.9% increase to a 0.4% decrease per year. An emerging HIV epidemic, concentrated in young, male, injection drug users, is responsible for increased TB reporting rates in urban Vietnam.

Patients who are co-infected with HIV and *Mycobacterium tuberculosis* are at high risk for active tuberculosis (TB) (*1*). In developing countries, such patients add to the load of already strained TB programs. Although the effect of HIV on the TB epidemic is extensively documented in sub-Saharan Africa (*2*), less is known about its effect in Southeast Asia (*3*,*4*). Although HIV rates in the general Southeast Asian population are still relatively low (*5*), many of these countries have high prevalence of latent TB infection, which makes them vulnerable to the effects of a combined epidemic.

Vietnam is listed by the World Health Organization (WHO) as a TB high-burden country and, through a strong National TB Program (NTP), has reached and exceeded the WHO targets of 70% case detection and 85% cure rates from 1997 onward (*6*,*7*). As a result, TB incidence was expected to decline (*8*), but thus far this has not happened (*9*). One possible explanation for this phenomenon is HIV infection.

The first case of HIV infection in Vietnam was recorded in 1990, and since then HIV infection has been mostly limited to men and high-risk groups such as injection drug users (IDUs) and commercial sex workers (*10*). In more recent years, however, rising HIV infection rates in TB patients have been documented in Ho Chi Minh City, the major urban area with the highest HIV prevalence in the country (*11*). Since 1997, Ho Chi Minh City has also reported increasing TB rates, particularly for young adults (*12*).

Our study objective was to describe the course of the HIV prevalence among TB patients in Ho Chi Minh City during 1997–2002. By combining our data with the NTP reporting data, we also quantified the effect of HIV on the TB reporting rates in this city.

Methods

Patient Enrollment

From 1997 through 2002, we performed a repeated cross-sectional survey of HIV prevalence among TB patients in the 12 most urbanized districts (districts 1, 3, 4, 5, 6, 8, 10, 11, Phu nhuan, Tan binh, Nha be, and Binh thanh) of Ho Chi Minh City. Until 1998, districts included all patients ≥15 years of age who had confirmed TB according to WHO criteria and who consented to HIV testing during the same quarter each year. Since 1999, enrollment was restricted to the last quarter of the year for all districts. Enrollment stopped after a quota was reached; the quota

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was proportional to the annual number of patients treated in the district, set to obtain a total sample size of ≈800 patients each year. Ethical approval was obtained from the Ho Chi Minh City Council Research Board.

Measurements

We determined HIV status by ELISA (Genolavia Mixt, Sanofi, Paris, France, until 1999; and Genscreen, Sanofi, Paris, France, from 1999 onward) and an independent confirmatory test (Serodia; Fujirebio, Tokyo, Japan, or Vironostika, Organon, Boxtel, the Netherlands) if the first test result was positive. District TB staff collected data for each patient on TB disease (diagnostic category, treatment history), age, sex, marital and employment status, education level, and risk factors for HIV infection. Patients who owned small businesses and seasonal workers were coded as "self-employed"; civil servants and patients under contract (e.g., drivers), as "employed." All data were entered twice in EpiInfo version 6 (Centers for Disease Control and Prevention, Atlanta, GA, USA), and discrepancies were checked against the raw data. TB reporting data were obtained from NTP quarterly district reports. Sex, age, and distributions of urban and rural population size were interpolated from the results of the 1994, 1999, and 2004 census; standard exponential population growth was assumed.

Statistical Analyses

For HIV trend analyses, we used 2-year blocks, increasing group size and power of the analyses. The Cuzick test for trend was used to identify monovariate time trends in HIV prevalence (*13*). To compare proportions, we used the χ^2 test.

Multivariable analysis was performed by logistic regression. After transformation (squaring), time of inclusion could be entered as a continuous variable (χ^2 for departure linear trend = 0.68 [df = 1], p = 0.41). Variables were included when the likelihood ratio χ^2 test was significant at the 0.1 level.

We identified multivariable time trends by entering time interaction variables (time of inclusion × variable x) in our logistic model (*14*,*15*). Compared with the baseline category, an odds ratio (OR) >1 indicates a faster rise in HIV prevalence in that category, thereby identifying high-risk groups. For the sake of interpretability, we present the multivariable model without interaction terms or time trends.

To describe the combined epidemic outside the known high-risk groups, we ran the final multivariable model after excluding all IDUs. The model goodness-of-fit was assessed by the Hosmer-Lemeshow test of goodness-of-fit and by visual inspection of the distribution of the model residuals (*16*,*17*).

We used the formula $p \times (1 - 1/RR)$ to estimate the fraction of TB in patients that was attributable to HIV (the population-attributable fraction, PAF) (*18*). In this formula, p represents the prevalence of HIV in TB patients, and RR represents the relative risk of active TB developing in patients with HIV versus in patients without HIV. The PAF thereby corrects for the fact that some of the TB cases among HIV-infected patients would also have occurred were the patients not HIV infected (*18*). Because this information cannot be known for individual patients, RR represents the average relative risk for HIV-infected patients. Lacking situational data, we conservatively assumed a constant RR of 5 from 1997 through 2002. We based this estimate on the literature and took into account the early stage and specific characteristics (focused in high-risk groups that overlap with TB risk factors such as injection drug use) of the combined epidemic in Southeast Asia (*3*). To test our assumption, the RR was also varied between 2 and 10 or gradually increased (from 2 to 10) over the study period to simulate a progressive fraction of HIV-infected patients in which active TB develops as a result of increased immune suppression.

The rate of TB observed without HIV was calculated as [(1 – PAF) × current TB rate]. We restricted this part of the analysis to new smear-positive TB patients from urban districts. The diagnosis of these patients' condition is highly standardized and the HIV/TB data came from urbanized districts, which ensured that combining the 2 datasets was as valid as possible. Exponential growth rates were estimated by using the least squares method.

In 2002, the Ho Chi Minh City Council started a mandatory rehabilitation program for IDUs. Because these rehabilitation centers were not included in our surveillance, we quantified potential resulting bias by estimating how including 50% more IDUs in 2002 would affect our results.

Analyses were performed by using Stata version 8 (Stata Corp., College Station, TX, USA). Excel 2003 (Microsoft Corp., Redmond, WA, USA) was used to asses the effect of HIV on reporting rates.

Results

HIV in TB Patients

A total of 5,701 patients consented to HIV testing (92% of those eligible) and were included in the study. Because of clerical error, 504 patients entered the study from July through December 1996. These were added to the subset analyzed for 1997. Apart from IDUs, patient numbers in the individual risk categories were too low to be analyzed separately and were therefore added to the category of "other."

HIV prevalence rose exponentially from 1997 through 2002 (Table 1). The mean age of HIV-infected patients decreased from 38 (standard deviation [SD] = 10) to 27 (SD = 8) years; the mean age of the HIV-negative group remained

Table 1. HIV prevalence in tuberculosis patients in Ho Chi Minh City, Vietnam, 1997–2002*

Variable	1997–1998, % (n/N)	1999–2000, % (n/N)	2001–2002, % (n/N)	p value†
Study population	1.5 (38/2,476)	2.9 (47/1,617)	9.0 (144/1,608)	<0.001
Age, y				
<24	0.6 (2/342)	5.9 (14/239)	19.9 (53/267)	<0.001
25–34	2.0 (14/711)	3.1 (14/452)	14.4 (65/450)	<0.001
35–44	2.4 (17/719)	2.4 (11/460)	3.6 (16/439)	0.100
45–54	0.7 (5/704)	1.7 (8/466)	2.2 (10/452)	0.020
Sex				
Male	2.0 (35/1,749)	3.8 (43/1,139)	11.6 (134/1,158)	<0.001
Female	0.4 (3/727)	0.8 (4/478)	2.2 (10/450)	0.001
Marital status				
Married	1.2 (19/1,538)	1.4 (14/989)	4.7 (46/975)	<0.001
Single	1.8 (14/775)	5.4 (28/521)	15.9 (87/549)	<0.001
Separated	3.1 (5/163)	4.7 (5/107)	13.1 (11/84)	0.010
Education level				
Illiterate	1.1 (3/281)	2.0 (3/151)	9.4 (10/106)	0.001
Primary	1.4 (18/1298)	3.8 (18/478)	10.7 (55/512)	<0.001
Secondary or higher	1.9 (17/897)	2.6 (26/988)	8.0 (79/990)	<0.001
Employment status				
Employed	0.9 (3/334)	2.3 (7/300)	5.8 (21/362)	<0.001
Self-employed	1.6 (22/1376)	2.9 (26/909)	8.6 (84/979)	<0.001
Unemployed	1.7 (13/766)	3.4 (14/408)	14.6 (39/267)	<0.001
Risk group				
Injection drug use	31.3 (5/16)	47.1 (8/17)	95.4 (41/43)	<0.001
Other	1.3 (33/2,460)	2.4 (39/1,600)	6.6 (103/1,565)	<0.001
Patient history				
New case	1.6 (33/2,018)	2.9 (39/1,338)	9.5 (129/1,358)	<0.001
Relapsed case	0.5 (1/223)	4.1 (5/122)	5.0 (6/119)	0.002
Other‡	1.7 (4/235)	1.9 (3/157)	6.9 (9/131)	0.030
Tuberculosis type				
Smear-positive	1.5 (27/1,799)	3.3 (38/1,145)	8.3 (91/1,100)	<0.001
Smear-negative	0.3 (1/362)	1.5 (4/260)	5.2 (10/194)	<0.001
Extrapulmonary	3.2 (10/315)	2.4 (5/212)	13.7 (43/314)	<0.001

*%, percentage of HIV-positive patients; n, no. HIV-infected patients; N, total no. patients.
†p value for Cuzick nonparametric test for trend across time periods.
‡Includes previously treated tuberculosis patients who did not respond to treatment, defaulted, or received their first treatment outside the National TB Program.

stable at ≈39 (SD = 14). The male:female ratio was higher for HIV-infected (9:1) than for other TB (7:1) patients (χ^2 = 53.6 [df = 3], p<0.001). HIV prevalence in young (<35 years) men rose to 22.3% (108/484) during 2001–2002.

The 12 districts did not differ significantly in HIV prevalence during the study period (χ^2 = 38.1 [df = 33], p = 0.25) (data not shown). HIV prevalence in reported IDUs rose to 95% in 2001–2002, which accounted for 28% of all HIV-infected patients.

Multivariable and Time-Trend Analyses

In the multivariable analysis, when time trends and other interactions were disregarded, HIV infection among TB patients was associated with age <45 years, male sex, not being married or employed, and being an IDU (Table 2). As the time-trend analyses show (Table 3), HIV prevalence increased faster in young (<35 years) patients, most prominently in the youngest age group (15–24 years), and in IDUs. Additional interaction (p value for excluding interaction from model = 0.002) between marital status and sex indicated that the high HIV prevalence in single TB patients was mainly attributable to HIV infection among male patients.

When IDUs were excluded, the multivariable model predicted the data less well (–2 log likelihood with IDUs = –686.7, without = –660.2), but this exclusion affected neither the direction of the ORs nor their size in a relevant way (data not shown). Also, the Hosmer-Lemeshow test of goodness-of-fit remained nonsignificant in both models (p = 0.72 with IDUs, p = 0.69 without). Although IDUs and non-IDU HIV-infected patients did not differ relevantly in age, sex, or marital status (χ^2 test, p = 0.93, 0.53, and 0.78 respectively), they did differ in their employment status and level of education (χ^2 test, p = 0.05 and <0.001, respectively).

TB Reporting Rates

PAF calculations show that 0.7%, 1.5%, 1.3%, 6.7%, 9.5%, and 9.7% of reported new smear-positive TB cases

Table 2. Multivariable model (without interaction terms and time trends) for HIV among tuberculosis patients in Ho Chi Minh City, Vietnam, 1997–2002*

Variable	Crude OR† (95% CI)	p value‡	Adjusted OR§ (95% CI)	p value‡
Year of inclusion	5.78 (4.2–7.9)	<0.001	5.80 (4.1–8.2)	<0.001
Age, y		<0.001		<0.001
≤24	6.16 (3.8–9.9)		5.15 (2.9–9.3)	
25–34	4.25 (2.7–6.8)		4.16 (2.5–7.0)	
35–44	1.94 (1.2–3.2)		1.96 (1.1–3.4)	
>45	1		1	
Sex		<0.001		<0.001
Male	5.33 (3.2–8.8)		5.79 (3.4–9.9)	
Female	1		1	
Marital status		<0.001		<0.001
Married	1		1	
Single	3.3 (2.4–4.3)		1.67 (1.2–2.4)	
Separated	2.7 (1.7–4.5)		3.93 (2.2–7.0)	
Employment status		0.18		0.020
Employed	1		1	
Self-Employed	1.31 (0.9–2.0)		1.62 (1.1–2.5)	
Unemployed	1.49 (1.0–2.3)		1.97 (1.2–3.2)	
Risk category		<0.001		<0.001
Injection drug use	76.44 (45.5–128.3)		46.06 (25.3–84.0)	
Other	1		1	

*OR, odds ratio; CI, confidence interval.
†Monovariate ORs.
‡p-value for likelihood ratio χ^2 test for excluding variable from the model.
§ORs adjusted for all variables in multivariable model.

were attributable to HIV in 1997, 1998, 1999, 2000, 2001, and 2002, respectively. After these cases were excluded from analysis, the rising trend in TB reporting rates reversed to a mild decline (Figure, panel A). When stratified for sex (Figure, panel B) and age (Figure, panel C), this effect seemed limited to men and was most prominent in the younger age groups. Increasing the RR over time did not affect the results (data not shown). Adding 50% more IDU patients to the 2002 population enhanced the effect of HIV, which reduced the corrected annual growth in TB reporting from –0.2% to –1.2% in men and from –1.8% to –2.9% in young (<35 years) persons.

Discussion

Our results show that Ho Chi Minh City is faced with a combined HIV/TB epidemic that is concentrated and expanding rapidly in young men; injection drug use is a high-risk factor. By 2002, 1 in 10 TB patients was HIV infected, and 1 in 5 men <35 years of age was HIV infected. Even after taking into account the effect of HIV, TB case-reporting rates do not show the decline that is expected if directly observed therapy short course (DOTS) targets are met.

Although the observed trends in HIV infection among TB patients are cause for concern, they are not unexpected. Since 1996, HIV rates have been rising in Vietnam (*10*,*19*). After the average 6-year delay between HIV infection and development of active TB as an opportunistic infection (*20*), HIV infection rates among TB patients were expected to start rising around 2000. Also, the HIV epidemic has mainly affected young men, of whom a large proportion were suspected to have been IDUs (*10*). That this group takes the brunt of the combined epidemic and shows the fastest increase in TB/HIV prevalence is therefore understandable.

The relatively low proportion (28%) of HIV-infected patients who reported injection drug use leaves 72% of HIV-infected TB patients without a clear risk factor. This finding would suggest that HIV has moved beyond the established risk groups and into the general population. However, the strong social stigma associated with injection drug use in Vietnam increases the chance of underreporting; the reported 28% may be lower than actual drug use. The lack of difference in multivariable models with and without reported injection drug use, as well as the similar age, sex,

Table 3. Time trends for HIV among tuberculosis patients in Ho Chi Minh City, Vietnam, 1997–2002*

Variable	OR† (95% CI)	p value‡
Age, y		0.001
≤24	4.49 (1.3–15.3)	
25–34	2.82 (0.9–8.6)	
35–44	0.67 (0.2–2.3)	
>44§	1	
Risk category		0.005
IDU	10.56 (1.6–66.6)	
Non-IDU§	1	

*OR, odds ratio; CI, confidence interval; IDU, injection drug user.
†OR from time × variable in model; OR>1 indicates a faster rise in HIV prevalence in that category than in the baseline category.
‡p value for likelihood ratio χ^2 test for excluding variable from model.
§Baseline category.

and marital status distributions of IDU and non-IDU HIV-infected patients, supports this possibility.

Under the assumption of a causal relationship between infection with HIV and the risk for active TB (*1*), PAF calculations show that HIV was directly responsible for >9% of TB cases during the last 2 years of our study. This finding explains the increase of TB reporting rates, especially for young men.

The relevance of our data goes beyond the explanation of increasing TB reporting rates in Ho Chi Minh City. Dye et al. predicted that in settings with no HIV, reaching the WHO targets for DOTS would result in an annual decrease of ≥7% in TB reporting rates (*8*). However, correction for HIV only resulted in a small (0.3%) decline, showing that direct effect of HIV provides only partial explanation for the observed lack of effect of DOTS in Ho Chi Minh City. An additional explanation that we have not studied may be indirect effect of the HIV epidemic, i.e., through increased transmission of *M. tuberculosis* by HIV-infected TB patients. Although recent studies from sub-Saharan Africa have shown mixed results on this issue (*21*–*23*), those data are from settings with a generalized HIV epidemic, and the effect of an epidemic that is concentrated in IDUs may be different, especially in inner city areas.

Other explanations for the lack of decline in TB reporting rates in Ho Chi Minh City include private sector involvement (*24*), internal migration, and perhaps emergence of the Beijing genotype (*25*). In addition, the case detection rate reported by WHO for Vietnam may overestimate that for Ho Chi Minh City. These explanations may apply especially to other parts of Vietnam, where a similar lack of decline in TB reporting rates is observed in the absence of high rates of HIV infection among TB patients (*26*).

Limitations

Apart from underreporting of risk factors, other limitations may have affected our results. For patients in the districts included in this surveillance, TB may have been diagnosed outside the surveillance project, e.g., in the city's TB referral hospital or the private sector, which predominantly diagnose smear-negative and extrapulmonary TB. These diagnoses were reported for 29% of the patients in our study compared with 35% of all patients reported in Ho Chi Minh City over the study period. Our data may therefore underrepresent patients with smear-negative and extrapulmonary TB and may have underestimated or overestimated the HIV infection prevalence among them. Our estimates of the impact of HIV infection on TB reporting rates, however, will not be subject to such bias because these were based on new smear-positive patients only.

We have no data on levels of CD4+ lymphocytes and could not stage immune depletion in HIV-infected patients. Whether a case of TB in an HIV-infected patient was due to advanced immune depletion or would have occurred regardless of HIV infection is thus unknown. We have dealt with this possible bias by applying the PAF, which measures excess cases only (*27*). The PAF depends on the RR of TB for HIV-infected persons compared with non–HIV-infected persons and thereby on the level of immune deple-

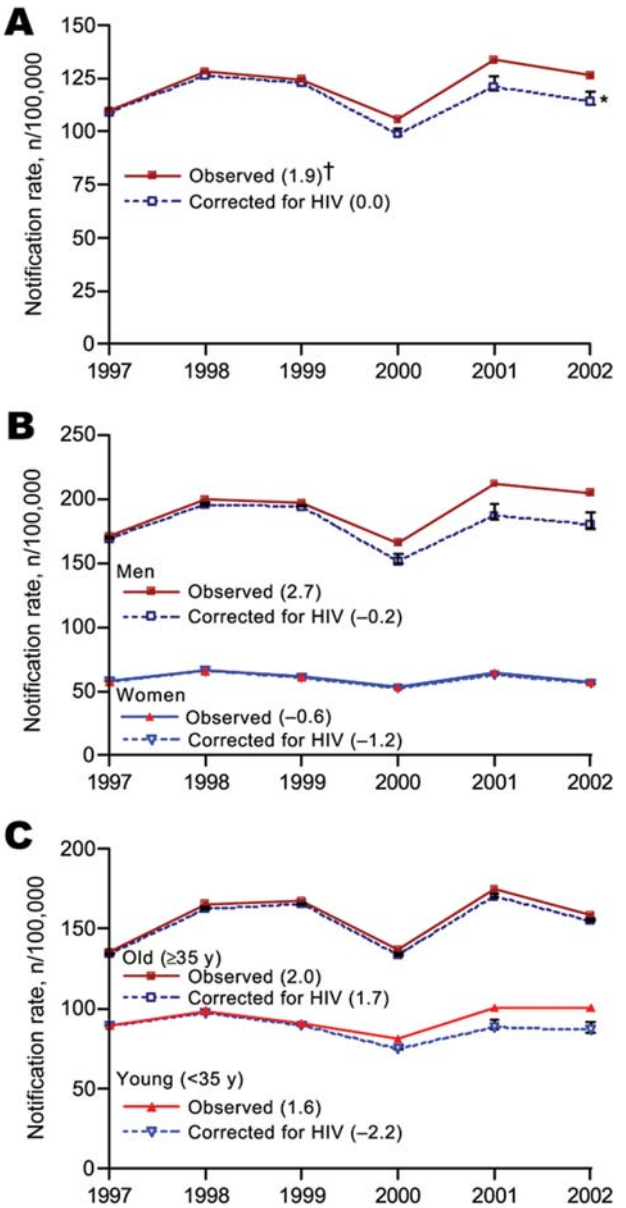

Figure. Trends in notification rates of new smear-positive tuberculosis (TB) cases in Ho Chi Minh City, Vietnam, observed and after correction for proportion of cases attributable to HIV infection. Total population (A), sex (B), and age specific (C). Correction of notification rates based on population attributable fraction to HIV infection assuming a risk ratio (RR) of 5 for risk for TB among HIV-infected compared with non–HIV-infected populations. *Error bars indicate corrected rates based on assumption that RR = 2 (top) or RR = 10 (bottom). †Exponential annual change (expressed as percentage) of TB notification rates.

tion. Because no estimates of this RR are known for the Vietnamese setting, we assumed a value of 5, which is in accordance with RRs found in several studies conducted elsewhere (*3*,*28*,*29*). We also applied values of 2 and 10 and increased the RR over the study period, simulating an increasingly vulnerable population. Neither affected our results in any relevant way.

The withdrawal of IDUs from regular surveillance in 2002 may also have caused bias. But as our simulations showed, the absence of 50% of IDUs reduced the size of the effect but not its direction.

Recommendations

We recommend that in Ho Chi Minh City all TB patients be tested for HIV because detection of HIV infections can help prevent some of the excess deaths in this population (cotrimoxazole preventive treatment and antiretroviral therapy) (*30*,*31*). To prevent active TB, prophylactic isoniazid treatment for HIV-infected patients could be considered (*31*,*32*). However, a recent study from Ho Chi Minh City showed that isoniazid resistance levels might be too high (>25% in new TB patients) for successful implementation (*33*). Highly active antiretroviral therapy is being introduced and is expected to reduce the risk for TB disease in HIV-infected patients (*34*–*36*). Injection drug use clearly remains a potent source of health problems; efforts to reach out to the vulnerable population of IDUs should be sustained and increased. In addition to the interventions mentioned, TB screening for HIV-infected IDUs and TB treatment for those found to have TB disease should be considered.

Conclusions

Ho Chi Minh City is now faced with a combined HIV/TB epidemic, predominantly among young men, which reduces the success of TB control. However, HIV alone does not fully explain the lack of a strong decline in TB reporting rates.

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Epidemiology of Schistosomiasis in the People's Republic of China, 2004

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Results from the third nationwide cluster sampling survey on the epidemiology of schistosomiasis in the People's Republic of China, conducted by the Ministry of Health in 2004, are presented. A stratified cluster random sampling technique was used, and 239 villages were selected in 7 provinces where *Schistosoma japonicum* remains endemic. A total of 250,987 residents 6–65 years of age were included in the survey. Estimated prevalence rates in the provinces of Hunan, Hubei, Jiangxi, Anhui, Yunnan, Sichuan, and Jiangsu were 4.2%, 3.8%, 3.1%, 2.2%, 1.7%, 0.9%, and 0.3%, respectively. The highest prevalence rates were in the lake and marshland region (3.8%) and the lowest rates were in the plain region with waterway networks (0.06%). Extrapolation to all residents in schistosome-endemic areas indicated 726,112 infections. This indicates a reduction of 16.1% compared with a nationwide survey conducted in 1995. However, human infection rates increased by 3.9% in settings where transmission is ongoing.

Schistosomiasis, which is caused by *Schistosoma japonicum*, is one of the most serious parasitic diseases in the People's Republic of China despite a documented history >2,100 years. The first reported clinical case in modern China was made by an American physician in 1905 (*1*). On the basis of limited hospital-based data and fragmentary epidemiologic survey reports, schistosomiasis japonica in 1947 was endemic in 138 counties in China. The rural population at that time in those counties was ≈25.3 million, which was the at-risk population. The estimated number of people infected with *S. japonicum* was 5.3 million (*2*). Mao estimated that 32.8 million Chinese were infected with *S. japonicum* in the late 1940s (*2*). However, use of different sources and province-specific prevalence data showed higher estimates of the number of people infected and at-risk populations (*3*,*4*).

Since 1949, after the founding of the People's Republic of China, large-scale epidemiologic surveys were conducted by Chinese health workers to identify schistosomiasis-endemic areas, prevalence and incidence of this disease, and number of deaths caused by *S. japonicum* infections. Results showed that schistosomiasis was endemic in 12 provinces, with an estimated 11.6 million people infected. There were 1.2 million infected cattle and an area of 14,300 km^2 was infested by the intermediate host snail, *Oncomelania hupensis* (*5*).

Over the past 50 years, the ongoing national control program has made great progress in controlling this disease. To date, 5 of 12 formerly *S. japonicum*–endemic provinces and >60% of disease-endemic counties have reached the national criteria of transmission interruption, and the number of human infections has been reduced by >90% (*6*–*8*). However, in 2003, 110 counties had not yet reached the criteria for transmission control, i.e., the overall prevalence in disease-endemic villages of these counties was >1% (*9*,*10*). The epidemiology of *S. japonicum* in China and achievements made in its control have been reviewed (*11*). New data suggest that progress has stalled since the termination

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of the World Bank Loan Project (WBLP) for schistosomiasis control at the end of 2001 (*7*,*9*,*12*,*13*).

In 1989, the first nationwide schistosomiasis sampling survey was conducted by the Chinese Ministry of Health to determine the prevalence of *S. japonicum* in all regions of the country where transmission occurs (*14*–*16*). An estimated 1,638,103 people were infected (*8*,*16*). Six years later, the second nationwide schistosomiasis survey was completed. The number of people infected with *S. japonicum* had been reduced by >40% to 865,084 (*15*). Nevertheless, there is considerable concern that schistosomiasis has reemerged in some adjacent areas of hyperendemic regions in the new millennium (*9*,*17*–*19*).

The third nationwide cluster sample survey was conducted in 2004 to update epidemiologic data for schistosomiasis in China. The data generated can serve as benchmarks for design of a new framework of a national control program that includes mid-term and long-term goals and a more flexible strategy. The National Institute of Parasitic Diseases (IPD) at the Chinese Center for Disease Control and Prevention in Shanghai (*10*) was entrusted by the Ministry of Health to design, manage, and supervise this survey in close collaboration with specialized provincial institutions in the 7 provinces where *S. japonicum* was endemic.

Materials and Methods

Sampling Strategy and Study Population

The third nationwide schistosomiasis sampling survey covered all 7 schistosome-endemic provinces (Anhui, Hubei, Hunan, Jiangsu, Jiangxi, Sichuan, and Yunnan). The sampling unit was the administrative village (i.e., basic level of administration, often comprising >1 natural village), with only those villages selected where transmission was ongoing.

A stratified cluster random sampling technique with 3 strata was used for village selection. The 7 schistosome-endemic provinces represented the first stratum. Within each province, the second stratum was categorized by characteristics of environmental ecosystems defined and widely used by Chinese health workers, which includes 8 subtypes: 1) fork beach, 2) islet without embankment, 3) islet with embankment, 4) inner embankment in the lake and marshland region, 5) plateau, 6) mountain, 7) hill in the hilly and mountainous region, and 8) waterway networks in the plain region (*20*). Within each ecosystem, estimated local prevalence of *S. japonicum*, which was based on results of recent parasitologic examinations, served as the third stratum and used cut-off prevalences of 1%, 5%, and 10%. In each disease-endemic province, ≈1% of administrative villages of the same prevalence class and ecosystem type were randomly selected. One administrative village was randomly selected if the total number of villages was <100 (Figure 1).

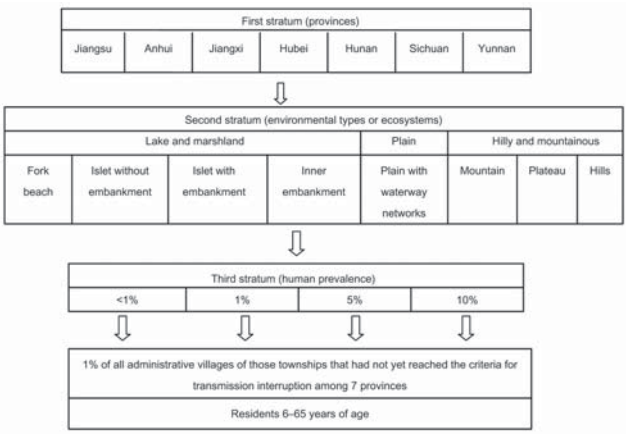

Figure 1. Design of the cluster sampling survey for schistosomiasis, People's Republic of China.

All residents 6–65 years of age in selected villages were invited to participate in the study. If the total number of eligible persons was <1,000, residents from neighboring villages with similar ecoepidemiologic characteristics were recruited until ≥1,000 persons were included.

Diagnostic Approach

All participants were screened for *S. japonicum*–specific immunoglobulin G by using a standardized ELISA (Shenzhen Kangbaide Biotech Co., Shenzhen, People's Republic of China). Seropositive persons were tested by stool examination. One stool specimen was obtained from each participant, and 3 Kato-Katz thick smear slides were prepared and examined under a light microscope by experienced laboratory technicians (*21*). The number of *S. japonicum* eggs was counted on each slide, and the arithmetic mean value was calculated for each person.

In a subsample of villages (n = 25), study participants were interviewed regarding previous infection with *S. japonicum* and previous treatment history. Common symptoms, e.g., abdominal pain and diarrhea, were investigated by using a recall period of 2 weeks. Liver and spleen enlargement and the degree of hepatic fibrosis were assessed with a portable ultrasound device. Assessment of illness was performed on supine persons during baseline respiration who had fasted. The fibrotic degree of liver parenchyma was graded between 0 and 3 according to criteria of the World Health Organization–sponsored Cairo Working Group (*22*,*23*).

In 11 villages selected at random from different ecosystems and for different infection levels, seropositive study participants provided 1 stool specimen and 3 Kato-Katz thick smear slides were prepared. Miracidium hatching test after concentration of eggs with a nylon tissue bag was used. Persons with positive results in the miracidium hatching test or with eggs on the slides were identified as

having *S. japonicum* infections and served as the standard for the diagnosis (*24–26*). Comparisons of results of 2 diagnostic approaches enabled calculation of sensitivity of the Kato-Katz technique.

Data Management and Statistical Analysis

Samples of every new batch of ELISA kits used in the survey were randomly selected and tested with standard serum samples to determine their sensitivity and cross-reactivity with antibodies against hepatitis B virus and *Paragonimus* spp. Rates of false-negative results of serologic tests (compared with the Kato-Katz method) and Kato-Katz thick smears (compared with the hatching test) were calculated.

All data were checked for internal consistency before double entry into standardized databases at provincial institutes. Senior scientists from IPD validated data and established a masterfile from which coverage, compliance, prevalence, and intensity of *S. japonicum* infection and total estimates of persons infected were calculated. Statistical analyses were performed by using SAS version 8.0 (SAS Institute Inc., Cary, NC, USA). The following formulas were used to calculate the corrected human infection rate in the village: infection rate $p = (x/X)/(1 - Q) \times (f/F)/(1 - R)$ and variance $S^2_p = p(1 - p)/(X - 1)\ (1 - X/n)$, where p is the corrected human infection rate in a sampled village, S^2_p is the variance of p, x is the number of the seropositive persons, X is the number of eligible persons screened by the serologic test, f is the number of stool-positive persons by the Kato-Katz technique, F is the number of persons tested by the Kato-Katz technique, Q is the false-negative rate of the serologic test, R is the false-negative rate of Kato-Katz thick smears, and n is the total population in a sampled village.

The χ^2 test was used to compare *S. japonicum* infection rates between provinces, ecosystems, occupational groups, sex and age. Ordinal logistic regression analysis was used to investigate whether there was an association between serologic results and the degree of liver fibrosis, stratified by ecosystem.

Quality Control

A rigorous quality control system was implemented throughout the study. Blood samples and Kato-Katz thick smear slides from each survey were kept, and 5% of the samples were randomly selected for re-evaluation in the respective provincial institutes. Surveys were repeated in villages where quality control showed a sensitivity <90%. All original data were entered twice, and 10% of original data were compared with submitted databases at IPD. Data from sites where level of agreement was <95% were re-entered.

Results

Disease-Endemic Villages, Sampling Scheme, and Compliance

Overall, 17,542 administrative villages with an estimated population of 29,059,194 were classified as endemic for *S. japonicum* in 2004. Online Appendix Table 1 (available from www.cdc.gov/EID/content/13/10/1470-appT1.htm) summarizes the number of villages and persons stratified by different levels of endemicity. Prevalence of *S. japonicum* infection was <1% in >50% of villages (n = 9,243) inhabited by >15 million persons. A total of 1,334 villages (7.6%) were classified as settings where infection prevalence was >10%. Estimated population size in these settings was 2,059,211 (7.1%).

The present survey was conducted in 239 villages and included 291,167 persons 6–65 years of age. This corresponds to 1.4% of all schistosome-endemic villages and 1.0% of the population living therein. Compliance to undergo a serodiagnostic test and, among *S. japonucum*–positive persons, to submit a stool sample was high (86.2%).

Infection Rate and Estimated Number of Persons Infected

Overall, 30,680 (12.2%) of 250,987 participants were positive for *S. japonucum* by ELISA. Of these persons, 94.2% submitted a stool specimen and 9.1% of these specimens were egg positive. Estimated corrected *S. japonicum* prevalence, when sensitivity of the Kato-Katz method in *S. japonicum*–endemic villages was taken into account, was 2.5%. Prevalence varied considerably by province, from 0.3% (Jiangsu) to 4.2% (Hunan). Prevalence rates above average were found in Hubei (3.8%) and Jianxi (3.1%) Provinces, and prevalence rates in Anhui, Yunnan, and Sichuan Provinces were 2.2%, 1.7% and 0.9%, respectively.

Stratification by ecosystem showed that the highest corrected prevalence was found in the lake and marshland region (3.8%). Overall prevalence in the hilly and mountainous region was 1.1%. Human infection rates among different subtypes of disease-endemic areas showed that the highest rate (7.1%) was seen in the subtype mountain regions, followed by subtypes islets with embankment (4.3%), inner embankment (4.3%), fork beach (3.4%), and islet without embankment (2.7%). Lower prevalence rates were observed in subtypes hill (1.01%) and plateau (0.3%) in the hilly and mountainous regions. A lower prevalence rate of 0.06% was found in the plain region characterized by waterway networks.

As shown in Figure 2, prevalence of *S. japonicum* infection in male study participants (2.6%) was higher than that in female participants (2.2%). There was a tendency

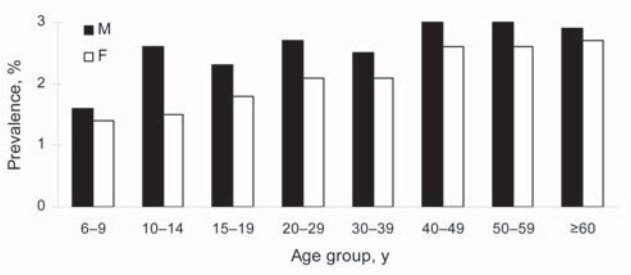

Figure 2. Corrected *Schistosoma japonicum* infection prevalence rates in humans stratified by age and sex, 2004, People's Republic of China.

for prevalence to increase with age, with the highest prevalence found in men 40–49 years of age. Prevalence of infection also varied with occupation. The prevalence in fishermen and boatmen was 3.3%, which was significantly higher than in other professional groups ($p<0.001$). However, no significant association was found between infection prevalence and educational level (Table).

On the basis of survey results, total number of persons infected with *S. japonicum* in China in 2004 was estimated to be 726,112 (95% confidence interval [CI] 714,497–737,728). More than 82% of infected persons lived in lake and marshland regions (596,599, 95% CI 585,910–607,287), and most of the remaining persons resided in hilly and mountainous regions (128,720, 95% CI 124,206–133,233).

Infection Intensity

The geometric mean of infection intensity was 33 eggs per gram (EPG) of feces among egg-positive persons and 0.4 EPG in the general population. Stratified by province, the highest geometric mean among egg-positive persons was found in Jiangxi (56 EPG) and Hunan (0.70 EPG) when the general population was considered. With regard to ecosystem stratification, the highest infection intensity in egg-positive persons was in the plain region with waterway networks (128 EPG), and the highest infection intensity in the entire population was in the lake and marshland regions (0.5 EPG).

Male study participants had a higher infection intensity than females, both among egg-positive persons (34 vs. 30 EPG) and the general population (0.4 vs. 0.3 EPG). No clear trend emerged when stratification was conducted by age, but intensity of infection was generally less variable between age groups in male participants and decreased somewhat with age in female participants. With regard to occupation, fisherman, boatmen, and preschool children had the highest geometric mean infection intensity (0.9 EPG), followed by farmers and students (0.4 EPG). The infection intensity generally decreased with higher educational level (Table).

Illness and Self-reported Symptoms

Results of *S. japonicum*–related illness, as assessed by ultrasonography, and self-reported signs and symptoms, are shown in online Appendix Table 2 (available from www.cdc.gov/EID/content/13/10/1470-appT2.htm). Seropositive persons were more likely to report diarrhea over the past 2 weeks than seronegative persons. Degree of liver fibrosis was positively associated with a positive serologic result and with residency in the lake and marshland region. Persons living in the plain region with waterway networks had lower levels of liver fibrosis. No association was found between serologic status and hepatomegaly or splenomegaly.

Discussion

Results of the third nationwide cluster sampling survey on the epidemiologic status of schistosomiasis provide a comprehensive update on the current extent and distribution of human *S. japonicum* infections in China. The epi-

Table. ELISA and Kato-Katz thick smear results for *Schistosoma japonicum* infection prevalence and intensity, stratified by occupation and education, People's Republic of China*

Characteristic	ELISA		Kato-Katz		Corrected prevalence, %	Geometric mean EPG (infected)	Geometric mean EPG (population)
	No. examined	Positive rate, %	No. examined	Positive rate, %			
Preschool children	848	8.7	65	18.5	0.4	31	0.9
Students	61,430	6.7	3,861	9.7	1.8	37	0.4
Farmers	184,325	14.0	24,285	8.9	2.6	32	0.4
Fishermen and boatmen	1,150	25.0	284	16.5	3.3	54	0.9
Others	3,234	14.0	413	6.5	2.5	30	0.3
Education level							
Illiterate	14,078	18.9	2,559	9.1	2.5	33	0.4
Primary school	119,016	12.2	13,655	9.2	2.5	35	0.4
Junior high school	101,283	11.4	10,917	8.5	2.6	31	0.3
Senior high school	11,616	10.4	1,160	9.3	2.6	31	0.4
Additional education	1,993	7.1	136	10.3	2.3	27	0.4
Unknown	3,001	20.5	481	18.3	2.3	25	0.8
Total	250,987	12.2	28,908	9.1	2.5	33	0.4

*EPG, eggs per gram of feces.

demiology of schistosomiasis japonica in China is closely related to local environmental conditions (*20*). Thus, schistosome-endemic areas in China have traditionally been classified according to local environment and local prevalence of infection. The Ministry of Health created different thresholds to classify areas into distinct stages of control. For example, once infection prevalence of *S. japonicum* decreases to <5%, this disease-endemic setting is considered to have reached infection control. Transmission control is declared once local prevalence decreases to <1%. Transmission interruption has been achieved if <2 of 1,000 stool samples examined are egg positive and no new cases have occurred for 5 years. Once this level has been achieved, local elimination can be declared after another surveillance period for 5 years without new infection (*8*,*12*,*27*,*28*).

The 2004 nationwide schistosomiasis survey, which included a correction factor because of the low sensitivity of the Kato-Katz technique, indicated an estimated 726,112 *S. japonicum* infections in humans with a small 95% CI. This estimate is lower than other recent estimates of other research groups (*29*–*32*). During the survey, the Kato-Katz technique and the miracidium hatching test were used for fecal examination. It is well known in China that the hatching test is more sensitive than the Kato-Katz technique because the volume of fecal material used for parasitologic detection is several hundred times higher than that used on Kato-Katz slides. However, because immature eggs of schistosomes cannot hatch and egg excretion of *S. japonicum* is not uniform, samples that showed no hatching may show eggs in Kato-Katz slides. The hatching test and Kato-Katz technique are mutually complementary and provide higher sensitivity in parasitologic diagnosis of *S. japonicum* infection. A combination of these 2 techniques was used in 11 villages as the standard for diagnosis in calculating the false-negative rate of the Kato-Katz technique and is believed to be the most sensitive method for individual and community diagnosis in determining true prevalence (*33*).

The WBLP for schistosomiasis control was started in 1992. It emphasized praziquantel-based control of illness, and progress in disease control was made (*12*). However, in subsequent years, data suggested that schistosomiasis has reemerged (*7*,*9*). Several factors have been suggested as underlying causes, such as unusually severe floods in 1998 (*34*), major ecologic transformations caused by water resource development (*35*), potential effects of climate change (*36*,*37*), market and health sector reforms (*38*), and termination of the WBLP for schistosomiasis control and insufficient attention to control efforts until 2001 (*29*).

Because of conceptual and technical differences, direct comparison is not possible between the current survey and the previous ones conducted in 1989 and 1995. In addition, the current survey covered a wider geographic area because it included all schistosome-endemic areas in which transmission has not yet been interrupted. In previous surveys, the focus was on comparatively smaller areas that had not reached transmission control status, i.e., had a prevalence ≥1%.

Notwithstanding these differences, several conclusions can be drawn, which in turn are relevant for future design, implementation, and monitoring of schistosomiasis control program in China. The main differences between the second and third nationwide schistosomiasis surveys are summarized in online Appendix Table 3 (available from www.cdc.gov/EID/content/13/10/1470-appT3.htm). First, estimated number of human cases decreased to 726,112 from 865,084 in the mid-1990s. Consideration of areas where transmission was not controlled showed that the number of infected persons decreased from 847,584 in the second survey to 710,790 in the current survey; a decrease of 16.1%. Second, the number of villages in the areas where transmission was still ongoing has been reduced by 40.3% from 13,911 in 1995 to 8,299 in 2004. Third, the number of people at risk in remaining disease-endemic villages was 13,937,235, a decrease from 22,209,662 in the mid-1990s and a reduction of 37.3%. Finally, areas where schistosomiasis transmission control had not been achieved decreased considerably (online Appendix Figure 1, available from www.cdc.gov/EID/content/13/10/1470-appG1.htm, and online Appendix Figure 2, available from www.cdc.gov/EID/content/13/10/1470-appG2.htm). These findings underscore that the national schistosomiasis control program in China has made further progress over the past decade. Conversely, human prevalence rates in the areas where transmission control has not been achieved increased from 4.9% in 1995 to 5.1% in the present survey, an increase of 3.9%.

Estimated corrected *S. japonicum* prevalence in the current survey averaged over all schistosome-endemic areas was 2.5%. Stratification by province and ecosystem showed spatial heterogeneities. Mean prevalence in villages with an infection rate >1% decreased in Hubei, Hunan, and Yunnan Provinces. In Yunnan, the decrease was most pronounced (–58.6%). Increased prevalences were observed in Jiangsu and Sichuan; prevalence increased from 0.03% to 2.9% in Jiangsu. A fairly constant prevalence was observed in Anhui. Highest corrected prevalence was in the lake and marshland region of eastern and central China, and a low prevalence was found in the mountainous region of Yunnan and Sichuan Provinces. Highest prevalence among all ecosystems was in the mountain ecosystem (7.1%) rather than in the lake and marshland region. This finding probably resulted from decreasing control efforts for schistosomiasis after the end of the WBLP. Corrected prevalence in the plain region with waterway networks was nearly zero. When compared with the previous nationwide survey, prevalence increased by 20.6%–59.0% in fork

beach and mountain and hill ecosytems, but decreased by 8.9%–39.7% in other ecosystems.

Typical features of chronic *S. japonicum* infection include pathologic changes in the spleen and liver (*39*). Illness assessed by ultrasonography showed changes in ≈70% of all seropositive persons in the plain region with waterway networks, inner embankment, islet without embankment, and hilly ecosystems. Lower rates were found in islets with embankment, plateaus, and mountain ecosystems. In addition, 174 cases of advanced schistosomiasis were detected. Most of these advanced cases were found in the lake and marshland region but 22.4% were in the mountainous and hilly region, a slightly higher percentage than the fraction of the total number of estimated cases in this area.

Additional progress has been made in control of schistosomiasis in China over the past decade. However, currently used control strategies and tools will not eliminate schistosomiasis in certain areas if these strategies are used at the same level of intensity. Applied research on schistosomiasis control is needed to develop new approaches to further reduce infection in these hotspots of transmission. Results of the third national schistosomiasis survey provide a comprehensive overview of the current epidemiology of the disease, which is crucial for the design of the next 5-year plan on schistosomiasis control. This overview will help create at the local level better control programs that include current epidemiologic and socioeconomic conditions to increase their efficiency, address remaining challenges, and avoid reemergence of *S. japonicum* infection in areas where it had been controlled (*28*,*31*). By consideration of these challenges and potential risks for transmission of schistosomiasis in China, such as ecosystem changes caused by construction of the Three Gorges Dams on the Yangtze River and effects of global warming (*7*,*18*,*19*,*29*), the Chinese central government has given high priority to control of schistosomiasis and new control goals have been made (*7*,*40*). One goal is to achieve transmission interruption in the hilly and mountainous regions and the plain regions with waterway networks by 2015. The same time frame has been set for transmission control in the lake and marshland region. To attain these goals, renewed efforts are needed to further improve available tools and develop additional control strategies; adapt programs to changing demographic, ecologic, and socioeconomic issues; implement new strategies; and achieve schistosomiasis control in China.

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etymologia

schistosomiasis

[shis''-, skis'' to-so-mi'ə-sis], from the Greek—s*khistos* (split) and *soma* (body)

Infection of the blood with a parasite of the genus *Schistosoma*. Originally thought a single organism with a split body, the parasite was eventually recognized as having male and female forms. Three main species cause human infection: *S. haematobium, S. mansoni,* and *S. japonicum*. Each species has its own range of host snails. The parasite releases eggs containing larvae through feces or urine; if the eggs reach water, the larvae are released and may penetrate a snail. A very large number of larvae are then produced inside the snail and released back into the water. Infection is acquired through skin contact with contaminated water.

Schistosomiasis, which leads to chronic hepatic and intestinal fibrosis of the urinary tract, was first identified in Egypt in 1851 by German pathologist Theodor Bilharz and is also called bilharzia. Approximately 160 million persons throughout the world are infected, particularly in Africa, the Middle East, South America, and Southeast Asia.

Source: Institute of Tropical Medicine of Antwerp: www.itg.be

Dengue Fever Seroprevalence and Risk Factors, Texas–Mexico Border, 2004

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Reported autochthonous dengue fever transmission in the United States has been limited to 5 south Texas border counties since 1980. We conducted a cross-sectional serosurvey in Brownsville, Texas, and Matamoros, Tamaulipas, Mexico (n = 600), in 2004 to assess dengue seroprevalence. Recent dengue infection was detected in 2% (95% confidence interval [CI] 0.5%–3.5%) and 7.3% (95% CI 4.3%–10.3%) of residents in Brownsville and Matamoros, respectively. Past infection was detected in 40% (95% CI 34%–45%) of Brownsville residents and 78% (95% CI 74%–83%) of Matamoros residents. For recent infection, only weekly family income ≤$100 was a significant predictor (adjusted odds ratio 3.2, 95% CI 1.3–8.0). Risk factors that predicted past dengue infection were presence of larval habitat, absence of air-conditioning and street drainage, and weekly family income ≤$100. Mosquito larvae were present in 30% of households in both cities. Our results show that dengue fever is endemic in this area of the southern Texas–Mexico border.

Dengue fever is the most prevalent mosquitoborne viral disease in the world, causing an estimated 50 million infections and 25,000 deaths annually, with at least 2.5 billion persons at risk for transmission (*1–4*). Reports of autochthonous dengue fever transmission on the US side of the Texas–Mexico border have been rare—only 64 cases were reported during 1980–1999, compared with 62,514 cases on the Mexican side of the border (*5–10*). In the debate over the potential for expansion of dengue and malaria with climate change, the border region has been cited as evidence that mosquitoborne diseases are largely determined by public health capacity and socioeconomic factors, and specifically that US affluence and lifestyle limit transmission of the disease (*5*,*11–13*). These conclusions, however, are largely based on incidence reports obtained from passive surveillance that contrast with the epidemiologic dengue situation on the ground.

Recent studies (*14*; J. Brunkard, unpub. data) suggest that dengue is substantially underreported on both sides of the border and prompted us to conduct an epidemiologic investigation in the neighboring cities of Brownsville, Texas, USA, and Matamoros, Tamaulipas, Mexico. Our primary objectives were to assess population seroprevalence of dengue and to identify the most important risk factors for regional transmission. Public health agencies from both countries at the local, state, and national levels collaborated on the project. To our knowledge, this is the first dengue seroprevalence study conducted in the lower Rio Grande Valley since 1980 (*6*).

Materials and Methods

Survey Design

In the fall of 2004, we conducted a binational, cross-sectional serosurvey at the household level in Brownsville and Matamoros to measure dengue prevalence in the region. We interviewed members of 300 households in each

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city for a total sample size of 600. Household selection was probability-based, using a stratified, multistage, cluster-sampling design. In the first stage, 50 census tracts and 50 basic geostatistical areas, the Mexican equivalent of the census tract, were selected by using probability-proportional-to-size sampling with replacement. In the second stage, 3 census blocks were randomly selected from each census tract, and for the final stage, households were randomly or systematically selected.

The sampling frame was based on year 2000 census data for both the United States and Mexico (*15*,*16*). However, at the final stage, we counted all houses in the block on-site and randomly selected starting points, allowing for the incorporation of population changes since the 2000 censuses were conducted.

Household Serosurvey

We collected a blood sample (5 mL intravenously) from 1 volunteer per household (≥15 years of age), conducted larval inspections in and around the house, and interviewed participants by using a household survey that measured risk factors for dengue and public perception about the disease. Two survey teams consisting of 2 interviewers, a medical professional, and an entomologist worked concurrently in both cities to control for seasonal and temporal variance. The survey was timed to coincide with the height of the traditional dengue season (August–December), with most cases occurring in September and October. The survey ran for 5 weeks in October and November 2004. We recorded age, sex, and length of residence in the area for all participants. We attempted to include only those residents who had lived in the region for ≥10 years, so that our seroprevalence measure would more accurately reflect regional transmission.

Before beginning the study, human subjects approval was obtained from the University of California Institutional Review Board. We pilot tested the survey questionnaire in neighborhoods in both cities in September 2004. Signed, informed consent was given by all survey participants in their preferred language (Spanish or English), including an additional consent form for the Health Insurance Portability and Accountability Act from US survey participants. Participants <18 years of age (n = 6) were required to obtain a parent's signed consent before giving their own. Participation in the survey was voluntary, and no gifts or financial incentives were offered. Survey participants were notified in person or by mail if they tested positive for recent dengue infection.

Laboratory Analysis

Serum samples were analyzed at the Laboratorio Estatal de Salud Pública Tamaulipas (State Laboratory in Cd. Victoria, Tamaulipas, Mexico) by using DUO immunoglobulin (Ig) M/IgG capture ELISA (Panbio Inc., Brisbane, Queensland, Australia) to identify recent primary and secondary infections and an indirect IgG ELISA for past dengue infection (Panbio). The Dengue Branch (San Juan, Puerto Rico) of the Centers for Disease Control and Prevention (CDC) conducted confirmatory testing on all samples that tested positive or equivocal for recent infection with capture IgM and IgG ELISAs (Panbio). CDC provided dengue-positive and -negative control serum specimens to test on the ELISA kits (Panbio) before testing the serum samples. CDC also tested a random subsample (n = 12) of serum samples that were negative for recent dengue infection.

Laboratory-based Classification

Only samples confirmed by CDC were classified as recent infections. CDC criteria included samples with presence of IgM antibodies ≥0.2 optical density (OD) or presence of IgG antibodies with titers >40,960 (*17*). The IgG ELISA performed by CDC is based on titration of the sera to determine the antibody titer of IgG in the sera. Values of 40,960 are equivalent to the hemagglutination inhibition (HI) titer of 2,560, which the World Health Organization classifies as recent secondary infections (*18*). Past infection was identified by presence of low-titer dengue IgG antibodies, as measured by indirect IgG ELISA (Panbio).

Plaque Reduction Neutralization Test (PRNT)

Additional confirmatory tests were performed by CDC on 12 positive or equivocal samples by using a 90% reduction in numbers of plaques ($PRNT_{90}$), as previously described (*19*), to determine the specificity of the antibody response to the infecting virus. Samples with a $PRNT_{90}$-positive titer for a single serotype with an IgG titer >10,240 were classified as recent infections.

Entomologic Survey

We conducted larval sampling in and around the households to identify the mosquito species present and to determine whether the presence of *Aedes aegypti*, the primary dengue vector, was associated with recent or past dengue infection. We surveyed water-holding containers inside and outside the house and collected larvae and pupae. They were identified by entomologists in both city health departments. The data were translated into house and Breteau indices, which are indicators of mosquito vector density (*20*).

Statistical Analysis

Adjusting the analysis to account for the survey design enables generalization across the statistical population. We used Stata version 9 (Stata Corp., College Station, TX, USA) for all survey design-adjusted descriptive and infer-

ential analyses. We used binomial survey–adjusted Wald tests or Wilcoxon-Mann-Whitney rank sum tests to determine significant differences in frequencies or distributions of key variables, respectively, between Matamoros and Brownsville. We conducted survey design–corrected, multivariate logistic regression based on a multivariate a priori hypothesis. We used the outcomes of recent and past dengue infection as dependent variables in separate models.

Independent variables in all models included *Ae. aegypti* and *Ae. albopictus* mosquito habitat (number of water-holding containers in and around the house), presence of air-conditioning and intact screens, household density, storage of water, street drainage, weekly family income, presence of immature *Ae. aegypti* on the premises, and history of crossing the border within the past 3 months. We constructed 3 models: separate models for recent and past dengue infection and a third model adding a dummy variable for city, which allowed us to identify the independent variables in the model most responsible for the different prevalence in past dengue infection in the 2 cities. Twenty-two exclusions were made because of missing data in the independent variables; all models contained 578 observations. We conducted Fisher exact tests to determine the effect of missing data on the dependent variables. All variables were entered into the model as a block without regard for significance level.

Results

Serologic Testing

Serologic evidence of past dengue infection was identified in 40% (95% confidence interval [CI] 34%–45%) of Brownsville residents and 78% (95% CI 74%–83%) of Matamoros residents. An additional 3% of residents in both cities tested equivocal for prior dengue infection. Seroprevalence of IgG dengue antibodies was remarkably consistent with citywide averages across all age groups within both cities except for younger persons (ages 15–24 years) in Brownsville and older persons (ages ≥65 years) in Matamoros. Seroprevalence was slightly higher in female participants in both cities, but differences were not statistically significant (Table 1).

Following a dengue infection, IgM responses are of limited duration, generally 1–2 months (*21*), and may not be elevated in secondary infections (*22*). Recent dengue infection—as indicated by presence of IgM antibodies ≥0.2 OD, IgG antibodies >40,960 (*17*), or $PRNT_{90}$ results—was identified in 2% (95% CI 0.5%–3.5%) of Brownsville residents and 7.3% (95% CI 4.3%–10.3%) of Matamoros residents. Most appeared to be secondary infections. Results from the $PRNT_{90}$ assay (n = 3) indicated that dengue serotypes 1 and 2 were circulating in the population (Table 2).

Table 1. Prevalence of IgG dengue antibodies by age and sex, Brownsville, Texas, and Matamoros, Mexico, 2004*

Characteristic	Brownsville, %	Matamoros, %
Age group, y		
15–24	8	79
25–34	45	75
35–44	43	72
45–54	45	80
55–64	35	79
65–74	43	95
≥75	38	90
Sex		
Male	35	72
Female	42	80

*IgG, immunoglobulin G.

Comparison of Panbio Inc. and CDC Test Results

The IgG capture ELISA (Panbio) calculates a positive result based in units. This test determines the sample absorbance compared to a calibrant absorbance. Based on the kit, the interpretation of a positive result is >22 units and the interpretation of this result is suggestive of a recent secondary dengue infection. The CDC IgG ELISA is based on the titration of the antibody present in the serum sample. This titration can be correlated with an HI value to determine a diagnosis of recent secondary dengue infection. When the 2 tests are compared based on the definition of recent secondary dengue infection, the Panbio test is 87.5% sensitive and 100% specific when using the CDC IgG ELISA as the accepted standard. All samples that tested positive for IgG antibodies by Panbio test kits were confirmed by CDC (*3*,*17*).

Demographics

Mean ages for Brownsville and Matamoros residents were 46.5 and 41.8 years, respectively (range 15–88 years). Most participants were female: 67% in Brownsville and 75% in Matamoros. Based on interviewer observations, we believe that the dominant reason for unequal representation of men in the survey was their reluctance to give blood. There was little difference in mean length of residence in the 2 cities (Brownsville, mean 25.6 years [range 3–77]; Matamoros, mean 29.3 years [range 8–77]). A large percentage

Table 2. Serologic test results for serosurvey, Brownsville, Texas, and Matamoros, Mexico, 2004*

Serologic test	Brownsville, n	Matamoros, n
Recent infection†	6	22
IgM ≥0.2 OD†	1	2
IgG ≥40,960	5	19
$PRNT_{90}$	1 (DEN-2)	2 (DEN-1)
Past infection‡	119	235

*IgM, immunoglobulin M; OD, optical density; PRNT, plaque reduction neutralization test; DEN, dengue virus.
†Laboratory-confirmed by the Dengue Branch, Centers for Disease Control and Prevention, defined by antidengue IgG titer >40,960 or IgM ≥0.2 OD.
‡Laboratory-confirmed by using indirect IgG ELISA (Panbio Inc., Brisbane, Queensland, Australia).

of the survey participants had lived in their respective cities their entire lives: 25.3% in Brownsville and 41.7% in Matamoros; 83% of survey participants in Brownsville and 99% in Matamoros had lived in their city ≥10 years.

Risk Factors

Many population characteristics were similar between the 2 cities: water and sewerage provision, household size, level of intact screens, and mosquito habitat and density. Key differences ($p<0.01$) included water storage practices, presence of air-conditioning, street drainage, income, presence of discarded tires, percentage of the population buying drinking water, and travel across the border (Table 3).

For recent infection, only weekly family income ≤$100 was significant as a predictor with an adjusted odds ratio (AOR) of 3.2 (95% CI 1.3–8.0), $p = 0.01$. All other variables were not significant (Table 4). Design effects for all variables included in the model ranged from 0.74 to 1.06, indicating near identical variance to a design using simple random sampling.We ran the same model using past dengue infection as the dependent variable and found several epidemiologic risk factors associated with previous dengue infection: street drainage, air-conditioning, *Ae. aegypti*, and *Ae. albopictus* larval habitat in the neighborhood, and weekly family income ≤$100 US (Table 5).

Past dengue infection was significantly different between the 2 cities (Pearson's design-based $F [1, 98] = 78.01$, $p<0.0001$). We added a city variable to the past infection model to determine its influence in explaining dengue prevalence in our model. In the model, city was highly significant (AOR 4.36, $t = 5.74$, $p<0.0005$), and the model F improved from $F (10, 89) = 5.42$, $p<0.0001$ to $F (11, 88) = 7.14$, $p<0.0001$ with the addition of city to the model. Several variables that predicted past dengue infection changed significantly with the addition of the city variable to the model including stored water, street drainage, air-conditioning, and income, indicating that the influence of these factors on past infection differed by city. We tested for collinearity among all independent variables and found none; variance inflation factors (VIF) for all tests were ≤1.82, mean VIF = 1.26, far lower than the accepted VIF >10 value for significant collinearity (*23*).

Entomologic survey

We found mosquito larvae in 30% of households in both cities, but the relative abundance of the species differed between the 2 cities (Table 6). The house index for *Ae. aegypti* differed substantially between the 2 cities (14% and 25% in Brownsville and Matamoros, respectively). *Ae. albopictus*, an exotic species first detected in Texas in the 1980s, was more abundant in Brownsville (13%) than in Matamoros (4%), while *Culex quinquefasciatus* was present at the same level in both cities. Breteau indices for all species were the same as house indices in both cities or differed by <1%.

Discussion

Brownsville and Matamoros are contiguous cities separated by the Rio Grande (Figure). Of the 6 persons with recent dengue infections in Brownsville, 4 had not crossed the border or traveled outside of the United States in the preceding 3 months and therefore acquired the infections locally (United States). Based on year 2000 census population estimates of 161,546 and 376,279 for Brownsville and Matamoros, respectively, our point prevalence for dengue infections translates to 3,231 undocumented annual dengue infections in Brownsville (95% binomial Wald CI 751–5,711) and 27,581 annual dengue infections in Matamoros (95% binomial Wald CI 16,180–38,757). The dengue season came late in 2004, with several probable cases occurring in Matamoros in December and January after the conclusion of the survey, so our seroincidence rate was

Table 3. Population characteristics and risk factors for dengue in Brownsville, Texas, and Matamoros, Mexico, 2004

Risk factor	Brownsville, %	Matamoros, %	p value*
Piped water	98	98	1.000
Buy water	95	99.7	<0.001
Sewerage	91	88	0.495
Street drainage	82	48	<0.001
Store water	4	34	<0.001
Screens present	76	64	0.009
Intact screens	40	32	0.054
Air-conditioning (room and central)	83	32	<0.001
Discarded tires	44	20	<0.001
Larval habitat	88	92	0.284
Mosquito larvae present	31	30	0.764
Crossed border (1 mo)	54	38	<0.001
Crossed border (3 mo)	66	45	<0.001
Median household weekly income ($ US)	300	100	<0.001
Mean persons/household	3.9	4.2	0.028
Mean hours/day at home	18.3	19.4	0.022

*Probability values of variables with percentages by adjusted Wald test; the remainder by 2-sample Wilcoxon-Mann-Whitney rank-sum test.

Table 4. Logistic regression results for recent dengue infection in Brownsville, Texas, and Matamoros, Mexico, 2004*

Variable	Adjusted odds ratio	p value	95% Confidence interval	Deff
Income ≤$100	3.22	0.012	1.31–7.95	0.95
Missing income	1.35	0.671	0.34–5.42	1.00
Street drainage	0.69	0.395	0.29–1.65	1.00
Larval habitat	2.20	0.381	0.37–13.07	0.74
Air-conditioning	0.74	0.543	0.28–1.96	0.94
Intact screens	0.98	0.959	0.41–2.32	1.06
Store water	1.17	0.709	0.51–2.68	0.90
Aedes aegypti	1.05	0.912	0.47–2.31	0.92
Cross border, 3 mo	0.95	0.900	0.40–2.24	1.05
People/household	0.97	0.727	0.80–1.17	0.88

*Missing data in independent variables (n = 22) did not significantly change prevalence of recent or past dengue infection (p>0.10) in the remaining 578 observations used in subsequent models. Deff, design effect, the ratio of variance between the survey design and simple random sampling.

likely an underestimate of dengue transmission for that year (*24*).

Based on our seroprevalence results for past infection, dengue infections are clearly not being identified by passive surveillance. This result was found in the outbreak of dengue in 1980 in which passive surveillance failed to detect any dengue infections, while Hafkin et al. (*6*) found 63 dengue infections through active surveillance.

Several factors may mask the region's endemic dengue transmission. One possibility is that the dengue strains circulating in the region result in mostly subclinical infections and mild diseases that do not require hospitalization and are managed through outpatient self-medication such as acetaminophen. Another reason dengue is underreported on the US side of the border may be that a large percentage of these residents cross the border into Mexico for medical diagnoses and treatments. According to our surveys, 59% of Brownsville residents regularly cross the border for medical purposes; however, only 2% of Matamoros residents went to Brownsville for their medical needs. Lack of laboratory resources to confirm dengue infection is another possible explanation. During our survey, physicians in Matamoros reported seeing a large number of patients with suspected dengue, but they were treated with acetaminophen and bed rest because resources were insufficient to conduct laboratory confirmation tests for dengue infection. The most commonly reported illness in the region is the flu.

Risk Factors

Low income across both cities was the dominant risk factor for both recent and past dengue infection. Poverty is a proxy for many risk factors that make people vulnerable to infectious diseases; some poverty-related factors were measured in this study while others were not. Our specific finding of the protective effect of air-conditioning has been found in another area of the US-Mexico border (*14*). Lack of street drainage appears to limit the ability of mosquito abatement and garbage collection trucks to enter these neighborhoods after a heavy rain. Also, the presence of water-holding containers facilitates vector proliferation in close contact to human hosts.

Epidemiologic Dynamics

Recent seroepidemiologic studies conducted in dengue-endemic countries have found high dengue seroprevalence: 29.5% in the Brazilian state of Goiás (*25*); 65.7% and 71%, respectively, among schoolchildren in Vietnam (*26*) and Thailand (*27*); 79.5% in Veracruz, Mexico (*28*); and 91% in Managua, Nicaragua (*29*). Historical accounts report widespread outbreaks affecting up to 500,000 people in the US Gulf Coast states during the Galveston, Texas, epidemic in 1922 (*30*) and outbreaks in 1934 and 1941 (*31*). However, very few population-based studies on dengue seroprevalence have been conducted in the United States. The most recent, conducted by Reiter et al. (*14*) in 1999,

Table 5. Logistic regression results for serologic evidence of past dengue infection in Brownsville, Texas, and Matamoros, Mexico, 2004*

Variable	Adjusted odds ratio	p value	95% Confidence interval	Deff
Income ≤$100	2.59	0.000	1.58–4.26	0.92
Missing income	0.90	0.679	0.54–1.50	0.83
Street drainage	0.57	0.009	0.37–0.87	1.07
Larval habitat	2.35	0.008	1.26–4.41	1.00
Air-conditioning	0.58	0.014	0.38–0.89	1.04
Intact screens	1.35	0.111	0.93–1.95	0.90
Store water	1.62	0.079	0.95–2.76	1.19
Aedes aegypti	0.84	0.476	0.53–1.35	1.05
Cross border, 3 mo	0.90	0.581	0.62–1.31	0.93
People/household	1.06	0.300	0.95–1.19	1.31

*Missing data in independent variables (n = 22) did not significantly change prevalence of recent or past dengue infection (p>0.10) in the remaining 578 observations used in subsequent models. Deff, design effect, the ratio of variance between the survey design and simple random sampling.

Table 6. House index: percentage of premises positive for a given mosquito species in Brownsville, Texas, and Matamoros, Mexico, 2004

Species	Brownsville, %	Matamoros, %	p value*
Aedes aegypti	14	25	0.003
Ae. albopictus	13	4	0.0001
Culex quinquefasciatus	5	4	0.69

*Probability values by adjusted Wald test.

found 23% seroprevalence in Laredo, Texas, and 48% seroprevalence in Nuevo Laredo, Tamaulipas, Mexico, ≈200 miles (320 km) northwest of our study area. Our population-based study reports the highest seroprevalence of dengue documented in the continental United States since at least 1950.

Demographic factors that could facilitate regional dengue transmission include immigration, which potentially introduces new strains of dengue from dengue-endemic regions in Latin America, and a high local birth rate, which introduces a steady stream of newly susceptible persons. Cocirculation of multiple dengue serotypes has been previously documented in the region (*10*) and suggested from our results by the $PRNT_{90}$, and cases of dengue hemorrhagic fever have increased in Mexico in the past 2 decades (*24*,*32*,*33*). This, coupled with the high background seroprevalence identified in this study, places the border population at greater risk of future dengue hemorrhagic fever outbreaks (*34*,*35*), although the role of sequential infections in disease severity is contested (*36*,*37*).

This study was motivated in part by the climate–dengue debate. While the role of climate change on future dengue transmission is unclear, we find that dengue is already a problem in this area of the US–Mexico border. Because dengue infections are not being identified through local surveillance efforts, we recommend proactive physician outreach emphasizing the potential for dengue infections and increased access to dengue diagnostic tests, especially on the Mexican side of the border, where a large proportion of US and Mexican border residents seek their primary medical care. Improved systems of active binational surveillance for dengue infections are needed, and sentinel sites should include the network of high-volume private clinicians practicing at the border. Ultimately, investments in local infrastructure, improvements in household screening, economic assistance for air-conditioning in dengue-endemic areas, and sustained community education about the importance of reducing larval habitat around the home will be necessary to reduce dengue transmission in this region.

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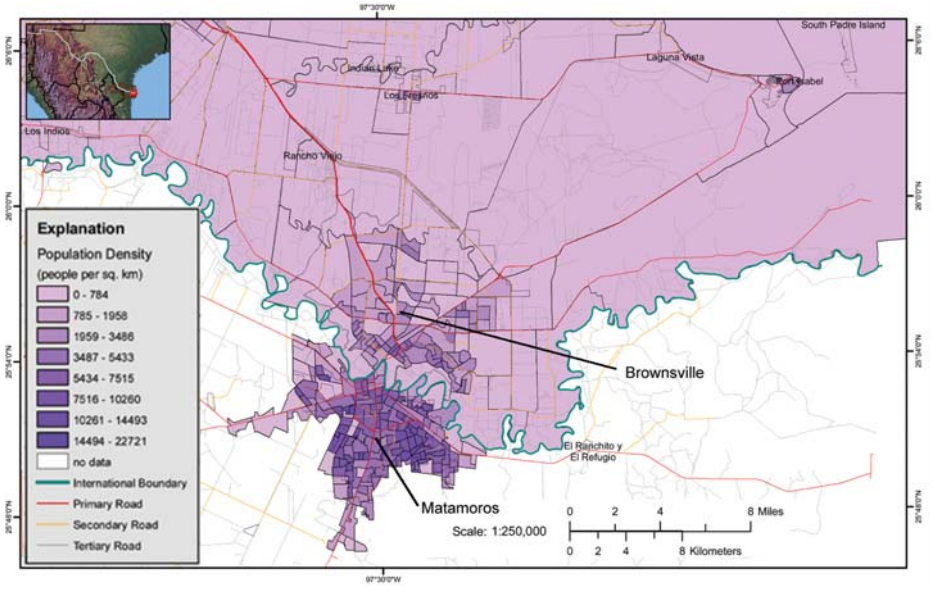

Figure. Map of Brownsville, Texas, and Matamoros, Mexico, contiguous cities on the US–Mexico border. Source: US Geological Survey; available from http://borderhealth.cr.usgs.gov/staticmaplib.html

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Cost-effectiveness of Algorithms for Confirmation Test of Human African Trypanosomiasis

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The control of *Trypanosoma brucei gambiense* human African trypanosomiasis (HAT) is compromised by low sensitivity of the routinely used parasitologic confirmation tests. More sensitive alternatives, such as mini-anion exchange centrifugation technique (mAECT) or capillary tube centrifugation (CTC), are more expensive. We used formal decision analysis to assess the cost-effectiveness of alternative HAT confirmation algorithms in terms of cost per life saved. The effectiveness of the standard method, a combination of lymph node puncture (LNP), fresh blood examination (FBE), and thick blood film (TBF), was 36.8%; the LNP-FBE-CTC-mAECT sequence reached almost 80%. The cost per person examined ranged from €1.56 for LNP-FBE-TBF to €2.99 for LNP-TBF-CTC-mAECT-CATT (card agglutination test for trypanosomiasis) titration. LNP-TBF-CTC-mAECT was the most cost effective in terms of cost per life saved. HAT confirmation algorithms that incorporate concentration techniques are more effective and efficient than the algorithms that are currently and routinely used by several *T.b. gambiense* control programs.

Human African trypanosomiasis (HAT) is a parasitic disease that affects 36 countries in sub-Saharan Africa. The most recent World Health Organization (WHO) prevalence estimates are 50,000–70,000 cases worldwide, based on a total number of 17,500 new HAT cases per year worldwide (*1*). *Trypanosoma brucei gambiense* HAT control activities are based principally on the active detection of cases by population screening and subsequent treatment of infected patients. Because of the relative toxicity of HAT drugs, a correct diagnosis is essential before the treatment can begin (*2*).

The specificity of the card agglutination test for trypanosomiasis (CATT) used in screening is not 100% accurate, so HAT control programs use a variable sequence of parasitologic tests as confirmation tests. In the Democratic Republic of Congo (DRC), this sequence, called the standard algorithm, comprises lymph node puncture (LNP), followed by fresh blood examination (FBE) and thick blood film (TBF). Several authors have reported on the low sensitivity levels of HAT confirmation tests (*3*,*4*). Paquet et al. reported that mobile HAT screening teams in Uganda detected only 39% of all HAT cases (*5*). HAT cases missed by population screening will later be diagnosed by fixed health services operating in the same areas as the mobile teams, but almost invariably not until the late stages of the disease. HAT confirmation is more straightforward (*6*). Late-stage detection is problematic because it carries a much poorer prognosis for the patient and forgoes the principal public health objective of HAT control, which is a rapid reduction in transmission. Technical solutions to increase the sensitivity of screening algorithms do exist. Several concentration tests have been proposed, notably the mini-anion exchange centrifugation technique (mAECT) (*7*), capillary tube centrifugation (CTC) (*8*), and the quantitative buffy coat (QBC) (*9*). A recent study of 436 case-patients conducted in Kwamouth, DRC, showed that 154 had parasitologic-confirmed HAT cases. Although good sensitivity was reported, and mAECT and CTC were relatively simple to implement, it is not economically feasible to use these innovative tools in the field.

Other authors have proposed recourse to serology using higher cut-offs of the CATT test to increase specificity (referred to as CATT titration, in contrast with CATT

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whole blood, which is the test used in the first screening step) as part of the algorithm for use in screening, or as a test to decide whether to treat. The present study is an analysis of the cost-effectiveness and value for HAT control policy of different HAT confirmation algorithms, including serologic algorithms.

Methods

A decision-analysis model was used to estimate the effectiveness and cost-effectiveness of a number of HAT screening-treatment algorithms; different HAT confirmation test sequences were compared, including concentration techniques and CATT titration. Decision analysis is a method that quantifies the value of several alternative options in a complex choice. This technique requires the construction of a decision tree, which shows detailed options, estimation of the node probabilities, and an evaluation of the economic or public health consequences of each option (M. John, unpub. data) (*10*,*11*). A health service perspective was taken for this analysis, which includes all screening and treatment costs generated by the choice of HAT test algorithms.

Decision Tree

Fourteen HAT control experts were asked to identify all relevant test sequences that would confirm HAT in a given person (i.e., positive in the initial screening test, CATT). The availability of these tests was an important factor in the expert poll. At the time of this study, QBC was no longer manufactured, and for this reason, it was not included in our analysis. We analyzed 7 algorithms as shown in Table 1. All algorithms imply that only parasitologic positives will be put on stage-dependent treatment except for the fourth group (serologic algorithm), which indicates stage-dependent treatment of persons who are negative for parasites but positive by CATT titration.

We constructed a decision tree comparing the algorithms mentioned above (Figure 1); the entry point is a person who participates in HAT population screening conducted by a mobile team. There are 4 possible outcomes for such a person: a true HAT case-patient is treated, a true HAT case-patient remains untreated, a non-HAT case-patient receives HAT treatment, or a non-HAT case-patient remains untreated. These 4 outcomes were evaluated in terms of lives saved.

Probabilities

Table 2 shows the probabilities used in this decision analysis. The baseline values were generated in a study carried out in Kwamouth between February and May 2004 (*4*) or were retrieved from the literature. In the baseline scenario, we assumed HAT prevalence in the community to be 1%. This value is a limit used by HAT-control programs to distinguish between severe and nonsevere HAT foci. A literature search was performed by using the Medline database to find information reported between 1950 and 2005 to identify baseline values of parameters with a plausible range. A sensitivity analysis was performed to test consistency of our conclusions over the range of plausible values.

Effectiveness

The effectiveness of each HAT screening-treatment algorithm was estimated, taking into account all steps, including screening, confirmation, and treatment. The results were quantified in terms of the number of lives saved (confirmed case, treated, and cured) by each algorithm. HAT treatment decision depends on disease staging (*20*). A diagnostic algorithm can theoretically generate 4 different outcomes: true positive, false positive, true negative, and false negative.

Effectiveness values were assigned to each of the alternatives. A true HAT case-patient who is treated is equal to 0.9 lives saved because the efficacy of HAT treatment is estimated at 90%, according to data from the Programme National de Lutte contre la Trypanosomiase Humaine Africaine (PNLTHA) between 1996 and 2002. For a few persons, treatment of non-HAT case-patients with toxic drugs will lead to iatrogenic death. Therefore, an effectiveness value was assigned to this endpoint of –0.001 lives saved

Table 1. Screening algorithms for human African trypanosomiasis*

Label	Algorithm	Abbreviation
Algorithm 1 or standard	Lymph node puncture, fresh blood examination, and thick blood film	LNP-FBE-TBF
Algorithm 2	Lymph node puncture and capillary tube centrifugation	LNP-CTC
Algorithm 3	Lymph node puncture, CATT titration, capillary tube centrifugation, and mini-anion exchange centrifugation technique	LNP-CATT titration-CTC-mAECT
Algorithm 4	Lymph node puncture, capillary tube centrifugation, and mini-anion exchange technique	LNP-CTC-mAECT
Algorithm 5	Lymph node puncture, thick blood film, capillary tube centrifugation, and mini-anion exchange technique	LNP-TBF-CTC-mAECT
Algorithm 6	Lymph node puncture, capillary tube centrifugation, and CATT titration	LNP-CTC-CATT titration
Algorithm 7	Lymph node puncture, thick blood film, capillary tube centrifugation, mini-anion exchange technique, and CATT titration	LNP-TBF-CTC-mAECT -CATT titration

*CATT, card agglutination test for trypanosomiasis; CATT titration, CATT titration at end-titer 8.

Table 2. Probabilities used in baseline scenario and plausible range*

Characteristic	Baseline value, %	Reference	Plausible range, %	Reference
HAT prevalence	1.0	Annual reports PNLTHA (1995–2002)	0.5–5.0	Annual reports PNLTHA (1995–2002)
LNP sensitivity	18.8	(*12*)	18.8–58.6	(*3,4*)
LNP specificity	100.0	By convention	NA	
FBE sensitivity	3.9	(*4*)	3.9–22.4	(*3,4*)
FBE specificity	100.0	By convention	NA	
TBF sensitivity	27.3	(*4*)	27.3–34.5	(*3,4*)
TBF specificity	100.0	By convention	NA	
CTC sensitivity	56.5	(*4*)	29.0–73.0	(*3,7*)
CTC specificity	100.0	By convention	NA	
mAECT sensitivity	75.3	(*4*)	43.0–88.0	(*3,7*)
mAECT specificity	100.0	By convention	NA	
CATT whole blood sensitivity	90.4	(*13*	68.8–99.2	(*13–16*); M. John, unpub. data
CATT whole blood specificity	96.5	(*13*)	83.5–98.4	(*21,23,24*)
CATT titration sensitivity†	78.8	(*4*)	78.8–100.0	In absence of data in literature, we considered the maximum of 100%
CATT titration specificity†	59.0	(*4*)	59.0–100.0	In absence of data in literature, we considered the maximum of 100%
Pentamidine efficacy	98.0	‡	98.0–99.0	(*17*)
Melarsoprol efficacy	90.0	‡	70.0–96.3	(*17,18*)
Latrogenic mortality of pentamidine	0.1	‡	0.1–0.7	(*17*)
Latrogenic mortality of melarsoprol	2.0		2.0–7.0	(*17–19*)

*HAT, human African trypanosomiasis; PNLTHA, Programme National de lutte contre la Trypanosomiase Humaine Africaine; LNP, lymph node puncture; NA, not applicable; FBE, fresh blood examination; TBF, thick blood film; CTC, capillary tube centrifugation technique; mAECT, mini-anion exchange centrifugation technique; CATT, card agglutination test for trypanosomiasis.
†Conditional values to CATT whole blood positive. The plausible range included the value at different end-titers (4, 16, 32).
‡Data extracted from PNLTHA/RDC annual report 1999.

for first-stage drugs and –0.020 lives saved for second-stage drugs. A non-HAT case-patient and a true HAT case-patient who was untreated were each assigned an effectiveness value of 0 lives saved. Effectiveness was expressed as the percentage of HAT deaths averted by a strategy. To obtain the actual number of lives saved by the strategy, this value must be multiplied by HAT prevalence.

Costs

While assessing the different algorithms, we distinguished between 3 steps in the process: 1) screening, 2) confirmation, and 3) treatment. Using the ingredient approach, we estimated the cost of screening by the mobile team for HAT on the basis of observations made in 2003. A detailed overview of cost items is given in Table 3. The cost of the screening step includes all equipment required to set up a mobile team: vehicles, depreciation, operating costs, and CATT reagents.

For the second step (HAT confirmation), each confirmation test was assessed in terms of resources consumed, equipment depreciation, and time taken by a mobile team to realize each test. Data on costs and time were collected during a validation study conducted in Kwamouth in 2004 (*4*). The time for each diagnostic test was estimated by using a stopwatch for a sample of 50 procedures, and the results were measured in terms of minutes elapsed. The cost of LNP has been estimated as €0.19, FBE at €0.21, TBF at €0.54, CTC at €0.76, and mAECT at €2.82 (*4*).

The third step, treatment, includes the cost of hospitalization care (fixed cost) as well as the cost of drugs and medical supplies. Preferential drug prices that are currently applicable were used in the calculations (pentamidine €1.54/vial, melarsoprol €5.3/vial), and the number of doses was 8 for pentamidine and 9 for melarsoprol. We assumed

Table 3. Annual cost of the operations of a mobile team for human African trypanosomiasis (HAT) active case finding, Democratic Republic of Congo, 2003

Input category	Annual cost, €*	% Total cost
Capital		
Vehicles	5,125.00	11
Medical and lab equipment	2,760.00	6
Training	671.66	1
Other equipment	1,416.75	3
Subtotal	9,973.41	21
Recurrent		
Personnel	11,520.00	25
Medical and lab supply	14,798.52	32
Essential drugs, not for HAT	2,100.00	4
Stationary	2,842.36	6
Vehicles, operation and maintenance	5,200.00	11
Other operating input	300.00	1
Subtotal	36,760.88	79
Total	46,734.29	100

*US $1 = €0.86, May 2003.

Table 4. Cost, incremental cost, effectiveness, incremental effectiveness, cost effectiveness, and incremental cost effectiveness ratio of HAT screening-treatment algorithms in baseline scenario*

Algorithm	Effectiveness†	Incremental effectiveness‡	Cost per examined person, €	Incremental cost, €‡	Efficiency§	Incremental cost effectiveness ratio¶
LNP-FBE-TBF	36.80		1.56		423.91	
LNP-CTC	55.00	18.20	1.74	0.18	316.36	98.90
LNP-CATT titration-CTC-mAECT	64.50	9.50	1.96	0.22	303.88	231.58
LNP-CTC-mAECT	77.60	13.10	2.06	0.10	265.46	76.34
LNP-CTC-CATT titration	77.80	0.20	2.82	0.76	362.47	Dominated
LNP-TBF-CTC-mAECT	79.60	2.00	2.1	0.04	263.82	200.00
LNP-TBF-CTC-mAECT-CATT titration	83.00	3.40	2.99	0.89	360.24	2617.65

*HAT prevalence 1%. HAT, human African trypanosomiasis; LNP, lymph node puncture; FBE, fresh blood examination; TBF, thick blood film; CTC, capillary tube centrifugation; CATT, card agglutination test for trypanosomiasis; CATT titration, CATT titration at end-titer 8; mAECT, mini-anion-exchange centrifugation technique. Cost/effectiveness ratio calculated as cost / (effectiveness × prevalence). Differences due to rounding in table. US $1 = Euro 0.79, Feb 2004.
†% HAT deaths averted.
‡Cost and effectiveness calculated incremental to next least effective nondominated algorithm after ranking all algorithms by effectiveness.
§Cost effectiveness per life saved.
¶Incremental cost effectiveness ratio calculated as incremental cost / (incremental effectiveness × HAT prevalence). Differences due to rounding in table. All incremental changes expressed in comparison with LNP-FBE-TBF algorithm.

that 50% of case-patients were in stage 1 (needing pentamidine) and 50% were in stage 2 (needing melarsoprol).

Analysis

The efficiency of each algorithm was evaluated on the basis of the cost-effectiveness ratio expressed in terms of €/life saved. This ratio was obtained by dividing the cost (in €) per person examined for each algorithm by the strategy's effectiveness (in % HAT deaths averted per person examined), multiplied by HAT prevalence.

We used the following equations:

€/life saved = (€/person examined) / [(HAT prevalence) × (sensitivity of test sequence) × (effectiveness of HAT treatment) – (1–HAT prevalence) × (1–specificity of test sequence) × (probability of iatrogenic death)]

Cost per person examined = [(total annual operating costs of a mobile team) / (number of persons screened per year)] + [cost of confirmatory test$_i$ × probability confirmatory test$_i$] + [cost of treatment$_k$ × probability treatment true positive)] + [cost of treatment$_k$ × probability treatment false positive]

where cost of confirmatory test$_i$ = cost of material + execution time + depreciation of equipment; i = LNP, FBE, TBF, CTC, mAECT, or CATT titration; cost of treatment$_k$ = cost of hospitalization care + cost of drugs + cost of medical supplies; k = therapy of a true first-stage case, a true second-stage case, or a false-positive person; and probability confirmatory test$_i$ = the probability of executing confirmatory test in position i in the test sequence of the evaluated algorithm. This probability was derived from the decision tree as follows:

[prevalence × sensitivity$_{\text{CATT whole blood}}$ × (1–sensitivity$_{\text{confirmatory test 1}}$) × ...× (1–sensitivity$_{\text{confirmatory test i –1}}$)] + [(1 – prevalence) × (1 – specificity$_{\text{CATT whole blood}}$)]

Probability of treatment of a true-positive person was calculated per stage z (1,2) as follows:

prevalence × proportion of cases$_{\text{stage z}}$ × sensitivity$_{\text{CATT whole blood}}$ × sensitivity$_{\text{confirmatory test 1}}$ × ... × sensitivity$_{\text{confirmatory test i–1}}$ × sensitivity$_{\text{confirmatory test i}}$

Probability of treatment of a false-positive person was generally defined as:

(1 – prevalence) × (1 – specificity$_{\text{CATT whole blood}}$) × (1 – specificity$_{\text{confirmatory test 1}}$) × ... × (1 – specificity$_{\text{confirmatory test i–1}}$) × (1 – specificity$_{\text{confirmatory test i}}$)

However, for algorithms including CATT titration without subsequent confirmation, the probability of treatment of a false-positive person was defined as:

(1 – prevalence) × (1 – specificity$_{\text{CATT whole blood}}$) × (1 – specificity$_{\text{confirmatory test 1}}$) × ... × (1 – specificity$_{\text{confirmatory test i–1}}$) × (1 – specificity$_{\text{confirmatory test i}}$) + (1 – specificity$_{\text{CATT whole blood}}$) × (1 – specificity$_{\text{CATT titration}}$).

Effectiveness of HAT treatment was defined for true cases as:

[(proportion of stage 1 cases) × (efficacy pentamidine)] + [(proportion of stage 2 cases) × (efficacy melarsoprol)]

Finally, we calculated the incremental cost-effectiveness ratio (ICER) of saving 1 additional HAT patient by comparing each alternative algorithm to the strategy immediately above it after ranking the order of effectiveness. The ICER was calculated as incremental cost / (incremental effectiveness × HAT prevalence).

HAT prevalence, sensitivity, and specificity of different tests were the subject of a sensitivity analysis. A series of 1-way sensitivity analyses were conducted to examine

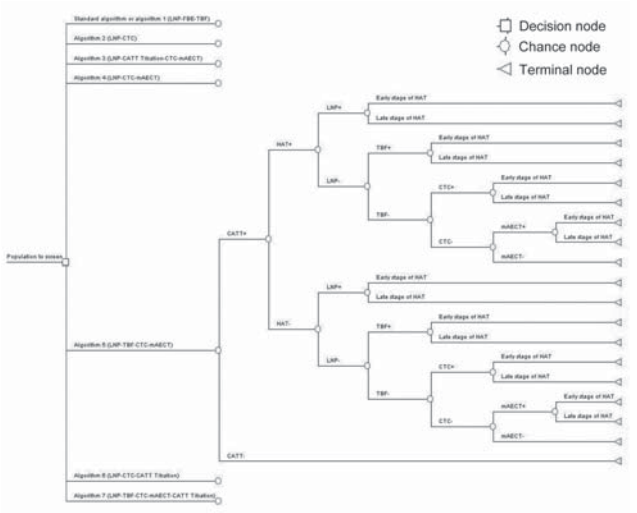

Figure 1. Decision tree comparing algorithms used to analyze human African trypanosomiasis (HAT). LNP, lymph node puncture; FBE, fresh blood examination; TBF, thick blood film; CTC, capillary tube centrifugation; CATT, card agglutination test for trypanosomiasis; CATT titration, CATT titration at end-titer 8; mAECT, mini-anion-exchange centrifugation technique.

the effect of changes in those parameters over the plausible range mentioned in Table 2 on the efficiency ranking of strategies. DATA Pro 2004 software (TreeAge, Williamstown, MA, USA) was used for this analysis.

Results

We estimated the cost of population screening for HAT conducted by a mobile team in 2003 (excluding the cost related to confirmation and treatment) at €1.17/per person examined (Table 4). The treatment cost was estimated at €51.32/person treated with pentamidine and €129.92/person treated with melarsoprol.

Table 4 shows the cost and effectiveness per person examined for the complete screening-treatment process. Furthermore, it presents the incremental cost, the incremental effectiveness, and the incremental cost-effectiveness ratio of each algorithm compared with the next least effective. Table 4 shows that algorithm 5 is the most cost effective algorithm (€264.02/life saved), with algorithm 4 a close second-best option (€265.98/life saved). Although the cost per person examined for the 2 algorithms does not differ substantially, algorithm 5 is slightly more efficacious, saving 79.60% of avoidable deaths versus 77.60% for algorithm 4.

The standard algorithm (algorithm 1) had an effectiveness of 36.80% with a €/person examined of €1.56 and an efficiency of €424.94/life saved. From column 7 in Table 4, the incremental cost-effectiveness ratio to save 1 additional life ranged between €76.34 and €200/life saved for the concentration technique algorithms and €2,617.65/life saved if the decision to treat was based on serologic evidence. Algorithm 6 was dominated by algorithm 5 because algorithm 6 costs more and is less effective.

The sensitivity analysis showed that our conclusion remained robust to variation over the range of uncertainty in all parameters included in the model (Figure 2). Changes in the specificity of CATT and CATT titration, cost of pentamidine, and HAT prevalence decreased the difference in the cost-effectiveness ratio with the next most efficient algorithm. Figure 3 shows the variation of the cost-effectiveness ratio in function of the prevalence.

Discussion

Current observations suggest that parasitologic confirmation tests of inadequate sensitivity lead to suboptimal effectiveness of HAT active case-finding programs (*4*). Our analysis shows that concentration techniques, and to a lesser extent, serologic tests can substantially improve the efficiency of HAT confirmation algorithms. The currently used algorithm (standard algorithm) was the least cost effective of all those compared**,** mainly because its sensitivity is so low. Low effectiveness of the standard algorithm was also reported by Paquet et al. (*5*), Pépin et al. (*6*), and recently by Robays et al. (*13*) who put it respectively at 39.5%, 20–30%, and 50%. The greater effectiveness of concentration techniques (currently €2.82/test for mAECT) more than compensates for the higher cost. Serologic algorithms were not more cost effective than the algorithms that included concentration techniques, but the difference in efficiency decreased at a higher prevalence (Figure 3). Algorithms combining CATT titration with subsequent confirmation by concentration techniques were not competitive in this analysis; efficiency always remained lower than that of algorithms based exclusively on concentration techniques. This is due to the loss of patients caused by the suboptimal sensitivity of CATT titration.

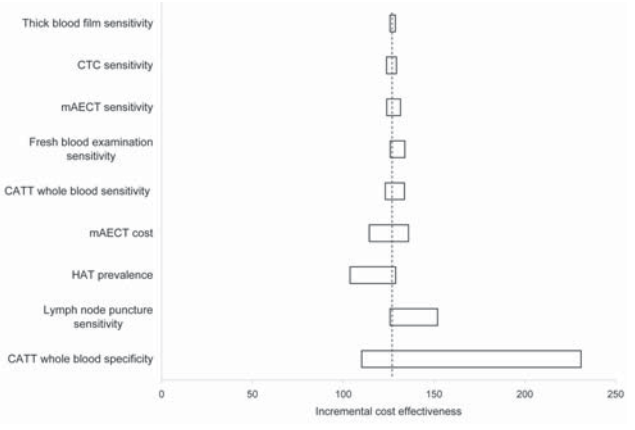

Figure 2. Sensitivity analysis of cost effectiveness (€/life saved) according to variation in prevalence of human African trypanosomiasis (HAT). CTC, capillary tube centrifugation; mAECT, mini-anion-exchange centrifugation technique; CATT, card agglutination test for trypanosomiasis.

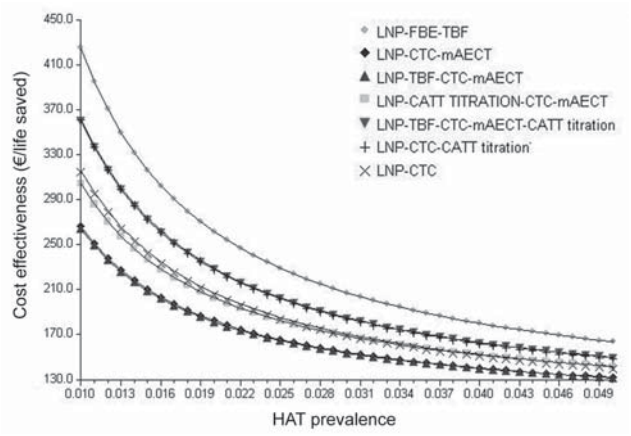

Figure 3. Variations in cost effectiveness ratios as a function of prevalence of human African trypanosomiasis (HAT). LNP, lymph node puncture; FBE, fresh blood examination; TBF, thick blood film; CTC, capillary tube centrifugation; mAECT, mini-anion-exchange centrifugation technique; CATT, card agglutination test for trypanosomiasis; CATT titration, CATT titration at end-titer 8.

Our study could shed some light on the controversial issue of treating for HAT on the basis of serologic evidence. In a similar discussion in the field of kala-azar case management, Boelaert et al. (*14*) argued that a serologic algorithm was more cost effective, and therefore a better choice, than a parasitologic algorithm with poor sensitivity.

In this study, we also find that treating patients suspected by serologic tests to have HAT is a better option than using the current algorithm without concentration techniques. This may be a valuable strategy when introduction of mAECT or CTC is not yet feasible. A limitation of the study is that the specificity values of CATT and CATT dilution are based on data from 1 region and might be underestimated given findings from routine data from the national program (*4*). However, this regional variation remains poorly documented, and better estimations of the specificity of CATT in different regions are needed. The use of CATT titration has primarily been evaluated in the group of CATT whole blood–positive persons who were negative for parasites. Chappuis et al. (*15*), Simarro et al. (*16*), Van Nieuwenhove and Declercq (*17*), Frézil et al. (*18*), and Bruneel et al. (*19*) evaluated the strategy of treating persons who had positive serologic test results (indirect immunofluorescent antibody test or CATT) and who were negative for parasites and found this strategy more effective than that based on parasitologic confirmation.

Our model did not give different weights to iatrogenic deaths of noninfected persons compared with infected persons. This point should be carefully considered because the serologic algorithm will expose some noninfected persons to toxic drugs. However, as no treatment with second-stage HAT drugs will be given unless a lumbar puncture and parasitologic confirmation in cerebrospinal fluid are obtained, the exposure of noninfected persons to the highly toxic second-stage HAT drugs will be minimal, even under a serologic algorithm.

Our decision analysis is based on a model that depends on certain assumptions made for the purpose of simplification. An important assumption is that a true HAT case missed by the mobile teams will eventually die; in practice, if treatment is sought, this patient's condition might be diagnosed and cured, or alternatively, HAT could be detected later by the mobile team on a second visit. We examined whether this assumption would change our conclusions by hypothesizing that 40% of such HAT case-patients would be detected and treated at a later stage. In this scenario, the differences between algorithms in terms of cost-effectiveness are reduced without altering the relative order (data not shown).

Second, confirmation tests in the sequence were used as if they were independent, but in reality, this is unlikely. For example, mAECT sensitivity could be different in a group of TBF-positive persons compared to a group of TBF-negative persons. We have examined the effect of this conditional relationship on the findings of our study by means of sensitivity analysis (data not shown). Once again, these variations did not affect the ranking of the results.

The cost of the mobile team per person screened was estimated on the basis of 40,000 examinations per year (*20*). Even if the program could screen 60,000 persons per mobile team per year, it would not change the rank order of efficiency. Finally, better confirmation algorithms for HAT may also have a beneficial effect on transmission because there will be fewer undetected cases to spread the disease in the community. If such an effect is considered, it might favor the efficiency of treatment based on serologic markers, which may lead to a faster reduction of the human reservoir similar to the chemoprophylaxis campaigns of the 1950s. Unfortunately, no sufficiently validated models for *T. b. gambiense* sleeping sickness transmission allow for the estimation of this potential benefit at population level. Our analysis disregarded this potential future benefit.

A policy change in HAT population screening seems definitely needed, and there is ample scope for improving the sensitivity of the confirmation stage. Introducing algorithm 5 has an incremental cost-effectiveness ratio of €200.00/(additional) life saved. This ratio represents the cost to HAT control programs of shifting to algorithm 5 (the most cost effective) to save an additional life. This choice seems very rational. The incremental cost-effectiveness ratio was €76.34 if HAT control programs chose algorithm 4. TBF is a lengthy procedure, and dropping it from the sequence has logistic and organizational advantages. Our calculations were based on an estimate of 47 minutes of staff time required for TBF, obtained in a previous study

(*4*). However, because labor costs are so low in the DRC and TBF does not require expensive reagents or equipment, it remains a very affordable test whenever there are no time constraints for staff.

In conclusion, the standard HAT screening algorithm has low sensitivity and is inefficient. Inclusion of concentration techniques in HAT screening algorithms can be recommended as cost effective alternatives. The use of serologic algorithms should be studied further before being recommended for HAT population screening.

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Dr Lutumba is head of the research unit of the national sleeping sickness control program of the Democratic Republic of Congo. He has extensive experience in sleeping sickness control in the DRC and participates in clinical research programs on diagnosis and treatment of HAT.

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Public Transportation and Pulmonary Tuberculosis, Lima, Peru

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The association between public transportation for commuting and pulmonary tuberculosis (TB) was analyzed in workers in Lima, Peru. Traveling in minibuses was a risk factor for pulmonary TB. Preventive measures need to be taken by health services to prevent spread of this disease.

Tuberculosis (TB) continues to be an important public health problem in impoverished areas (*1–4*). It is spread through the air by patients with pulmonary TB (*5*). Because those most affected by pulmonary TB are persons 15–50 years of age, employment-related characteristics of these persons must be taken into account when studying this disease. In Lima, Peru, residents of peripheral neighborhoods generally use minibuses to travel to work or school and have long commute times. Because public transportation in Latin America routinely carries more passengers than permitted by law, it is plausible to assume that in areas with endemic pulmonary TB, daily use of public transportation may be a risk factor for acquiring TB (*6–9*).

The greatest amount of expectoration (productive coughing) occurs during the morning commute (6:00 AM–7:00 AM) because of accumulation of bronchial secretions at night (*10*). Given the conditions in which persons travel to work in Lima (long travel times and overcrowding on minibuses with closed windows), we analyzed whether use of minibuses was associated with the spread of pulmonary TB as part of a larger study to assess pulmonary TB in the Ate-Vitarte District of this city.

The Study

The study was conducted in the Ate-Vitarte district (population 365,473), which is located ≈12 km from the center of Lima. It is a marginal urban area that receives immigrants who come to Lima with high rates of TB. The study was reviewed and approved by the ethics committee of the East Lima Health Directorate IV.

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During July–August 2004, interviews were conducted with a random sample of 150 commuters ≥15 years of age who had productive coughs for >15 days and who came to health services for treatment. A total of 142 persons agreed to participate: 96 were treated at hospitals and 46 were treated at health centers. Informed consent was obtained from all participants. Interviews were conducted in the health services that persons visited. We obtained demographic and socioeconomic information, as well as information on perceived health, and means of transportation used in commuting.

All persons with productive coughs were requested to provide 3 sputum samples (the first immediately after the interview and the other 2 on 2 consecutive days) for smear testing. Samples were tested by using the Ziehl-Neelsen method, which is used in all epidemiologic surveillance in Peru (*11*). Study participants were considered positive for pulmonary TB if ≥1 acid-fast bacilli (AFB) were found (*11*).

Results were analyzed by using bivariate and multivariate logistic models with SPSS version 12 software (SPSS Inc., Chicago, IL, USA). Statistical significance was defined as $p<0.05$.

Demographic and socioeconomic characteristics of the study group are shown in Table 1. Variables analyzed for persons tested for pulmonary TB are shown in Table 2. Seventeen (11.9%) of 142 study participants were smear positive (29.6% 1 cross, 41.0% 2 cross, and 29.4% 3 cross). Two persons had been discharged from a TB treatment program <6 months earlier.

None of the demographic and socioeconomic indicators analyzed (Table 1) were associated with pulmonary TB. Table 2 shows crude associations between variables and pulmonary TB. The use of a minibus and a commuting time ≥1 hour had the highest associations with pulmonary TB. Adjusted relationships obtained by logistic regression that controlled for all variables shown in Table 2 confirmed that commuting to work by minibus was associated with a positive test result for pulmonary TB (adjusted odds ratio 4.90, 95% confidence interval 1.04–23.04).

Conclusions

We observed a pulmonary TB prevalence rate of 12% in persons with chronic productive coughs who came to health services in the study area. This rate was similar to a prevalence of 11% reported in a similar study conducted in an area of poor socioeconomic status in Chiapas, Mexico (*12*).

The proportion of persons 15–44 years of age with pulmonary TB in our study was consistent with data of the World Health Organization and the Peruvian Ministry of Health, which show that this age group has the highest prevalence of this disease (*5,13,14*). Our results also

Table 1. Demographic and socioeconomic characteristics of 142 persons tested for pulmonary tuberculosis, Lima, Peru

Characteristic	Value
Women, %	55.6
Mean age, y	35.7
Indigenous (Quechua), %	38.7
15–44 years of age, %	77.5
Immigrants, %	60.6
Education, %	
Illiterate	8.5
Primary school	20.4
Secondary school or higher	71.1
Household indicators, %	
Only 1 room	18.3
Roof of solid material	41.5
Electricity	97.8
Connection to public water supply	56.3
Toilet with running water	58.5
Overcrowded conditions*	39.4
Social security	15.5
Occupation, men (n = 63), %	
Street peddlers	68.3
Students	17.5
Another job	12.7
Not working	1.6
Occupation, women (n = 79), %	
Street peddlers	39.2
Housewives	43.0
Students	13.9
Another job	3.8
Means of transportation, %	
Public transportation	45.7
Individual transportation	26.8
Do not travel to work	27.5
Time spent commuting,† %	
30 min to 1 h	60.0
≥1 h	40.0

*More than 3 persons sleeping in the same room.
†Of those using public transportation, n = 65.

showed that there were no sex-related differences in the frequency of pulmonary TB (*13*,*14*).

Socioeconomic variables showed no association with pulmonary TB. However, this finding should be interpreted cautiously because of the small sample size, particularly the number of persons who lived in extreme poverty. Another factor that could limit our conclusions is accessibility of persons in areas of extreme poverty to public transportation. The fact that the field work phase of our study could not be increased because of shortages of resources and health center personnel time is also a limitation.

The relationship of having pulmonary TB with working at home or away from home showed a positive prevalence ratio of 6.06. Among persons working outside the home, commuting by minibus increased the risk of having pulmonary TB by a factor of 4.09 compared with persons who used individual forms of transportation. A commuting time ≥1 hour on a minibus also increased the risk for pulmonary TB by a factor of 2.07 (Table 2).

Minibuses in Lima increase the risk for pulmonary TB because they are usually overloaded (capacity is often doubled) in the early morning and late evening. Overcrowding, exposure to persons with productive coughs while commuting 2 times a day 5 days a week, and closed windows on minibuses, combined with a high prevalence of pulmonary TB in Lima, increase the risk of acquiring this disease. Because persons with cases of pulmonary TB have more productive coughs in the morning (when more bacilli are released because of their accumulation at night), there is increased risk for transmission of TB to other passengers (*15*), as has already been suggested by other studies in developing and industrialized countries (*6*–*9*). The findings that 41% of persons tested were positive with 2 crosses and 29.4% were positive in 3 crosses indicate poor TB prevention and control programs in the study area and higher probabilities of transmission (*15*).

Despite the limitations of our study, commuting in minibuses was a risk factor for pulmonary TB. Consequently, preventive measures need to be taken by health services to encourage persons with productive coughs to avoid this type of public transportation and to come to health services for diagnosis and treatment. Health services should also be more accessible to persons with pulmonary TB who, for whatever reason, cannot use other forms of transportation. This increased accessibility would include home treatment during the infectious phase of this disease.

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Table 2. Variables analyzed in 142 persons tested for pulmonary tuberculosis, Lima, Peru*

Variable	AFB smear–positive, n/N (%)	AFB smear–negative, n/N (%)	Total, n/N (%)	OR (95% CI)	Positive prevalence ratio
Occupation away from home	16/17 (94.1)	87/125 (69.6)	103/142 (72.5)	6.99 (0.89–54.61)	6.06
Commuter transport by minibus	14/16 (87.5)	51/87 (58.6)	65/103 (63.1)	4.9 (1.06–23.09)	4.09
Commuting time ≥ 1 h	8/16 (50)	20/87 (23)	28/103 (27.2)	3.35 (1.12–10.10)	2.07
History of pulmonary tuberculosis	4/17 (23.5)	24/125 (19.2)	28/142 (19.7)	1.29 (0.38–4.33)	1.25
Previous contact with tuberculosis cases (family)	7/17 (41.2)	40/125 (32.0)	47/142 (33.1)	1.49 (0.53–4.20)	1.41
Overcrowded conditions	6/17 (35.3)	50/125 (40)	56/142 (39.4)	0.818 (0.28–2.35)	0.83

*AFB, acid-fast bacilli; OR, odds ratio; CI, confidence interval.

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Prevalence of *Plasmodium falciparum* Infection in Rainy Season, Artibonite Valley, Haiti, 2006

Thomas P. Eisele,* Joseph Keating,* Adam Bennett,* Berlin Londono,* Dawn Johnson,† Christina Lafontant,† and Donald J. Krogstad*

We conducted a population-based survey to estimate the prevalence of *Plasmodium falciparum* infection among persons older than 1 month in the Artibonite Valley of Haiti during the high malaria transmission season in 2006. Results from PCR for 714 persons showed a prevalence of 3.1% for *P. falciparum* infection.

Lying just 700 miles from the United States, Haiti is 1 of only 2 countries in the Caribbean with endemic transmission of *Plasmodium falciparum* malaria. Reportedly, 74%–80% of Haiti's population live in malarious areas <300 m elevation (*1*,*2*). *Anopheles albimanus* has been identified as the vector responsible for nearly all malaria transmission in Haiti (*3*). However, reliable population-based estimates of the distribution and impact of malaria in Haiti are scarce (*4*); existing data are primarily from confirmed malaria cases reported through the health system. The seasonal peak in malaria transmission typically occurs from November through January, following the main rainy season (*5*). We conducted a population-based survey to estimate the prevalence of *P. falciparum* infection among persons older than 1 month in the Artibonite Valley of Haiti during the high transmission season in 2006.

The Study

This research was conducted in the Artibonite Valley. Urban areas were excluded. This site was chosen because of its low altitude and abundant rainfall, as well as the large number of malaria cases historically seen at hospitals in the area (*5*–*7*). The Artibonite Valley is heavily farmed; 80% of the area is irrigated for cultivation of rice and other crops.

A 2-stage cluster design, probability proportional to cluster size, was used to generate a probability sample of 200 households within the study area; 20 primary sampling units were selected at the first stage and 10 households at the second stage (Figure 1). Fieldwork was conducted by trained data collectors from November 20 to December 10, 2006. Ethical approval was obtained from Tulane University and Hôpital Albert Schweitzer (HAS).

After informed consent was obtained, blood samples were collected and axillary temperature determined for all persons older than 1 month within each selected household. Thick and thin blood films were prepared for each person, as well as 4 blots of blood on filter paper for PCR. Up to 3 return visits were made to each household to limit nonresponse. Basic personal and household demographic data were collected through an interview with the designated head of household; a standardized questionnaire was used for all 200 households eligible for inclusion in the survey.

Using standard methods (*8*), a trained laboratory technician at HAS interpreted the malaria blood slides. Positive persons were treated with chloroquine. Filter paper blots were transported back to the laboratory at Tulane University for PCR analysis to test for *P. falciparum* parasites. Blood samples on filter paper from microscopy-confirmed infections and respondents with temperatures >37.5°C were analyzed individually by PCR for *P. falciparum*. Pooled PCR analysis of 10 samples was used to detect *P. falciparum* infections in filter paper samples from respondents with negative microscopy results; positive pools were then analyzed individually.

Positive specimens were identified on the basis of PCR for conserved sequences in 18S small subunit RNA, with a single reverse primer for all *Plasmodium* species and a *P. falciparum*–specific forward primer (*9*); expected amplicon size was 276 bp for *P. falciparum.* The positive control contained DNA from a culture of the *P. falciparum* Haiti strain. The negative control contained water instead of DNA. The

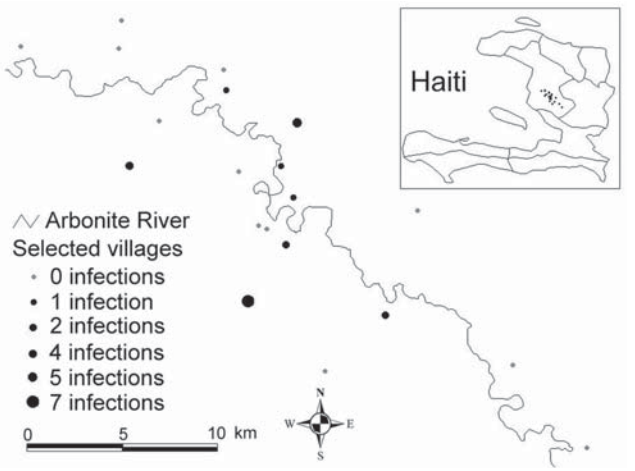

Figure 1. Map of selected villages for estimating the prevalence of *Plasmodium falciparum* infection, including number of infections identified within each village, Artibonite Valley, Haiti, 2006.

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amplified electrophoresis products were visualized on a 1% agarose gel and stained with ethidium bromide.

Prevalence of *P. falciparum* infection was calculated as the proportion of sampled persons with a positive PCR result divided by the number of persons who provided blood samples. All point estimates were weighted, with empirically estimated standard errors used to account for clustering.

A total of 804 persons older than 1 month were eligible for inclusion in the survey; 714 agreed to provide a blood sample. This resulted in a nonresponse rate of 11.2% for estimating malaria parasite prevalence. Ages of persons in the sample ranged from <1 to 92 years; 46.1% were male. Ninety-one children <5 years of age (12.7%) were included in the sample. A total of 8.6% of the persons were considered febrile (axillary temperature >37.5°C).

Microscopy at HAS identified 7 malaria infections among the 714 persons who had provided a blood sample; all were confirmed by PCR. Diagnosis by individual and pooled PCR of the remaining blood samples on filter paper identified an additional 16 *P. falciparum* infections, totaling 23. Thus the total prevalence was estimated to be 3.1% (95% confidence interval 0.60%–5.7%) (Table). The resulting sensitivity and specificity of microscopy were 30.5% and 100%, respectively. Persons with infections ranged in age from 1 to 62 years; 65.2% were male. A total of 14.2% of febrile persons had positive malaria results, compared with only 2.1% of nonfebrile persons. Of the 20 villages included in the sample, all 23 persons with malaria infections came from 8 villages, which were 26–319 m above sea level.

Table. Malaria parasite prevalence by demographic characteristics, Artibonite Valley, Haiti, 2006

Characteristic	No. malaria infections identified*	No. respondents tested	Parasite prevalence, %†
Age, y			
<5	2	91	2.2
5–9	5	100	4.8
10–19	2	186	1.5
20–29	6	95	4.7
30–39	2	59	3.8
40–49	3	69	4.0
50–59	1	42	5.1
≥60	1	50	1.6
Unknown	1	22	3.5
Sex			
Male	15	329	3.9
Female	8	385	2.3
Temperature			
Febrile (≥37.5°C)‡	9	61	14.2
Nonfebrile	14	647	2.1
Total	23	714	3.1§

**Plasmodium falciparum* infections only. Results based on PCR, which includes all microscopy-confirmed infections.
†Prevalence point estimates are weighted.
‡Sample size for febrile versus nonfebrile; 6 missing data records.
§95% confidence interval 0.6–5.7.

Conclusions

Results from the survey show the prevalence of *P. falciparum* infection to be 3.1% in this area of Haiti. To our knowledge, this is the first population-based estimate of malaria parasite prevalence in Haiti that used PCR diagnosis. Among febrile persons, whose prevalence was 14.2%, our results are substantially higher than previous estimates from passive surveillance of suspected malaria case-patients (*5*,*6*,*10*).

While moderate, a 3.1% prevalence represents a substantial level of illness, especially when one considers that the severity of the disease is likely high given the low level of acquired immunity among the Haitian population. Furthermore, based on passive case detection of confirmed malaria cases identified by HAS in the Artibonite Valley from 2004–2006 (Figure 2), transmission in 2006 appears to have been substantially lower than in previous years. Thus the population-based prevalence estimate of 3.1% likely represents the lower bound of the malaria impact in this area.

Malaria transmission was highly localized; all 23 infections were in persons from 8 villages (40%), which suggests that transmission is potentially based on a set of discrete ecologic determinants (Figure 1). Such clustering is consistent with the observed tendency for *Anopheles* mosquitoes to overdisperse (*11*). Surprisingly, 7 (30%) of the 23 infections were in persons from a village 319 m above sea level, although the exact location of inoculation cannot be confirmed. If transmission occurred at this elevation, it is above what has commonly been understood as the upper bound for transmission in Haiti.

The observed low level of sensitivity of microscopy compared with that of PCR for identifying *P. falciparum* infections is similar to findings observed elsewhere (*12*–*14*). We surmise that such a low level of sensitivity was attributable to 2 factors: 1) many of the infections likely occurred at low parasite densities, and 2) the laboratory technician was responsible for reading a large number

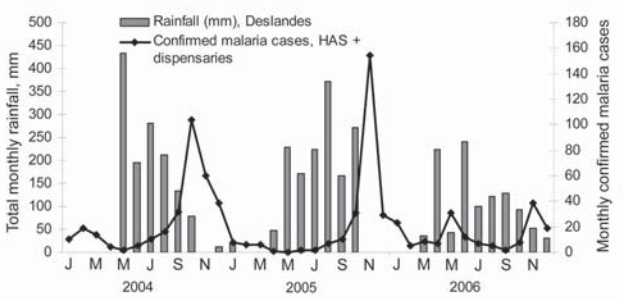

Figure 2. Microscopy-confirmed malaria cases at Hôpital Albert Schweitzer (HAS) and total monthly rainfall, 2004–2006, Deslandes, Artibonite Valley, Haiti.

of slides with low parasite prevalence over a relatively short period.

We argue that future malaria interventions in Haiti should be directed toward controlling malaria in the context of a moderate transmission setting; thus, large-scale distribution of insecticide-treated nets or widespread use of indoor residual spraying may be less cost-effective than enhanced surveillance with effective case management or focused larval control. A key aspect of future research in Haiti should therefore focus on understanding treatment-seeking behavior, barriers to accessing health services among febrile persons, and quantifying patterns of malaria transmission.

Acknowledgments

We are grateful to Matt Ward, the data collection team at Hôpital Albert Schweitzer, the population of the Artibonite Valley, and the Haitian Ministry of Health for allowing us to conduct this research in Haiti.

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Evaluating Tuberculosis Case Detection in Eritrea

Marieke J. van der Werf,*† Mineab Sebhatu,‡ and Martien W. Borgdorff*†

We used results from a national tuberculosis prevalence survey in Eritrea to calculate case detection rate (CDR) and compared it with the published CDR. The CDR obtained from the survey was ≈40%, whereas the CDR published by the World Health Organization was 3× lower (14%).

During the World Health Assembly in 1991, 2 targets were set for tuberculosis (TB) control: to detect 70% of all new sputum smear–positive cases arising each year and to successfully treat 85% of these cases (*1*). For assessment of the first target, case detection rate (CDR) is used; CDR is the number of cases reported divided by the number of incident cases estimated for that year. In Africa in 2004, the range of CDRs for new smear-positive TB patients was 14%–115% in different countries (*2*). The CDR is uncertain for many African countries because information for estimating the incidence is outdated or unavailable. The most recent national TB prevalence surveys were performed from 1955 through 1960; they covered 11 countries and a population of ≈40,000 (*3*). Since then, TB treatment has become widely available, and the emergence of HIV has had a substantial effect on TB incidence (*4*,*5*).

Recently, a TB prevalence survey was performed in Eritrea, a country with a population of 3 million, located in the Horn of Africa (*6*). The survey determined the prevalence of sputum smear–positive TB by examining sputum samples of persons ≥15 years of age. To assess the performance of Eritrea's TB program, we calculated the CDR by using information obtained from the survey and compared this CDR to published estimates.

The Study

The national TB prevalence survey in Eritrea was conducted from February through October 2005 (*6*). In 40 selected villages, a census (which included information about sex and age) was taken of ≈875 persons in each village. All persons ≥15 years of age were asked to provide a morning and a spot sputum sample. Persons were informed about the survey and could refuse participation. The study protocol for the prevalence survey was approved by the Ministry of Health.

The specimens were examined by fluorescence microscopy. Samples positive by fluorescence microscopy were reexamined by light microscopy for confirmation. Persons who had 2 positive sputum samples were informed about the test results and referred for treatment. Those who had 1 positive sputum sample were referred to a nearby healthcare facility for further smear examination. If results of smear examination were negative, thoracic radiographs were taken and evaluated by 2 experienced radiologists. The case definition for a sputum smear–positive case was at least 2 sputum specimens positive for acid-fast bacilli by Ziehl-Neelsen staining and microscopy or at least 1 sputum specimen positive for acid-fast bacilli and radiographic abnormalities consistent with active pulmonary TB (classification of the National Tuberculosis Control Program in Eritrea).

Using the prevalence estimate obtained from the survey and 2 different models, we calculated the CDR for 2004. In model 1, described by Styblo, CDR = (notification rate/prevalence rate) / (0.5 + 0.83 × [notification rate/prevalence rate]) (*7*,*8*). In model 2, described by Dye et al., CDR = (notification rate/prevalence rate) / ([notification rate/prevalence rate] + 0.5) (*9*,*10*). We then compared the calculated CDR with the CDR estimated by the World Health Organization (WHO) to evaluate whether comparable conclusions about TB case detection would be obtained.

A total of 38,047 persons were included in the prevalence survey. Of those ≥15 years of age, 18,152 (94.6%) provided at least 1 sputum sample (Figure). The prevalence of new smear-positive TB was estimated at 90/100,000 (95% confidence interval [CI] 35–145/100,000) in persons ≥15 years of age. In 2005, 44.7% of the Eritrean population was <15 years of age (*11*), which resulted in an overall new smear-positive TB prevalence of 50/100,000 (95% CI 19–80/100,000) under the assumption of no cases in persons <15 years of age.

In 2004, 17/100,000 new smear-positive cases were reported (*2*). The calculated CDR from model 1 was 43% and from model 2 was 40%. The 2004 CDR published by WHO was 14%.

Conclusions

For Eritrea, the CDR provided by WHO is considerably lower than that calculated from the results of the national TB prevalence survey. Both estimates indicate that Eritrea has not reached the 70% target for case detection. However, the WHO estimate suggests that the program needs to improve case detection by a factor of 5, whereas the survey estimate suggests that case detection needs to be improved by a factor of 1.6. Two explanations may account for the large difference: 1) the CDR derived from the TB

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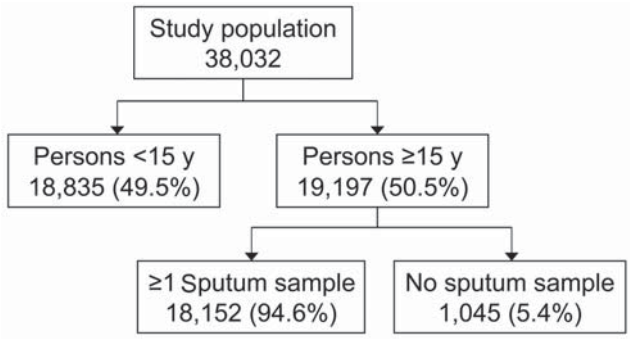

Figure. Summary of tuberculosis prevalence survey in Eritrea, 2005.

prevalence survey is too high because of an underestimation of the prevalence of smear-positive TB, or 2) the CDR estimate published by WHO is too low because of an overestimation of the incidence of smear-positive TB.

In the national TB prevalence survey, measures were taken to ensure high quality of the results; e.g., training of data collectors, repeat census taking, reexamination of all slides found positive on fluorescence microscopy, and reexamination of a 5% random sample of the negative slides. Persons who had smear-positive TB may have been missed because they did not provide a specimen; however, because only 5% of eligible persons did not provide a specimen, this can explain only a slight underestimation. Furthermore, recorded reasons for not providing a specimen seem to be unrelated to a higher chance of having TB. The quality of the provided specimens may have been suboptimal because instructing and motivating persons to provide a sputum sample is challenging. For diagnosis of TB, microscopic examination of saliva is less sensitive than examination of sputum; however, in ≈50% of saliva samples from patients with a positive sputum sample, bacilli can be demonstrated (*12*,*13*). For 27,647 samples that appeared to be saliva, smear–positive results were obtained for 12. Assuming that only 50% were detected, a maximum of 12 smear-positive TB patients may have been undetected. Taking this into account results in a prevalence of 87/100,000. Using this estimate, model 1 provides a CDR of 30% and model 2 a CDR of 28%; both figures are still substantially higher than the WHO CDR of 14%. The possibility that persons who provided a saliva sample were not able to produce a sputum sample because they did not have pathologic pulmonary changes should also be taken into consideration. If so, the estimated prevalence is correct.

Estimation of the incidence of smear-positive TB in Eritrea is complicated by the fact that no data from tuberculin or prevalence surveys were available. The only data available for Eritrea were reporting data, which experts assessed as being of low quality (*14*). Use of this limited information will result in an uncertain incidence estimate, which may result in an unreliable CDR.

For most countries in Africa, little information is available for estimating the prevalence of disease and progress towards the Millennium Development Goals (http://unstats.un.org/unsd/mi/mi_goals.asp, accessed 2006 Aug 30). On the basis of case reporting, TB was rightly declared an emergency by African health ministers at the WHO Africa Regional Committee in Maputo in 2005 (*15*). To be able to fight this emergency, more reliable information about the prevalence of TB in Africa is needed. Furthermore, for global TB control, reliable information about the TB epidemic in Africa is needed because 28% of the incident smear-positive cases occurred in the WHO African region in 2004 (*2*).

In conclusion, the example of Eritrea shows that a large gap may exist between available estimates of TB prevalence and actual TB prevalence in Africa. National TB prevalence surveys in Africa would help provide better information on TB prevalence and case detection.

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West Nile Virus Infection among the Homeless, Houston, Texas[1]

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Among 397 homeless participants studied, the overall West Nile virus (WNV) seroprevalence was 6.8%. Risk factors for WNV infection included being homeless >1 year, spending >6 hours outside daily, regularly taking mosquito precautions, and current marijuana use. Public health interventions need to be directed toward this high-risk population.

West Nile virus (WNV) was first identified in Houston in 2002 (*1*). From 2002 through 2004, 6% of patients hospitalized with WNV infection were homeless (*2*), which raised concerns that the homeless population might be at increased risk for infection. This study was conducted to determine the seroprevalence of WNV in Houston's homeless population after 2 transmission seasons and to determine risk factors for infection.

The Study

A cross-sectional survey was conducted by using convenience sampling of homeless shelters, soup kitchens, homeless camps, and mobile outreach organizations. Participants gave consent and were assigned a unique study number to preserve anonymity. An interviewer-administered questionnaire collected information on demographics, social and medical histories, housing status during the 2002 and 2003 WNV transmission seasons, length of time homeless, and outdoor exposures. The study was approved by the University of Texas Health Science Center at Houston Committee for the Protection of Human Subjects (HSC-SPH-03-111).

A Mini–Mental State Examination (MMSE) was performed to evaluate the cognitive status of the participant. Participants who scored <75% on the MMSE were considered cognitively impaired, and therefore their interview responses were excluded.

As incentive, participants were provided free onsite testing and counseling for HIV, hepatitis B, hepatitis C, and WNV infections. Blood samples were collected and later tested for WNV antibodies by immunoglobulin G (IgG) ELISA and hemagglutination inhibition (HI) test. Samples were considered WNV antibody–positive if both the IgG ELISA and HI assay gave positive reactions. Data were entered into a Microsoft (Redmond, WA, USA) Access database and analyzed by using Stata 8.0 (Stata Corp., College Station, TX, USA). WNV prevalence and risk of becoming infected were calculated for each variable. Univariate odds ratios (ORs) with $p<0.25$ were included in a logistic regression model. A backward stepwise approach was used to eliminate variables with $p>0.10$ to determine a final model. Interactions between variables were assessed for significance ($p<0.10$), and the Hosmer-Lemeshow goodness-of-fit statistic (*3*) was used to evaluate the fit of the final model.

During the spring of 2004, 424 participants were enrolled from 13 sites; 8 were excluded due to low MMSE scores. Of the 416 participants, 397 had complete interviews, adequate blood samples, and were included in the analysis. This sampling represents ≈4% of Houston's estimated 10,000 homeless population (*4*).

Of the 397 participants, 27 were WNV positive (seroprevalence 6.8%; 95% confidence interval [CI] 4.5–9.7). Men represented 72% of the participants, with 8.4% found to be positive for WNV, compared with 2.7% of women (OR 3.3; 95% CI 0.96–11.0) (Table 1). The study population was 59% black, 30% white, and 11% "other" or not stated; 13% were of Hispanic ethnicity. Mean age was 42 years (range 18–69 years).

For both 2002 and 2003 transmission seasons, 278 (70%) participants reported having stable housing, and WNV seroprevalence was 4.7% (95% CI 2.5–7.9) (Table 2). For those who had unstable housing in both 2002 and 2003 (n = 45; 11%), we found a significantly higher WNV seroprevalence of 13.3% (OR 3.1, 95% CI 1.1–8.7). For those who reported being homeless >1 year (n = 73; 18%), seroprevalence for WNV was 16.4% (95% CI 8.8–27.0), with a significantly increased risk for WNV infection when compared with those who did not consider themselves homeless or were homeless <1 month (OR 3.2; 95% CI 1.3–7.7, p = 0.01). When asked about the average length of time spent outdoors during the summer and fall, 38% reported ≤6 hours per day (seroprevalence 2.0%), 38% reported >6–12 hours (seroprevalence 8.0%), and 24% reported >12 hours (seroprevalence 12.5%). There was a positive trend (p value for trend 0.002) between number of hours spent outside and increased risk for WNV infection.

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Table 1. Participant demographics and WNV prevalence from the 2004 Houston Homeless Seroprevalence Study*

Demographic characteristics	All participants, n = 397 (%)	WNV prevalence		Risk for WNV infection, OR (95% CI)
		No. (%)	95% CI	
Sex				
Female	110 (28)	3 (2.7)	0.6–7.8	Reference
Male	287 (72)	24 (8.4)	5.4–12.2	3.3 (0.96–11.0)
Age, y				
18–34	97 (24)	2 (2.1)	0.3–7.3	Reference
35–49	204 (51)	17 (8.3)	4.9–13.0	4.3 (0.98–19.1)
≥50	95 (24)	8 (8.4)	3.7–15.9	4.4 (0.9–21.1)
Unknown	1 (0.3)	0	–	–
Race				
White	120 (30)	8 (6.7)	2.9–12.7	Reference
Black	233 (59)	18 (7.7)	4.6–11.9	1.2 (0.5–2.8)
Other/unknown	44 (11)	1 (2.3)	0.06–12.0	0.3 (0.04–2.7)
Ethnicity				
Hispanic	52 (13)	2 (3.9)	0.5–13.2	Reference
Non-Hispanic	343 (86)	24 (7.0)	4.5–10.2	1.9 (0.4–8.2)
No response	2 (0.5)	1 (50.0)	1.3–98.7	–

*WNV, West Nile virus; OR, odds ratio; CI, confidence interval.

Current marijuana use was also associated with WNV infection (OR 2.5; 95% CI 1.0–6.0).

Univariate analysis identified the following variables as significantly ($\alpha<0.05$) associated with risk for WNV infection: unstable housing in 2002 and 2003, being homeless >1 year, spending >6 hours outside per day during the summer and fall, and current marijuana use. The final logistic regression model identified the following independent risk factors ($p<0.10$) for WNV infection: being homeless >1 year (OR 3.8, $p = 0.002$), spending >6 hours outdoors (OR 4.3, $p = 0.02$), normally taking mosquito precautions (OR 2.8, $p = 0.04$), and current marijuana use (OR 2.4, $p = 0.07$). The Hosmer-Lemeshow goodness-of-fit-test statistic was 12.4 ($p>0.19$), which suggests that the model is a good fit. When interaction terms were entered into the model, the interaction between marijuana smoking and spending >6 hours outdoors was significant (likelihood ratio $p = 0.04$) and increased the strength of the association with WNV infection.

Conclusions

We believe this is the first study to determine the prevalence of WNV in homeless adults and to determine risk factors for becoming infected among this high-risk urban population. Findings from this study will help public health authorities determine appropriate intervention and prevention strategies.

We found a seroprevalence of 6.8% in our sample of homeless persons, with a seroprevalence of 16.4% in persons reporting being homeless >1 year. Other studies have assessed the prevalence of WNV in general populations in the United States (*5*–*10*), with estimates of 0%–14%.

To our knowledge, this is the first report of WNV seroprevalence in a population with high-risk outdoor exposures. Only 3 studies have evaluated risk factors for infection in the United States and found that increased time outdoors (*5*,*8*), inconsistent use of mosquito repellant (*5*), and age (*9*) were predictors for infection. In Houston's homeless population, spending >6 hours outside per day during the summer and fall and being homeless >1 year independently predicted risk for infection. Although being homeless >1 year was highly associated with increased time spent outdoors, this variable also independently predicted infection. This finding is important in a public health context because it highlights a strong potential for further cases of WNV infection in this population.

We found that regularly using mosquito precautions was associated with an increased risk for infection, which differs from the findings in New York (*5*). This finding was surprising since, in theory, use of mosquito precautions should reduce the risk for WNV infection. However, when asked about the types of mosquito precautions used, many participants reported methods that may be ineffective such as using candles or fire as a deterrent or swatting at mosquitoes. Education regarding appropriate preventive methods would be valuable in this population.

In addition, we found that marijuana use predicted WNV infection, which is difficult to explain. To our knowledge, this is the first report of marijuana use being a risk factor for WNV infection. Several explanations are possible, however: 1) this finding was due to chance, 2) persons using marijuana may spend more time outdoors between dusk and dawn when the *Culex* mosquito is most active, 3) the mosquito vector could be attracted to marijuana smoke, or 4) marijuana use could affect cognition, thereby preventing the user from interrupting a mosquito taking a blood meal. The relationship between marijuana use and WNV infection deserves further investigation.

Table 2. Self-reported social histories and prevalence of WNV infection from the 2004 Houston Homeless Seroprevalence Study*

Participant characteristics	All participants, n = 397 (%)	WNV prevalence		Risk for WNV infection, OR (95% CI)
		No. (%)	95% CI	
Housing status†				
Stable housing, 2002 and 2003	278 (70)	13 (4.7)	2.5–7.9	Reference
Unstable housing, 2002 or 2003	69 (17)	7 (10.1)	4.2–19.8	2.3 (0.9–6.0)
Unstable housing, 2002 and 2003	45 (11)	6 (13.3)	5.1–26.8	3.1 (1.1–8.7)‡
Unknown	5 (1)	1 (20.0)	0.5–71.6	–
Homelessness status				
Does not consider himself or herself homeless	111 (28)	8 (7.2)	3.2–13.7	Reference
Lives mostly on the streets	50 (13)	6 (12.0)	4.5–24.3	1.8 (0.6–5.4)
Lives in temporary shelter	125 (31)	10 (8.0)	3.9–14.2	1.1 (0.4–2.9)
Lives temporarily with friends/family	64 (16)	1 (1.6)	0.04–8.4	0.2 (0.02–1.7)
Other	47 (12)	2 (4.3)	0.5–14.5	0.6 (0.1–2.8)
Length of time homeless				
Does not consider himself or herself homeless or homeless <1 mo	171 (43)	10 (5.9)	2.8–10.5	Reference
1 mo–1 y	153 (39)	5 (3.3)	1.1–7.5	0.5 (0.2–1.6)
>1 y	73 (18)	12 (16.4)	8.8–27.0	3.2 (1.3–7.7)‡
Time spent outdoors on average each day during summer and fall§				
≤6 h	150 (38)	3 (2.0)	0.4–5.7	Reference
6–12 h	150 (38)	12 (8.0)	4.2–13.6	4.3 (1.2–15.4)¶
>12 h	96 (24)	12 (12.5)	6.6–20.8	7.0 (1.9–25.5)‡
Unknown	1 (0.3)	0 (0)	–	–
Substance use#				
Current tobacco use	273 (69)	20 (7.3)	4.5–11.1	1.3 (0.5–3.2)
>15 drinks containing alcohol/wk	70 (18)	3 (4.3)	0.9–12.0	0.6 (0.2–1.9)
Ever used street drugs	281 (71)	19 (6.8)	4.1–10.4	1.0 (0.4–2.3)
Ever used needles to inject street drugs	89 (22)	6 (6.7)	2.5–14.1	1.0 (0.4–2.5)
Current drug use (within past 6 mo)	108 (27)	11 (10.2)	5.2–17.5	1.9 (0.9–4.3)
Street drugs used in past 6 mo**				
CNS stimulants (crack/cocaine/ amphetamines)	84 (21)	5 (6.0)	2.0–13.3	0.8 (0.3–2.3)
Heroin/opiates	4 (1)	0 (0)	–	–
Marijuana	61 (15)	8 (13.1)	5.8–24.2	2.5 (1.0–6.0)¶

*WNV, West Nile virus; OR, odds ratio; CI, confidence interval; CNS, central nervous system.
†p value for trend = 0.02.
‡Significant at $\alpha = 0.01$.
§p value for trend = 0.002.
¶Significant at $\alpha = 0.05$.
#Alcohol and current drug use will most likely be underestimated because shelters did not allow use of these substances.
**Not mutually exclusive, hence univariate analysis compared specific drug use with no use of that drug.

For comparison, data on WNV prevalence in a nonhomeless population during the same time period and location would be useful. After the 2003 transmission season, a study at the University of Texas Health Science Center at Houston found a seroprevalence of 4.7% among 274 students, faculty, and staff (K. Murray, unpub. data).

This study provides important information on the magnitude and risk factors for WNV infection among homeless persons. Combining education with distribution of effective mosquito prevention aids such as mosquito repellent may help reduce the risk for WNV infection and other mosquitoborne diseases in this high-risk population.

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Schistosoma hematobium and *S. mansoni* among Children, Southern Sudan

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We conducted a survey of schistosomiasis among schoolchildren in 2 villages in Southern Sudan. In Lui (West Equatoria region), prevalence of *Schistosoma mansoni* infection was 51.5%; no cases of *S. hematobium* infection were detected. In Nyal (Upper Nile region), prevalence of *S. hematobium* infection was 73% and *S. mansoni* infection, 70%.

Schistosomiasis is a major communicable disease of public health and socioeconomic importance in the developing world. Both *Schistosoma hematobium* and *S. mansoni* are present in Sudan, a war-torn country with a population of ≈6 million persons and one of the world's most underdeveloped regions. Risk for schistosomiasis in Sudan is widespread, especially in the major irrigation systems in the Gezira area between the Blue and White Nile Rivers.

Early reports stated that schistosomiasis is endemic in southern provinces, but few sustained surveys have been conducted, particularly during the years overwhelmed by the civil war, and recent data on prevalence rates are lacking (*1–3*,*5–10*). We therefore conducted an epidemiologic survey in 2 villages in Southern Sudan.

The Study

This investigation was a cross-sectional study conducted in 2 separate regions of Southern Sudan from August through October 2002 (rainy season). The study population was recruited from the only primary schools in the village of Lui (Mundri County), West Equatoria Region, and from 3 randomly selected primary schools in the district of Nyal (for a total of 4 schools), Upper Nile Region, 40 km west of the Nile River (Figure). In 2006, an estimated 740,000 persons lived in the West Equatoria region, while an estimated 221,667 persons lived in Mundri County. An estimated 11,500–15,000 persons lived in Nyal village. The ecologic situation (rain, presence of water bodies, population density) and the presence of sanitation structures are homogenously distributed in Nyal District, and the populations of the schools we selected were representative of the parasitologic situation in this area. Lui has an estimated population of 7,000–9,000 persons and a primary school only.

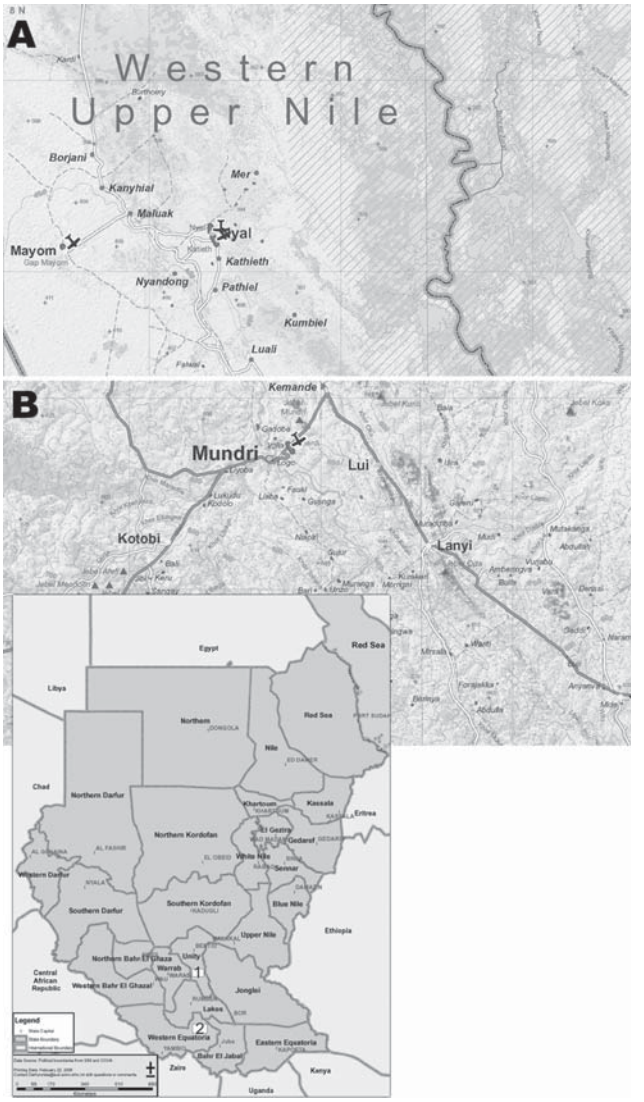

Figure. Location of the study areas in Southern Sudan: A) Western Upper Nile; B) West Equatoria Region, Mundri County (source: Centre for Development and Environment, University of Berne, Switzerland; available from www.cde.unibe.ch/sudan/maps), with an inset of the whole country (source: World Health Organization; available from www.emro.who.int/sudan/media/pdf/sud-states-2006.pdf).

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To assess the prevalence of schistosomiasis, we conducted a survey of stool and urine samples as recommended by the World Health Organization (WHO) (*11*). A sample of 200–250 children in each ecologically homogenous area was considered adequate to evaluate prevalence and intensity of *Schistosoma* infection in a disease-endemic area. Before the onset of the study, information meetings were held with the school staff. Schoolchildren were asked to provide a stool sample and at least 10 mL of urine. Consent was obtained directly from the children (or parents or teachers) before specimen collection. The study was part of the routine investigations conducted by the Sudan Ministry of Health and WHO to provide adequate treatment to the children in the area. Because the surveyed children were not exposed to any risk (they only provided stool and urine samples), no special approval was requested.

Specimens were collected at school and brought to the laboratory, where they were prepared and interpreted within 24 hours. This procedure was repeated over 2 days in each village surveyed. Urine samples were tested for *S. hematobium* infection by using the Urine Filtration Kit (Vestergaard-Frandsen, Nairobi, Kenya) provided by WHO. Fecal specimens were tested for *S. mansoni* infection by using the Kato Katz semiquantitative method (*11*,*12*). Infections were classified as high intensity if results were ≥50 eggs of *S. hematobium* per 10 mL of urine or at least 400 eggs of *S. mansoni* per gram of feces. Because, for technical reasons, urine and stool specimens were collected on separate days, we have no data on dual *S. hematobium* and *S. mansoni* infection.

Urine specimens were obtained from 400 children, 200 in each village; fecal specimens were obtained from 400 children, 200 in each village. The age range was 8–12 years; 75% of those who provided samples were boys. The disparity between the sexes is probably related to more unwillingness of the girls to provide urine or fecal specimens.

The prevalence and intensity of *S. hematobium* and of *S. mansoni,* by geographic area, are shown in the Table. Though the prevalence was higher for girls than for boys, these differences were not statistically significant by χ^2 test (data not shown). As a consequence of the high prevalence of schistosomiasis, praziquantel (40 mg/kg) treatment was offered to all children in the surveyed schools.

Conclusions

Most epidemiologic studies regarding schistosomiasis in Sudan have been carried out in the Gezira-Managil area and in other central or northern areas of economic importance, while relatively few studies have been conducted in other parts of the country. Our study was conducted in 2 ecologically different areas in Southern Sudan. The West Equatoria region is a savannah area, topographically and ethnographically homogeneous with the bordering regions of Uganda, characterized by a prevalence of intestinal bilharziasis (*8*). The Upper Nile region is a swampy area and has been considered by the Sudanese authorities as being nonendemic for bilharziasis (*3*,*8*).

The long-running civil war has overshadowed Southern Sudan's recent history and devastated social and health services. Updated epidemiologic data are thus lacking, and most epidemiologic reports from these areas are scattered, incomplete, and outdated (1930–1970) (*1–10*). Data from annual reports of the Sudan Medical service in 1939–1949 and from other published or unpublished sources indicated that *S. mansoni* and S. *hematobium* were found in 1.9% and 2.5% of specimens in the Upper Nile region and in 4.9% and 0.25% of specimens in the Bahr El Ghazal region (close to the Northern Sudan border); in the West Equatoria region, the prevalence of *S. mansoni*–positive specimens has been reported as high as 44.3%, but no specimen was positive for *S. hematobium* (*3*,*8*). These data were based mainly on routine examination of urine and stools in hospitals and dispensaries. Subsequently, Amin and Omer suggested a 4.8%–6.8% prevalence of *S. mansoni* infection and a 0.4%–44% prevalence of *S. hematobium* infection in different areas of Southern Sudan (*9*).

Since the 1970s, few observations on the prevalence of schistosomiasis in southern Sudan have been made. Magambo et al. in 1998 reported a *S. mansoni* prevalence of 2.2% among children in 2 primary schools in the East Equatoria region, very close to the Ugandan border (*10*). A report conducted in the early 1990s in Juba (the largest and most developed town in Southern Sudan under the Northern government control) showed that 66% of refugees were harboring intestinal helminths, and in 26% of cases, *S. mansoni* (*13*). Data from Rhino camp, one of the major Ugandan camps accommodating Southern Sudanese refugees, showed a prevalence of *S. mansoni* as high as 77.8% (*14*).

Table. Prevalence and intensity of *Schistosoma mansoni* and *S. hematobium* in 2 villages in Southern Sudan

	S. hematobium		*S. mansoni*		
Location	No. positive specimens/total examined (%)	No. high intensity infections (%)*	No. positive specimens/total examined (%)	No. moderate intensity infections (%)†	No. heavy intensity infections (%)‡
Lui (West Equatoria)	0/200	0	103/200 (51.5)	33 (16.5)	16 (8)
Nyal (Upper Nile)	146/200 (73)	57 (28.5)	140/200 (70)	54 (27)	37 (18.5)

*>50 eggs/10 mL urine.
†100–399 eggs/g feces.
‡≥400 eggs/g feces.

This potential impact of population mobility on the transmission of schistosomiasis should be taken into consideration. Actually, the January 2005 peace agreement between Khartoum and the Sudan People's Liberation Movement is expected to trigger the return of half a million Sudanese who fled to nearby countries and the gradual resettlement of millions internally displaced. Increased efforts by the international community to help meet the basic needs of this rapidly growing population are urgently needed.

Our data confirm a high prevalence of *S. mansoni* infection in some areas in the West Equatoria region and show that both *S. mansoni* and *S. hematobium* are highly endemic in the Upper Nile region. The collected data facilitated schistosomiasis control interventions in the area. Mass distribution of praziquantel to all schoolchildren has been organized in the 2 districts since 2003.

When one considers the extremely high prevalence and the very low cost of the control measures (*15*), mass administration of praziquantel should be annually provided to the children in the resident population and to the children in the displaced population that will resettle to these areas as a consequence of peace agreements. These data also provide further evidence supporting the Centers for Disease Control and Prevention recommendation (available from www.cdc.gov/ncidod/dq/pdf/lost%20boys%20and%20girls%20presumptive%20treatment%20recommendations.pdf) that members of the Lost Boys and Girls of Sudan refugee group who have resettled in the United States and other Sudanese refugees receive presumptive therapy for schistosomiasis.

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Dr Deganello is an infectious diseases specialist. At the time of this study, he was an infectious diseases field medical officer for WHO in Southern Sudan. He is currently completing postgraduate training in medical radiology at the University of Verona. His research interests include tropical diseases.

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Influenza A and B Infection in Children in Urban Slum, Bangladesh

To the Editor: Influenza A and B viruses are associated with seasonal epidemics (*1*). Influenza is increasingly recognized as a cause of severe respiratory disease among healthy children in industrialized countries (*2–4*). However, little information is available from developing countries (*5*).

We assessed the contribution of influenza and other respiratory viruses to febrile respiratory illness among children enrolled at the ICDDR,B Kamalapur surveillance and intervention site (*6*) in an urban slum in Dhaka, Bangladesh. This study was reviewed and approved by the Research Review and Ethical Review Committees of ICDDR,B and the Institutional Review Board of the Centers for Disease Control and Prevention (CDC) (Atlanta, GA, USA).

Surveillance in Kamalapur has been described (*6,7*). Briefly, the site is divided into 7 geographic strata and subdivided into geographic clusters of 50–100 households. Eighty-six clusters were randomly selected and 5,000 households within those clusters were enrolled after written consent was obtained.

Prospective fever surveillance methodology has been described (*7*). To evaluate viral causes of febrile respiratory illness, paired (acute- and convalescent-phase) serum specimens were retrospectively selected from the surveillance period of December 6, 2000 to December 5, 2001, from patients with documented fever ≥38.5°C, a cough for ≥1 day but ≤4 days, and age <13 years, and who were negative for antibodies to dengue by immunoglobulin M (IgM) antibody-capture ELISA.

Sera were tested by hemagglutination inhibition (HI) for influenza A (H1N1 and H3N2) virus and B virus, and by ELISA for respiratory syncytial virus, parainfluenza virus types 1, 2, and 3, adenovirus, and human metapneumovirus at CDC by using standard methods (*8*). Acute infection was defined as IgM in serum sample or a ≥4-fold increase in IgG titer between acute- and convalescent-phase serum samples for noninfluenza viruses or a ≥4-fold increase in HI titer for influenza viruses.

Statistical analysis was performed by using Stata Statistical Software Release 8.2, 2003 (Stata Corporation, College Station, TX, USA). Continuous variables were compared by using analysis of variance. Univariate categorical analysis was conducted by using 2 × 2 tables to obtain odds ratios (ORs) and 95% confidence intervals (CIs). Multivariate analysis was conducted by using stepwise forward logistic regression and all covariates significant with a 5% precision in univariate analysis. We adjusted the model for clustering (multiple observations per patient) and tested for goodness of fit.

Of 889 patients who came to the ICDDR, B Kamalpur field clinic with fever during the surveillance period, 198 (22%) met inclusion criteria for retrospective sampling. Of these, 128 had adequate paired serum specimens for influenza testing. Only 107 (83.6%) pairs had sufficient serum remaining for testing for other viruses.

Of 128 children, 21 (16%) had acute influenza infections; 2 of these children had both influenza A and B. Overall, 10 influenza A (8 H1N1 and 2 H3N2) and 13 influenza B infections were detected (Table). Other respiratory virus infections were detected in 33 children and accounted for 35 noninfluenza virus infections (Table). One child was coinfected with both influenza A (H3N2) virus and HMPV. Seven (70%) of 10 influenza A cases occurred during April–June (pre-monsoon period), and 10 (77%) influenza B cases occurred during July–September (monsoon period).

Data for 107 serum pairs tested for both influenza and other viruses indicated that influenza-infected children were older than children without influenza (OR 3.1, 95% CI 1.1–9.3). Multivariate analysis indicated that only reported body pain was more common in influenza patients than in others (OR 3.3, 95% CI 1.5–7.1). Three influenza-infected children had clinical pneumonia (tachypnea defined by the World Health Organization) with crepitations.

We confirmed that influenza A and B were common causes of febrile illness among children in Dhaka. Because these infections were identified in 1 of 6 dengue-negative febrile children tested for influenza, these infections may play a substantial role in respiratory diseases in these children. Our study confirms findings of a previous hospital study (*9*) but provides additional information for nonhospitalized febrile children. Acute infections coincided with the warm pre-monsoon and monsoon periods.

Our study had several limitations. First, the surveillance system was not

Table. Virus infections detected by serologic analysis in children <13 years of age, December 2000–December 2001, Dhaka, Bangladesh*

Virus	No. infections (N = 56)
Influenza A†	8
Influenza B†	11
Influenza A and B†	2
Respiratory syncytial virus‡	2
Parainfluenza type 3‡	9
Adenovirus‡	4
Human metapneumovirus‡	20

*Virus infections were defined as a ≥4-fold increase in titers between acute- and convalescent-phase serum sample testing.
†From 128 serum pairs tested.
‡From 107 serum pairs tested.

originally designed to identify influenza and relied on fever for specimen collection. Our retrospective selection criteria reflected the classic initial manifestations of influenza (*1*,*4*), and thus could have missed nonfebrile cases. Second, the study was not designed to reflect age distribution of children with respiratory infection, but rather those with fever and who had adequate amounts of available sera. This feature potentially biases toward older children. Third, data describe only 1 year, and patterns of illness may differ in other years. Fourth, acute infection was determined by serologic analysis. Previous studies in Bangladesh reported nutrition-related impaired immune responsiveness (*10*). Thus, some influenza-infected children who showed a nondetectable immune response may not have been included.

These findings indicate that influenza and other respiratory viruses contribute to pediatric febrile illness in urban Bangladesh. They also justify prospective surveillance to better define epidemiology and clinical findings associated with these viruses.

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Identification of Rickettsiae, Uganda and Djibouti

To the Editor: Tickborne rickettsioses are caused by obligate intracellular gram-negative bacteria that belong to the spotted fever group of the genus *Rickettsia*. These zoonoses share characteristic clinical features, including fever, headache, rash, and sometimes eschar formation at the site of the bite (*1*). Although rickettsioses are important emerging vectorborne infections of humans worldwide, little is known about rickettsioses in sub-Saharan Africa (*1*,*2*).

In 2002, 94 ticks were collected in Djibouti: 5 *Amblyomma lepidum*, 1 *A. variegatum*, 5 *Hyalomma marginatum rufipes*, 40 *Rhipicephalus pulchellus*, and 10 *Rh. evertsi evertsi* from cattle that had just arrived from Ethiopia; 30 *H. dromedarii* from dromedaries; and 3 *Rh. sanguineus* group ticks from cheetahs. In 2003, 57 ticks were collected from dogs in Kampala, Uganda: 1 *A. variegatum*, 9 *Haemaphysalis punctaleachi*, 28 *Rh. praetextatus*, and 19 *Rh. sanguineus*. All ticks were partially or fully engorged adults. This convenience sample of ticks was obtained as part of other ongoing studies.

Ticks were identified by using taxonomic keys (*3*) and kept in 70% ethanol before being tested. DNA of each tick was extracted, and rickettsial DNA was detected by PCR that used primers Rp.877p and Rp.1258r, which amplify a 396-bp fragment of the citrate synthase gene (*gltA*) of rickettsia, as described (*4*). Rickettsia-positive samples were tested by a second PCR that used Rr.190.70p and Rr.190.701n primers, which amplify a 629–632 bp fragment of *ompA* gene (*4*). Controls (2 negative [DNA extracted from noninfected laboratory ticks and distilled water] and 1 positive [*R. montanensis* DNA]) were included in each test. The

sequences of PCR products were obtained and compared with those available in GenBank (*4*).

One specimen of *Ha. punctaleachi* from Uganda and 1 *A. lepidum* from Djibouti, as well as positive controls, were positive according to PCR using both primer pairs. No signal was obtained from negative controls. The sequence of a 474-bp fragment of o*mpA* obtained from *Ha. punctaleachi* showed 99.8% (473/474) similarity with *R. conorii* (GenBank accession no. AY346453); those of a 340-bp segment of *gltA* showed 100% similarity with that of *R. conorii* (AE008677). The sequences of a 517-bp segment of *omp*A and a 341-bp segment of *glt*A amplified from *A. lepidum* showed 100% similarity to the corresponding sequences of *R. africae* (U83436 and U59733, respectively).

To our knowledge, this is the first detection of *R. conorii*, the agent of Mediterranean spotted fever, in Uganda. Although the main vector of this rickettsia is *Rh. sanguineus*, the few ticks of this species we tested were negative (*1*). It is also the first detection of *R. conorii* in *Ha. punctaleachi*, although it has been detected in the closely related *Ha. leachi* in Zimbabwe (*5*). *Ha. punctaleachi* prefers warm and humid conditions but can exist wherever rodent hosts for its immature stages and canine hosts for its adult stages are present (*6*). Adults are found throughout the year; peak numbers occur either from winter to early summer or from spring to late summer (*7*). Although the detection of *R. conorii* in *Ha. punctaleachi* does not mean that this tick is an efficient vector (*8*), clinicians in Uganda should be aware of the presence of Mediterranean spotted fever in their country.

This is also, to our knowledge, the first detection of *R. africae*, the agent of African tick bite fever, in Djibouti. *R. africae* was also detected in 1 *A. lepidum* collected in Sudan (*4*), but it is more frequently detected in *A. variegatum* and *A. hebraeum* with high infection rates throughout sub-Saharan Africa (*7*). *A. lepidum*, which coexists with *A. variegatum* in limited locations, is chiefly a cattle parasite. It will also attach to smaller domestic animals and a few wild herbivores, but it attacks humans less frequently than *A. variegatum* or *A. hebraeum*. *A. lepidum* occurs in a variety of climatic regions but most commonly inhabits semiarid regions in eastern Africa. The cattle in our study had been imported from Ethiopia, and the ticks may have infested these animals before their arrival in Djibouti. Indeed, in 1973 Burgdorfer obtained an isolate from *A. variegatum* in Ethiopia, which was thereafter shown to be indistinguishable from the rickettsia described as *R. africae* (*7*,*9*). Again, clinicians should be aware of the presence of *R. africae* in Djibouti and that it could affect their patients, both local and international, including French and American soldiers based in this country (*10*).

Because we did not do systematic sampling, our results cannot address the prevalence and distribution of *R. conorii* and *R. africae* in Uganda and Djibouti, respectively. However, healthcare workers who treat persons who live in or have traveled to these countries should be alert for spotted fever group rickettsial infections in their patients (*1*).

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Skin and Soft Tissue Infections and Vascular Disease among Drug Users, England

To the Editor: The injecting of illicit drugs is associated with skin and soft tissue infections (SSTIs) and vascular disease (*1–3*). These conditions include the development of cutaneous abscess and cellulitis at injection sites, from subcutaneous or intramuscular injecting, known as skin and muscle popping, and intravenous injecting (*1–3*). Intravenous injection is associated with phlebitis or thrombophlebitis, in which the vein may become infected (*4*). Inadvertent arterial injection, particularly when attempting to inject into the femoral vein, that is, "groin injecting," may cause arterial pseudoaneurysm (*4*,*5*).

Drug-related conditions form a major part of the workload of some hospital emergency departments in the United Kingdom and elsewhere (*1–3*,*6*). We aimed to identify emerging trends in hospital admission for SSTIs and vascular disease arising from drug use and, specifically, where these may have occurred after injection of the femoral vein.

We extracted hospital admission data for drug users 15–44 years of age for the fiscal years April 1, 1997–March 31, 2004, from the UK Department of Health, hospital episode statistics (HES) database. Using the International Classification of Diseases, 10th revision (ICD-10) codes F11–16, F18, and, F19, we identified drug users by a record in any diagnostic field of mental and behavioral disorders due to psychoactive substances, excluding alcohol and tobacco. We identified the primary diagnosis on admission and whether the admission was as an emergency.

Over the study period, admissions of drug users for cutaneous abscess, L020–L029; cellulitis, L030–L039; and phlebitis or thrombophlebitis, I801–I809; increased substantially (online Appendix Figure, available from www.cdc.gov/EID/content/13/10/1510-appG.htm). Increases occurred in specific primary diagnoses from 1997–1998 to 2003–2004; the largest percentage increase was for phlebitis or thrombophlebitis of the femoral vein, I801, from 60 to 533 (788%). Increases were also observed for aneurysm or pseudoaneurysm of an artery of a lower limb, I724, from 9 to 62 (589%), cutaneous abscess of trunk or groin, L022, from 92 to 613 (566%), cellulitis of trunk or groin, L033, from 13 to 74 (469%), and, phlebitis or thrombophlebitis of deep vessels of the lower limb other than the femoral vein, I802, from 269 to 1,314 (388%).

No national data exist for the prevalence of injection drug use in England (*7*). Although the number of opiate injecting drug users may have increased in the 1990s (*8*), the rapid and substantial increase in admissions for SSTIs and vascular disease suggests that this has not resulted from an increase in the injecting population alone.

The contribution to the increase in admissions from subcutaneous or muscle injecting and intravenous injecting cannot be determined from these data. The increases for phlebitis or thrombophlebitis of the femoral vein and aneurysm or pseudoaneurysm of the lower limb suggest that groin injecting may have contributed to the study findings (*9*). The choice of drugs may have contributed to our findings. An association between injecting site infections in England has been reported with crack cocaine injection and elsewhere with cocaine injection (*2*,*3*,*7*).

This study has some limitations. The HES database does not distinguish between injection and noninjection drug users and whether injection was intravenous or subcutaneous and intramuscular. The study does not relate HES data entries to the conditions described directly by the physical examination of patients or the review of clinical notes. Those conditions associated with femoral vein injection do not exclusively result from this practice, and the proportion of these admissions not associated with injection drug use is unknown. The study period was limited by date of the introduction of ICD-10 coding; therefore, earlier trends could not be identified. Nevertheless, this analysis highlights a potentially important trend and the need for further quantitative and qualitative research in injection drug users.

The response to these problems could be addressed by changing behavior and improving access to healthcare (*1*). Ideally, injection drug users (IDUs) should have early entry to, and be retained on, substance abuse treatment, particularly methadone maintenance (*1*). Skin and muscle injecting, and injecting into the femoral vein should be discouraged (*3*). To inject safely, IDUs need access to clean equipment to prevent the use of shared and dirty needles and the reuse of syringes. Injection sites should be rotated, the skin should be cleaned with alcohol, and the licking of needles and booting should be discouraged (*1–3*). Patients were predominantly admitted through emergency departments, which suggests poor contact with health services and reluctance to seek treatment until the point of crisis (*10*). Early medical treatment is required, possibly with the creation of hospital-based SSTI clinics, as were successfully introduced in San Francisco, or improved community outreach (*1*).

In summary, this study identifies a rapid and important increase in the hospitalization of drug users in England for SSTIs and vascular conditions. Further work is required to obtain more information about these clinical problems and the patients' associated lifestyle, on admission and in the community. Means of discouraging risk-related behavior and treatment should be implemented before

the conditions require urgent hospital admission.

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Confronting Potential Influenza A (H5N1) Pandemic with Better Vaccines

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Influenza A (H5N1) viruses are strong candidates for causing the next influenza pandemic if they acquire the ability for efficient human-to-human transmission. A major public health goal is to make efficacious vaccines against these viruses by using novel approaches, including cell-culture system, reverse genetics, and adjuvant development. Important consideration for the strategy includes preparation of vaccines from a currently circulating strain to induce broad-spectrum immunity toward newly emerged human H5 strains. This strategy would be a good solution early in a pandemic until an antigenically matched and approved vaccine is produced. The concept of therapeutic vaccines (e.g., antidisease vaccine) directed at diminishing the cytokine storm frequently seen in subtype H5N1–infected persons is underscored. Better understanding of host–virus interaction is essential to identify tools to produce effective vaccines against influenza (H5N1).

Bird flu caused by the influenza A virus subtype H5N1 has spread with alarming speed across Europe, Africa, and parts of Asia in which the infection was not reported earlier. Establishment of the highly pathogenic avian influenza (H5N1) as an endemic virus within duck and poultry populations and its capacity to cross species barriers increase the possibility of adaptation to humans and a pandemic. Human influenza infections with subtype H5N1 viruses are often fatal. As of June 4, 2007, 309 laboratory-confirmed cases of human infection have been reported to the World Health Organization (WHO); 61% were fatal, mainly in persons 10–39 years of age (www.who.int/csr/caculatordisease/avian_influenza/en). If a pandemic is triggered by transmissibility of influenza (H5N1) from person to person, millions of people could die, and economies would likely be crippled for 6–24 months.

In the event of a pandemic, vaccination against influenza (H5N1) could limit the impact of infection at a public health level. However, no evidence exists that available vaccines would be protective against the pandemic strain of the virus. We comment on some of the limitations of currently available vaccines and propose novel strategies to improve vaccine formulations against influenza (H5N1).

Host's Immune Responses to Influenza (H5N1)

The host response to influenza (H5N1) infection has not been defined, which has proven a considerable challenge in epidemiology and public health research. To develop efficient vaccines, understanding how the virus interacts with the host in natural infection is necessary. Having insights into the hosts' responses to influenza (H5N1) would help define targets for therapeutic intervention. Whether humans can develop immunity during a primary infection that would control replication and spread of subtype H5N1 viruses has been questioned (*1*). However, marked inflammatory responses develop after infection with influenza (H5N1) in humans and other animals (*2*–*4*). This condition is associated with statistically significant synthesis of various proinflammatory cytokines, such as tumor necrosis factor-α, interleukin (IL)–6, interferon (IFN)-γ, IL-1α, and chemokines, including IP-10, MIG, monocyte chemoattractant protein–1 (MCP-1, IL-8, and RANTES. i.e., regulated on activation, normal T-cell expressed and secreted). If this is the case, these observations are consistent with the possible induction of innate immune responsiveness in the persons infected with influenza (H5N1). Most cases of influenza (H5N1) infection in humans have been described as clinical. However, whether subclinical or asymptomatic infections can develop in some persons is not known. Dis-

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ease in humans caused by influenza (H5N1) appeared to be milder in Turkey than in eastern Asia (*5*). The death rate of ≈25% was half that of previously known outbreaks, and 5 mild or completely asymptomatic cases have been reported. One theory holds that milder cases have been occurring elsewhere but are not being recorded. Recently, 3 persons among 120 apparently healthy volunteers from the People's Republic of China, showed detectable virus-neutralizing antibody response to subtype H5N1 before vaccination (*6*). Moreover, pigs infected with subtype H5N1 have become asymptomatic in Indonesia. Are these signs of development of some degree of immunity to virus, containing its replication and thus causing milder infection in naturally infected mammals?

Recently, clusters of bird flu cases were reported in Western Java, Indonesia (*7*); fatal disease developed in 6 persons there from the same family. Two other family members became ill but survived. All the family members likely had similar levels of exposure because they all lived in the same household. Other cases of nonfatal infections have been seen in Thailand and Vietnam. Unfortunately, there is little information about the immune response to the virus in those who survived, which would be valuable for understanding the mechanisms of protection. Indeed, following up the persons (cohort) living in the same affected villages, presumably mostly not exposed to virus, should clarify whether the maintained response reflects boosting through natural exposure. Persons with prior exposure, as measured by antibody or viral RNA at recruitment, would likely have substantially higher responses to the vaccine than those naïve at recruitment if the vaccinating antigen contains homologous or cross-reacting determinants. Conceivably, boosting the "natural" immunity is a desirable outcome to improve protective efficacy of any vaccine approach. Additional studies are required to evaluate the merits of priming populations in advance of an influenza (H5N1) pandemic.

After initial hesitation about using a wide-scale program of poultry vaccination, some European and Asian countries have begun vaccination. Inactivated vaccines are widely used in poultry but lack of critical potency testing, standardization, and quality control has led to variable and suboptimal immune responses. Moreover, a legitimate concern remains that the fowl vaccinated by attenuated live viruses may survive the disease but still carry the virus; thus, they would continue to spread influenza (H5N1) silently at the flock level (*8*) or to humans who come into contact with them. Vaccination that resulted in low levels of seroconversion facilitated the emergence of the Fujian-like sublineage of influenza (H5N1) in poultry (*9*).

The immune responses elicited by subpotent vaccines may exert selection pressure that favors antigenic drift and shift (Figure). Antigenic drift relies on the accumulation of mutations within the antibody-binding sites in the hemagglutinin (HA), neuraminidase (NA), or both that abrogate the binding of antibodies. This makes influenza A virus strains able to evade neutralizing antibody from prior infection or vaccination. Antigenic shift, which is seen only with influenza A viruses, is a more drastic change. It results from genetic shift by reassortment exchange of the HA, and sometimes the NA, with novel subtypes that have not been present in human viruses for a long time. Antigenic shift leads to replacement of circulating strains with new variants that are able to reinfect hosts immune to earlier types; the result is usually a pandemic. Antigenic shifts caused 2 of the major influenza A pandemics in the last century, including the 1957 subtype H2N2 and 1968 subtype H3N2 outbreaks (*10*).

Live Vaccines for Use in Humans

Most influenza vaccines used in the United States and Europe are produced in embryonated hens' eggs and are formaldehyde-inactivated preparations (*11*). Because highly pathogenic influenza (H5N1) subtypes may kill embryonated eggs, use of viruses that are no longer pathogenic, such as H5 (which lacks the polybasic cleavage site), to reduce the virulence of influenza (H5N1) vaccine strains so that these can be efficiently propagated in eggs for vaccine production is feasible (*10*). Virus particles that lack the gene for the nuclear export protein or are defective for the matrix (M2) gene were used as live vaccines in animal models (*12*,*13*); however, whether these replication-defective vaccines will work in humans is not known. Live attenuated (cold-adapted) influenza vaccines have long been used in Russia, and a similar product has been approved for use in the United States (*14*). These vaccines will replicate in the host, and thus lower doses may be effective; however, the

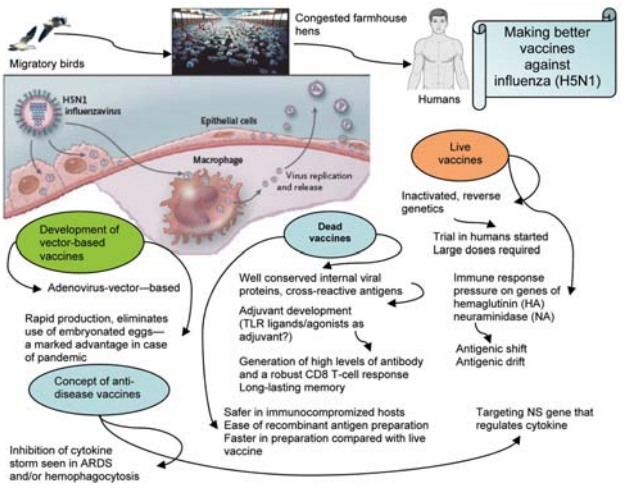

Figure. Spreading mode of influenza A (H5N1) viruses and efforts to make better vaccines for potential pandemic. ARDS, acute respiratory distress syndrome.

preexisting antibody to the virus is more likely to diminish the value of a live vaccine. Moreover, such live vaccines are reported to cause asthma-like reactivity in children (*15*). Monitoring live influenza vaccines is important because the risk for reversion to pathogenicity remains.

With the use of a technique known as reverse genetics, a prototype of influenza virus (H5N1) has been produced for the development of an inactivated subvirion vaccine. The gene segments encoding HA and NA were derived from A/Vietnam/2004, and all other genes were derived from the backbone (A/PR/8/34) virus, commonly used as a platform for influenza vaccine production. The HA gene was further modified to replace the stretch of 6 basic amino acids at the cleavage site, and the resulting virus was avirulent in chickens. In a recent trial, healthy adult volunteers were given 2 intramuscular doses of this inactivated influenza (H5N1) vaccine. This split vaccine induced an antibody response predictive of protection in 54% of healthy adults tested, but only when given intramuscularly at high doses (two 90-μg shots) (*16*). The large amounts needed (2 doses of vaccine, each 6 times the dosage of that used in a standard influenza shot) means that hundreds of millions of doses are needed to tackle a pandemic. Dose-sparing approaches, including the use of an efficient nontoxic adjuvant to boost persons' immune responses, may improve the vaccine. Another trial was performed with 300 healthy participants 18–40 years of age, in which aluminum hydroxide adjuvant was used with similar split-virus vaccine (*17*). However, the alum-adjuvanted vaccines did not improve the immunogenicity or percentage of seroconversion at lower vaccine doses and only slightly improved immunogenicity at the 30-μg dose. This difficulty underscores the importance of vigorous fundamental research to address the question of how to increase the immunogenicity of such vaccines, whether by better antigen presentation or by choosing alternative routes of administration, so that lesser amount of antigen could be given to induce protective response. The present annual global production capacity is ≈300 million doses of trivalent vaccine containing 15 μg HA per strain. This is equivalent to 900 million doses of monovalent vaccine, a quantity markedly insufficient for the world's 6.5 billion people. Clearly, dose-sparing formulations are urgently needed.

Inactivated Vaccines for Immunizing Humans

To test the hypothesis that whole-virion would be more immunogenic than conventional split-virion or subunit vaccines and may be adaptable to the antigen-sparing strategy, an inactivated, monovalent influenza A (H5N1), whole-virion vaccine was prepared from a highly virulent strain A/Vietnam/1194/2004 strain by removing the polybasic amino acids at the cleavage site, making the virus no longer pathogenic. The seed virus was grown to a high titer in embryonated eggs, inactivated with formalin, and purified. These viruses were then adjuvanted with aluminum hydroxide and used in a phase 1 trial (*6*). The highest immune response of 78% seropositivity was observed in the group given 2× 10 μg HA, which is equivalent to that elicited by higher doses of nonadjuvanted (90 μg) or adjuvanted (30 μg) split-virion vaccines (*16*,*17*).

Not knowing which particular genetic variant will sustain human-to-human transmission makes our ability to formulate a vaccine in advance all the more difficult. An inactivated vaccine that induces not only high levels of neutralizing antibody to surface proteins but also CD8 T-cell response against well-conserved antigens derived from internal viral proteins might provide superior protection in an epidemic or pandemic. In cases of established intracellular influenza A infection, infected cells are mainly eliminated by effector CD8+ T cells (CTLs) (*18*). Any vaccine that will induce and direct these CTLs to the site of infection and generate a long-lasting memory response will be more effective for mounting protection against a pandemic form of influenza (H5N1). Inactivated vaccines need to be presented to the host's immune system with an appropriate adjuvant, but inactivated vaccines that use an adjuvant currently approved for human use (alum or MF-59) usually have lower immunogenicity than live attenuated vaccines (*10*). Therefore, the pursuit for other nontoxic adjuvants, including TLR ligands and agonists that could effectively activate dendritic cells for the presentation of viral antigens to CD4 and CD8 T cells, should vigorously be continued. Use of cytokines such as IL-12 or IL-18 may enhance the immunogenicity of antiviral vaccines. Recombinant fowlpox vaccines coexpressing HA of subtype H5N1 and chicken IL-18 have been shown to induce complete protection in vaccinated chickens (*19*). Use of adjuvants may enhance broader cross-reactive immune responses among influenza viruses (*20*).

Vaccines that Generate Broad-spectrum Immunity

The evolution of many sublineages of influenza (H5N1) with antigenic diversity in Southeast Asia and southern China favors the wisdom of developing broadly cross-reactive vaccines for protection against an epidemic or pandemic (*21*). Genetically engineered viruses could be constructed; these would express several variant antigens or determinants, thereby generating a broader immune response. The goal would be to develop vaccines that would induce broad-spectrum immunity-conferring protection to influenza including subtype H5N1. Ferrets vaccinated with A/PR/8/34 single-gene reassortants that differed only in their H5s were protected against a lethal challenge with A/Vietnam/1203/04 virus, suggesting generation of cross-protection (*22*). Vaccination of mice with a live attenuated influenza vaccine or an alum-adjuvanted inactivated influ-

enza vaccine based on a related H5 HA from a nonpathogenic avian influenza virus, A/Duck/Pottsdam/1042–6/86 (H5N2), limited the disease severity and reduced deaths following challenge with a current highly pathogenic influenza (H5N1) (*23*). Such cross-protective vaccines may provide clinical protection and prevent deaths in the early stages of a pandemic.

Genes of highly conserved proteins such as the nucleoprotein or M2 proteins could be included in adenovirus vector–based vaccines because immune responses against these influenza viral antigens provide protection in animal models (*24*,*25*). Recently, human adenoviral vector–based HA subtype 5 influenza vaccine induced protection in mice against influenza (H5N1) viruses isolated from humans (*26*,*27*). However, pre-existing immune response to human adenoviruses could be a potential problem in the generation of immune response against a foreign gene of interest. Delivering the vaccine nasally could largely overcome this problem because there appears to be no pre-existing immunity in the upper airways. Moreover, a robust CD8 T-cell response would likely be flexible and able to fight influenza (H5N1).

Ideally, we need an effective vaccine for persons of all ages. However, if the vaccine is in short supply, priming first those persons at high risk (e.g., young children, persons >50 years of age, healthcare workers) may be justifiable. During the early stages of an emerging H5 pandemic, such persons at high risk might be given an adjuvanted vaccine produced from a currently circulating strain, even if it is antigenically distinct, until an optimally matched and approved vaccine is available. This strategy is to produce a vaccine from an antigenically distant influenza (H5N1) strain that could induce broad-spectrum immunity capable of neutralizing newly emerged human H5 strains.

Cell Culture–based Vaccines

Vaccine development based on a cell culture system has advantages over egg-based technology because H5 strains are highly pathogenic for chickens and supplying large numbers of embryonated eggs could be difficult in a pandemic. In addition, potential allergic reactions to egg components would be avoided by growing the vaccine virus in tissue culture cells. Recently, mammalian cell culture was used for propagating viruses to prepare killed influenza vaccine (*28*). Inactivated influenza vaccines produced with Madin-Darby canine kidney (MDCK) and Vero cells, which served as vaccine substrates, have been licensed in the Netherlands. Of note, the human cell line PER.C6 may provide a useful cell-based system because, unlike MDCK and Vero cell systems, it does not require a solid matrix support for the growth of cells. Selecting background viruses that grow well in these cell cultures and monitoring them for antigenic changes and contaminating microbes during propagation of the virus in cell culture need to be considered.

Development of "Universal" Vaccines

For the development of a universal influenza vaccine, a possible target is the relatively conserved M2 homotetramer. The concept is based on identifying alternative influenza antigens that are not as susceptible to antigenic shift and drift. Some degree of protection was induced in mice by priming with an M2 ectodomain peptide in adjuvant (*29*). Studies that used the M2eA peptide conjugated to keyhole limpet hemocyanin and *Neisseria meningitidis* outer membrane protein illustrated good immune responses not only in mice but also in ferrets and rhesus monkeys (*30*). In a recent study, 3 M2eA sequences, representing a range of epidemic strains and the (H5N1) strain, were fused to a proprietary hydrophobic protein domain. The resulting fusion proteins, formulated in liposomes, stimulated a protective response in mice challenged with subtypes H1N1, H5N1, H6N2, or H9N2 (*31*). Previous studies have shown that when M2e is linked to hepatitis B virus core (HBc) particles, it becomes highly immunogenic, eliciting protective antibody response in mice (*25*). Recently, a series of M2e–HBc constructs were made by increasing the copy number of M2e inserted at the N terminus from 1 to 3 per monomer. The best protection was seen when mice were vaccinated intranasally with these constructs combined with CTA1-DD, a cholera toxin A1–derived mucosal adjuvant (*32*).

M2 serves as a pH-induced proton channel on the surface of all influenza A viruses but is present in low quantities. Further studies are warranted for understanding the mechanism of immune response to M2eA and for defining the appropriate immunization conditions for humans.

Vaccination and Correlates of Immune Protection

The lack of established correlates of immunity in animals and humans poses challenges to developing consistent immunologic endpoints for clinical trials and appropriate criteria for vaccine efficacy. Serum antibody titers, mainly those determined by hemagglutination inhibition (HI) or virus neutralization (VN) assays, or both, are considered surrogate measures of protection. However, the HI test is insensitive for the detection of antibody to avian HA; there also are no recognized clinical correlates of immune protection for neutralization antibody (*33*,*34*). Recently, HI or VN assay failed to detect antibodies in ferrets protected by vaccination with whole-virus vaccines containing internal protein from Dk/Sing virus against a heterotypic virus (*34*). Whether the cross-protection reported is mediated by T-cell response is not known.

In recent years, attempts were made to improve the sensitivity of the HI test. More sensitive detection of anti-

body to avian HA was seen when horse erythrocytes were used in place of turkey erythrocytes in the HI test because influenza virus was better able to bind to a2,3Gal-specific receptor sites on these erythrocytes (*35*). The presence of asparagines at aa223 (H5 numbering) in H5 HA leads to improved sensitivity of the HI test (*22*).

Often the immunogenicity of H5 vaccine candidates is assessed by HI or VN assays, but the basis of protection remains unclear. Nevertheless, the tests that are used to evaluate efficacy of candidate vaccines are based on the assumption that antibody would mediate the protection against infection induced by vaccination, although this has yet to be critically established.

On the basis of initial evidence, inflammation has been proposed as a possible cause or driving force of avian influenza (H5N1). However, components of the inflammatory response might even be beneficial. To address these possibilities, we need to determine whether inflammation in avian influenza is an early event and a manifestation of innate immune response. If it is, some of the mediators of innate immune response, such as cytokine/chemokine levels, can be included in the evaluation of the potency of candidate vaccines. Further humoral response as a correlate for protection can be fine-tuned by determining the titer and isotype of antibody after vaccination. Several issues concerning vaccine efficacy are unresolved: What are the consequences of vaccination for existing influenza (H5N1) infection, the extent of serologic cross-reactivity between the most closely related types of the virus, and the role in clinical protection? Vaccine administration may provide some therapeutic effects for infected persons who have not yet made an immune response but provide none for those with persistent infection associated with measurable humoral immunity.

Clearly, more studies are warranted to establish a highly reproducible assay to measure immunogenicity of a candidate vaccine and to determine adequate correlates of immune protection. Safety and immunogenicity of adjuvanted vaccines or new formulations should be critically assessed, and any fast-track approval of marketing vaccines must not compromise safety.

Development of Therapeutic (Antidisease) Vaccines

The marked virulence of the 1997 outbreak suggests that influenza A (H5N1) infection may have novel pathogenic mechanisms not seen in human influenza strains. To attempt to understand pathogenicity of this virus, an influenza virus bearing all 8 gene segments of the 1918 pandemic virus, which claimed at least 20–40 million lives, was recently generated in cultured cells. The reconstructed 1918 influenza viruses displayed accelerated activation of host immune response in mice with high levels of chemokines and cytokines in the lungs, resulting in infiltration of inflammatory cells and extensive damage to the lungs with severe hemorrhaging (*36*). The pathogenicity induced by the reconstructed virus showed marked similarity to that reported with influenza (H5N1).

Increasing evidence from mouse models and humans suggests that certain inflammatory mediators are potent drivers of the disease. If this is true, this could have important implications for developing new therapeutics. Acute respiratory distress syndrome, hemophagocytosis, or both, develop in a substantial fraction of patients with influenza (H5N1) infection; both of these conditions are thought to be promoted by overproduction of proinflammatory cytokines (known as a "cytokine storm") (*37*). Consistent with these observations, cytokine release was markedly enhanced in human macrophages after infection with influenza (H5N1) (*38*). Further, marked enhancement of chemokine and cytokine levels was observed in influenza (H5N1)–infected persons, particularly in those who died, and these correlated with high and disseminated viral replication (*4*). Additionally, influenza (H5N1) viruses appear relatively resistant to the inhibitory effects of host antiviral cytokines, such as interferons (IFNs) (*39*). Thus, the severity of human influenza (H5N1) infection may be related to the induction of excessive proinflammatory responses that can accompany a primary infection and high viral shedding. Increased inflammation was associated with viral replication in the respiratory and extrarespiratory organs of cats experimentally infected with influenza (H5N1) (*3*). Mice infected with the highly pathogenic influenza (H5N1) strain A/HK/156/97, originally obtained from diseased chickens and an ill child in Hong Kong, China (HK), showed reduced ability to activate transforming growth factor–β (TGF-β), a potent anti-inflammatory cytokine, compared to mice infected with less virulent A/Env/HK437/99 viruses (*2*). The reduced ability to activate TGF-β may produce greater inflammation at the site of infection and thus cause more severe disease. Alternatively, the low levels of activated TGF-β in the sera of A/HK/156/97-infected mice may allow the viruses to replicate and spread unchecked in the respiratory tracts of the mice, causing more severe disease. Recently, the impact of the nonstructural (NS) gene variation of Hong Kong (H5N1)/97 on cytokine production was illustrated (*40*). The NS gene reassortant induced elevated pulmonary concentrations of the inflammatory cytokines IL-1α, IL-1β, IL-6, IFN-γ, and chemokine KC and decreased concentrations of the anti-inflammatory cytokine IL-10. This cytokine imbalance is reminiscent of the clinical findings in humans infected with influenza (H5N1)/97 virus and may explain the unusual severity of the disease.

The ability to site specific engineering changes in the virus genome allows us to consider a novel vaccine approach. By engineering a virus with site-specific changes

in the genome (for example in NS gene), we may produce influenza virus vaccine that favors the production of beneficial anti-inflammatory cytokines but remains highly immunogenic. In another approach, a human replication-incompetent, adenoviral vector–based influenza vaccine could be developed, in which genes of anti-inflammatory cytokines are coexpressed, which will inhibit overproduction of proinflammatory cytokines. Such vaccines would be considered therapeutic vaccines (e.g., antidisease vaccines), which would inhibit inflammation at the site of infection and protect against severe disease (Figure). Excessive production of anti-inflammatory cytokines may result in an inappropriate suppression of the host immune response. Further studies will validate the beneficial effect of the anti-inflammatory response for temporizing the cytokine storm seen in influenza (H5N1). Development of an immunization protocol that uses an adjuvant that allows selective priming of an antigen-specific immunoregulatory cytokines (e.g., IL-10, TGF-β) would be a major advance in the development of a vaccine for bird flu with a substantial inflammatory component. The search for potential adjuvants, such as TLR ligands and agonists that will favor the synthesis of inhibitory cytokines including IL-10, should be pursued. By testing whether manipulation of inflammatory pathways changes the pathologic course, we would identify new targets for disease intervention.

Conclusions

Vaccination is the best option by which to prevent the spread of a pandemic virus and reduce the severity of disease. Defining the host response to influenza (H5N1) in natural infection is urgently needed to better understand the basis of protection and subsequent development of efficacious vaccines. Improved vaccine strategies, which will require less antigen and be more robust in inducing both antibody and cell-mediated immunity for neutralizing influenza (H5N1) viruses, should be considered. To create an effective vaccine, a combination of factors must be optimized—such as number of doses, formulation without or with better adjuvant, and dose range. We also need to develop a reproducible assay that measures immunogenicity of a vaccine and to establish adequate correlates of protection. The efficacy of potential cross-reactive vaccine candidates to induce broad-spectrum immunity to influenza (H5N1) viruses should be assessed critically; stockpiling of such vaccines may be justified in the absence of optimally matched and approved vaccine during early stages of an H5 pandemic. Search for therapeutic vaccines (antidisease vaccines) aimed at controlling innate immune responses should be pursued, given the clinical evidence that the H5N1 subtype elicits a cytokine storm that contributes to disease pathogenesis. Vaccine development and deployment need to be undertaken by a partnership of academia, government, and industry. The risk for dissemination of pandemic virus will remain if the disease is controlled in 1 area but not in others. A global approach is vital for combating the next influenza pandemic, a monumental public health challenge.

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Antigenic Diversity of Human Sapoviruses

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Sapovirus (SaV) is a causative agent of gastroenteritis. On the basis of capsid protein (VP1) nucleotide sequences, SaV can be divided into 5 genogroups (GI–GV), of which the GI, GII, GIV, and GV strains infect humans. SaV is uncultivable, but expression of recombinant VP1 in insect cells results in formation of viruslike particles (VLPs) that are antigenically similar to native SaV. In this study, we newly expressed SaV GII and GIV VLPs to compare genetic and antigenic relationships among all human SaV genogroups. Hyperimmune antiserum samples against VLPs reacted strongly with homologous VLPs. However, several antiserum samples weakly cross-reacted against heterologous VLPs in an antibody ELISA. Conversely, an antigen ELISA showed that VLPs of SaV in all human genogroups were antigenically distinct. These findings indicate a likely correspondence between SaV antigenicity and VP1 genogrouping and genotyping.

The family *Caliciviridae* contains 4 genera (*Sapovirus*, *Norovirus*, *Lagovirus*, and *Vesivirus*), which include *Sapporo virus*, *Norwalk virus*, *Rabbit hemorrhagic disease virus*, and *Feline calicivirus*, respectively. Sapoviruses (SaVs) and noroviruses (NoVs) are etiologic agents of human gastroenteritis. The prototype strain of human SaV, Sapporo virus, was originally discovered in an outbreak in an orphanage in Sapporo, Japan, in 1977 (*1*). SaV infects children and adults and has been found to cause outbreaks of gastroenteritis in daycare centers, healthcare facilities, and elementary schools. Detection methods include reverse transcription–PCR (RT-PCR), real-time RT-PCR, enzyme immunoassays, and ELISAs (*2*–*6*). Recently, we detected SaV in untreated wastewater samples, treated wastewater samples, and river samples (*7*).

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The SaV genomes are predicted to contain either 2 or 3 main open reading frames (ORF1–3). SaV ORF1 encodes for nonstructural proteins and the major capsid protein, and ORF2 (VP2) and ORF3 encode proteins of yet unknown functions. On the basis of VP1 nucleotide sequences, SaVs have been divided into 5 genogroups (GI–GV), of which GI, GII, GIV, and GV strains infect humans and GIII strains infect porcine species (*8*). SaV genogroups can be further subdivided into genotypes. Recently, we identified several recombinant SaV strains (*8*,*9*).

Human SaV and NoV strains are uncultivable, but expression of a recombinant subgenomic-like construct (i.e., VP1 to the end of the genome) or VP1 alone in insect or mammalian cells results in the formation of viruslike particles (VLPs) that are morphologically similar to native SaV (*10*–*16*). However, production of VLPs of SaV remains difficult, usually only resulting in low yields of VLPs compared with norovirus (*10*,*12*,*16*,*17*). Cryoelectron microscopy and x-ray crystallography analyses of NoV VLPs identified the shell (S) and protruding domains (subdomains P1–1, P1–2, and P2) (*18*). Also, Chen et al. described strictly and moderately conserved amino acid residues in the capsid protein among the 4 genera in the family *Caliciviridae* (*13*).

Previously, we reported that SaV GI/1 (strain Mc114) and GV/1 (strain NK24) were antigenically distinct (*5*,*10*). More recently, we discovered that SaV GI/5 (strain Yokote1) VLPs were antigenically distinct from SaV GI/1 Mc114 and GV/1 NK24 VLPs (*19*). Other than these few studies, little is known about the genetic and antigenic relationships among the 4 human SaV genogroups. For classification of NoV, distinct genotypes have been defined as having bootstrap values >950 (VP1 sequences); at least 14 GI and 17 GII genotypes have been identified (*20*). For SaV, genogroups have only been vaguely defined, mostly

because 2 of them (GIV/1 and GV/1) were only recently identified, few sequences exist in the database, and antigenic relationships among all genogroups are unknown. In addition, genetic recombination was only recently discovered and appears to be common within the genus *Sapovirus*.

The purpose of this study was to examine cross-reactivities among the 4 human SaV genogroups and compare results with those of genetic analysis. For this purpose, 2 other SaV strains, GII/3 Syd53 and GIV/1 Syd3, were expressed and antisera were produced against their purified VLPs. A total of 5 SaV strains (GI/1 Mc114, GI/5 Yokote1, GII/3 Syd53, GIV/1 Syd3, and GV/1 NK24) that include all 4 human genogroups and 2 GI genotypes were compared. Our results show that SaV genogroups were antigenically distinct and corresponded with results of genetic classification on the basis of full-length VP1 nucleotide sequences. Proper genetic classification of SaV strains is required, and a consensus of genogroups and genotypes that represent genetically and antigenically diverse strains, which include recombinant SaV strains, should be established to avoid conflicting grouping.

Materials and Methods

Specimens

Virus-positive stool specimens were collected from several sources. SaV strain Mc114 (GenBank accession no. AY237422) was isolated from an infant hospitalized with acute gastroenteritis in Chiang Mai, Thailand, in 2001 (*21*). SaV strain NK24 (AY646856) was isolated from an infant with gastroenteritis in Nong Khai, Thailand, in 2003 (*22*). SaV strain Yokote1 was isolated from an outbreak of gastroenteritis at a kindergarten in Yokote City, Japan, in 2006 (*19*). SaV strains Syd53 and Syd3 were isolated from infants hospitalized with acute gastroenteritis in Sydney, New South Wales, Australia, in 2001 (*23*). NoV strain Osaka659 was isolated from an outbreak of gastroenteritis in Japan, in 2006 (unpub. data). RNA extraction and RT-PCR were performed as described (*24*).

Sequence Analysis

Nucleotide sequences were determined by using the Terminator Cycle Sequence Kit version 3.1 and an ABI 3130 sequencer (both from Applied Biosytems, Boston, MA, USA). Nucleotide sequences were aligned with ClustalX (www.embl.de/~chenna/clustal/darwin) and the distances were calculated by the Kimura 2-parameter method (*24*). Phylogenetic trees with bootstrap analysis from 1,000 replicas were generated by the neighbor-joining method as described (*20*). Amino acid VP1 secondary structure predictions were made by using PSIPRED secondary structural prediction software (*25*).

Expression of Viruslike Particles

For the expression of VP1 in insect cells, all SaV constructs were designed to begin from the predicted VP1 start AUG codon and included the ORF2 and poly(A) sequences. SaV strains Syd53 and Syd3 were cloned as described (*10*) for strains SaV Mc114, NK24, and Yokote1 according to the protocol of the Baculovirus Expression System using Gateway Technology (Invitrogen, Carlsbad, CA, USA). Briefly, strains Syd53 and Syd3 were amplified with specific sense primers Syd53attb1 (5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGAGGGTGTGTCCCACCCAGA-3′)andSyd3attb1(5′-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAGAACCATGGAGGGCAATGGCCTACCCCAGGCTG-3′) and antisense primer TX30SXN (5′-GACTAGTTCTAGATCGCGAGCGGCCGCCCTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3′). PCR fragments were purified after electrophoresis on a 1.0% agarose gel. Fragments were cloned into donor vector pDONR201 (Invitrogen) and transferred into a baculovirus transfer vector pDEST8 (Invitrogen).

The recombinant pDEST8 was purified and used to transform *Escherichia coli* DH10Bac-competent cells (Invitrogen), which produced recombinant bacmids (baculovirus shuttle vectors) containing the VP1 gene. Recombinant bacmids were then transfected into Sf9 cells (Riken Cell Bank, Ibaraki, Japan), and recombinant baculoviruses were isolated. Recombinant baculoviruses were used to infect $\approx 3 \times 10^6$ confluent Tn5 cells (Invitrogen) at a multiplicity of infection of 5–10 in 1.5 mL of Ex-Cell 405 medium (JRH Biosciences, Lenexa, KS, USA), and the infected cells were incubated at 26°C. The culture medium was harvested 5–6 d postinfection, centrifuged for 10 min at 3,000× *g*, and further centrifuged for 30 min at 10,000× *g*. VLPs were concentrated by ultracentrifugation for 2 h at 45,000 rpm at 4°C (Beckman TLA-55 rotor; Beckman Coulter, Fullerton, CA, USA), and resuspended in 30 μL of Grace's medium. Samples were examined for VLP formation by electron microscopy as described (*10*), and large-scale production of VLPs was performed as described (*24*).

Antibody Production

Hyperimmune sera to newly expressed VLPs of SaV (Syd53 and Syd3) were prepared in rabbits and guinea pigs. The first subcutaneous injection was performed with purified VLPs (≈10 μg) in Freund complete adjuvant. After 3 weeks, the animals received 1 booster injection (intravenously in rabbits and subcutaneously in guinea pigs) of 10 μg of VLPs without adjuvant. Blood was collected from the animals 1 week after their last booster injection.

Antibody ELISA

Cross-reactivities among antiserum samples against SaV were examined by using an antibody ELISA with hyperimmune rabbit antibodies against VLPs. Briefly, wells of 96-well microtiter plates (Maxisorp; Nunc, Roskilde, Denmark) were each coated with 100 μL of purified VLPs (≈1.0 μg/ mL in carbonate-bicarbonate buffer, pH 9.6) (Sigma, St. Louis, MO, USA) and incubated overnight at 4°C. Wells were washed twice with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBS-T) and blocked with PBS containing 5% skim milk (PBS-SM) for 1 h at room temperature. Wells were then washed 4 times with PBS-T, 100 μL of 2-fold–diluted hyperimmune rabbit antibodies from an initial concentration of 1:500 in PBS-T-SM was added to each well, and the plates were incubated for 1 h at 37°C. Wells were then washed 4 times with PBS-T, and 100 μL of a 1:1,000 dilution of horseradish peroxidase–conjugated goat antirabbit immunoglobulin G diluted in PBS-T-SM was added to each well. Plates were incubated for 1 h at 37°C. Wells were then washed 4 times with PBS-T, and 100 μL of substrate (*o*-phenylenediamine) and H_2O_2 were added to each well, and the plates were left in the dark for 30 min at room temperature. The reaction was stopped by the addition of 50 μL of 2N H_2SO_4 to each well, and the absorbance was measured at 492 nm (A_{492}). The optical density (OD) cutoff point was determined to be 0.15, which was equal to 3 times the mean OD of preimmune serum (*5*).

Antigen ELISA

Cross-reactivities among VLPs were also examined by using an antigen ELISA. Briefly, wells were coated with 100 μL of a 1:8,000 dilution of hyperimmune rabbit antiserum diluted in PBS (except for Syd3, for which a 1:3,000 dilution was used), and the plates were incubated overnight at 4°C. Wells were washed 4 times with PBS-T and blocked with PBS-SM for 1 h at room temperature. Wells were then washed 4 times with PBS-T, 100 μL of VLPs (≈1.0 μg/mL in carbonate-bicarbonate buffer, pH 9.6) (Sigma) was added to duplicate hyperimmune rabbit wells, and the plates were incubated for 1 h at 37°C. Wells were then washed 4 times with PBS-T, 100 μL of a 1:8,000 dilution of hyperimmune guinea pig antibody diluted in PBS-T-SM was added to each well (except for Syd3, which used a 1:3,000 dilution), and the plates were incubated for 1 h at 37°C. Wells were washed 4 times with PBS-T, and 100 μL of a 1:1,000 dilution of horseradish peroxidase–conjugated rabbit antiguinea pig immunoglobulin G diluted in PBS-T-SM was added to each well. The plates were then processed as described above. On the basis of our previous study, a specimen with an A_{492} (P – N) >0.1 and a P/N ratio >1.34 (where P is hyperimmune antiserum and N is preimmune antiserum) was considered significantly positive (*4*).

Results

Sequence Analysis

The sequence of the 3′ end of the genome (≈2,600 nt), i.e., VP1 to poly(A), for the newly expressed SaV strains (Syd53 and Syd3) was determined. Genetic analysis was performed with only complete VP1 sequences, which included sequences from our epidemiologic studies and other sequences available on the database (Figure 1). Five SaV GI and 6 GII genotypes were observed, but only 1 genotype for SaV GIV and 1 for GV was found. This result suggests that SaV GI and GII strains were more genetically diverse, prevalent, or more virulent than SaV GIV and GV strains. However, because the SaV GIV and GV strains were only recently detected (*26*,*27*), this result may reflect only the specificity and sensitivity of the detection methods used. On the basis of our previous classifications, SaV Mc114 and Yokote1 sequences both belonged to GI, but to different genotypes, GI/1 and GI/5, respectively; Syd53 be-

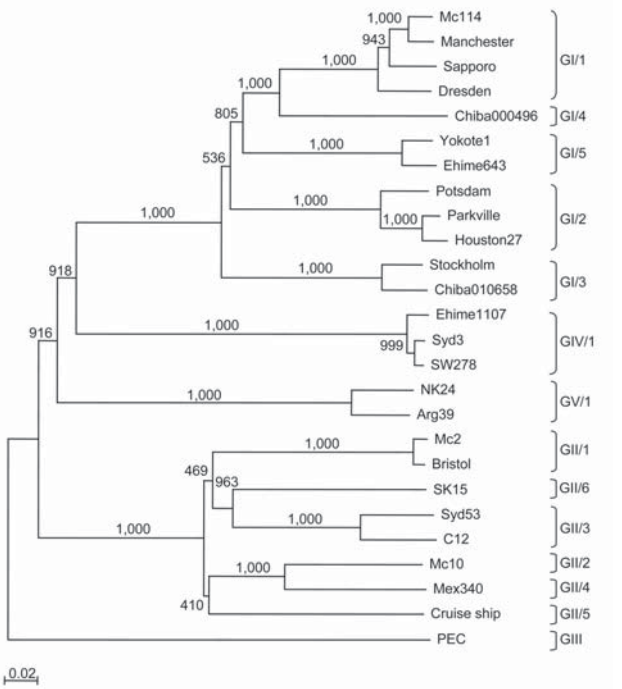

Figure 1. Phylogenetic tree of sapoviruses on the basis of entire nucleotide sequences of the capsid protein. Different genogroups and genotypes are indicated. The numbers on each branch indicate the bootstrap values for the genotype. The scale bar at the lower left represents nucleotide substitutions per site. GenBank accession nos. for reference strains: Arg39, AY289803; Bristol, HCA249939; C12, AY603425; Chiba000496, AJ412800; Chiba010658, AJ606696; Cruise ship, AY289804; Dresden, AY694184; Ehime643, DQ366345; Ehime1107, DQ058829; Houston27, U95644; Manchester, X86560; Mc2, AY237419; Mc10, AY237420; Mc114, AY237422; Mex340, AF435812; NK24, AY646856; Parkville, U73124; PEC, AF182760; Potsdam, AF294739; Sapporo, U65427; SK15, AY646855; Stockholm, AF194182; SW278, DQ125333; Syd3, DQ104357; Syd53, DQ104360; and Yokote1, AB253740.

Table 1. Nucleotide similarity (values below blank diagonal) and amino acid identity (values above blank diagonal) of complete capsid (VP1) sequences of sapovirus strains*

Strain	Mc114 (GI/1)	Yokote1 (GI/5)	Syd53 (GII/3)	Syd3 (GIV/1)	NK24 (GV/1)
Mc114 (GI/1)		79	46	52	51
Yokote1 (GI/5)	76.5		46.8	50.3	50.9
Syd53 (GII/3)	56.1	56.9		48.3	48.6
Syd3 (GIV/1)	58.1	57.4	55.9		54.2
NK24 (GV/1)	58.2	57.3	56.4	58.6	

*Values are percentages.

longed to GII/3; Syd3 belonged to GIV/1; and NK24 belonged to GV/1. SaV GI/1 Mc114 and GI/5 Yokote1 VP1 sequences shared 76.5% and 79% nucleotide similarity and amino acid identity, respectively (Table 1). The nucleotide similarity and amino acid identity among the genogroups was low, i.e., <60% (Table 1).

Expression of VP1

We previously expressed SaV GI/1 Mc114, GI/5 Yokote1, and GV/1 NK24 in insect cells, which resulted in the formation of VLPs morphologically similar to native SaV (*5*,*10*). In this study, we newly expressed SaV GII/3 Syd53 and GIV/1 Syd3 in insect cells to analyze the cross-reactivity among all human SaV genogroups. SaV GII/3 Syd53 and GIV/1 Syd3 successfully formed VLPs with a diameter of 41 to 46 nm and were morphologically similar to native SaV (Figure 2). Hyperimmune sera against these purified VLPs were prepared in rabbits and guinea pigs.

Antibody ELISA Analysis

Our previous antibody ELISA result showed that SaV GI/1 Mc114 and GV/1 NK24 antisera had no cross-reactivities against heterologous GV/1 NK24 and GI/1 Mc114 VLPs, respectively (*5*). In the current study, cross-reactivities among 5 VLPs of SaV (GI/1 Mc114, GI/5 Yokote1, GII/3 Syd53, GIV/1 Syd3, and GV/1 NK24) were analyzed by using the antibody ELISA with 2-fold serial dilutions (1:500–1:1,024,000) of hyperimmune antiserum (Figure 3). The OD cutoff point was 0.15, which was equal to 3 times the mean OD of preimmune serum (*5*). Hyperimmune rabbit antiserum reacted strongly against the homologous VLPs (Table 2, Figure 3). SaV GII/3 Syd53 and GIV/1 Syd3 antisera titers were 512,000 and 2,056,000, respectively (Table 2). Several antisera weakly cross-reacted with heterologous VLPs. SaV GI/1 Mc114 antiserum cross-reacted weakly with GI/5 Yokote1 and GII/3 Syd53 VLPs, i.e., their cross-reactivities were 8- and 16-fold lower than that of the homologous VLP titer, respectively. SaV GI/5 Yokote1 antiserum cross-reacted weakly with GI/1 Mc114 and GII/3 Syd53 VLPs, i.e., its cross-reactivity was 16-fold lower than that of the homologous VLP titer. SaV GII/3 Syd53, GIV/1 Syd3, and GV/1 NK24 antisera appeared to have no cross-reactivities against any of the heterologous VLPs, i.e., their cross-reactivities were >32-fold lower than those of the homologous VLP titer. These results suggested that SaV GI/1 Mc114 and GI/5 Yokote1 antiserum had weak 2-way cross-reactivities against GI/5 Yokote1 and GI/1 Mc114 VLPs, respectively. The negative control NoV Osaka659 antiserum showed no cross-reactivities against VLPs of SaV at any dilution of antiserum, which indicates that the antiserum was specific for the VLPs and not the insect cell proteins.

Antigen ELISA Analysis

On the basis of a previous study, a specimen with an A_{492} (P – N) >0.10 and a P/N ratio >1.34 was considered significantly positive (*4*). Our recent antigen ELISA results showed that SaV GI Mc114, GI/5 Yokote1, and GV/1 NK24 VLPs were antigenically distinct (*19*), i.e., GI/1 Mc114 P– N 0.41, P/N 9.19; GI/5 Yokote1 P – N 0.93,

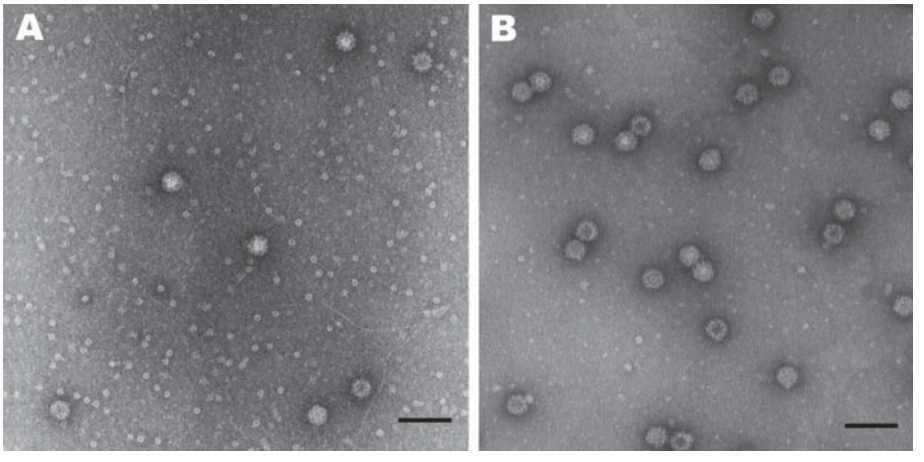

Figure 2. Electron micrographs of A) Syd53 and B) Syd3 viruslike particles of sapovirus. Scale bars = 100 nm.

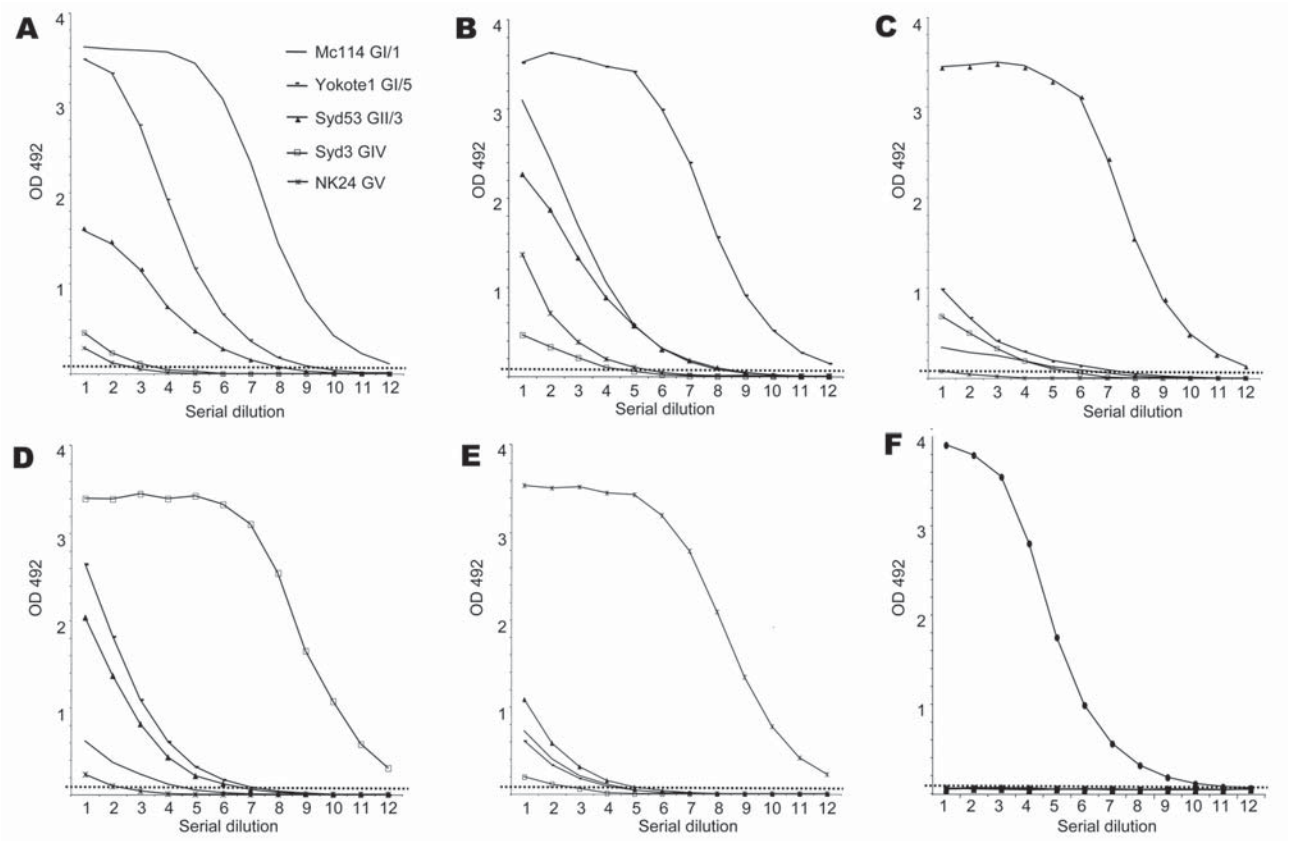

Figure 3. Antibody ELISA absorbances at 492 nm of 6 viruslike particles (VLPs) of sapovirus. A) Mc114 GI/1; B) Yokote1 GI/5; C) Syd53 GII/3; D) Syd3 GIV/1; E) NK24 GV/1; F) NoV Osaka659. Wells were coated with ≈100 ng of purified VLPs. Antiserum was used in 2-fold dilutions from 1:500 to 1,024,000 in phosphate-buffered saline, 0.1% Tween 20, 5% skim milk shown as 1–12 along the x-axis. OD, optical density; NoV, Norwalk virus.

P/N 19.59; and GV/1 NK24 P – N 1.03, P/N 21.58. In the current study, we examined the cross-reactivity among the newly expressed VLPs and those previously prepared by using the antigen ELISA. The antiserum samples reacted only with homologous VLPs, i.e., SaV GII/3 Syd53 P – N 1.02, P/N 21.33; and GIV/1 Syd3 P – N 1.44, P/N 29.74 (Table 3). Several antisera appeared to cross-react with heterologous VLPs, i.e., where the P – N was <0.10, but the P/N ratio was >1.34. SaV GI/5 Yokote1 antisera appeared to cross-react with GII/3 Syd53 VLPs (P/N 1.97); GIV/1 Syd3 antisera appeared to cross-react with GI/5 Yokote1 VLPs (P/N 1.42); and GV/1 NK24 antisera appeared to cross-react with GII/3 Syd53 VLPs (P/N 1.50). However, all of these cross-reactivity results were considered negative because P – N was <0.10.

Amino Acid Alignment and Secondary Structure Prediction

An amino acid alignment of the 5 SaV VP1 sequences (GI/1 Mc114, GI/5 Yokote1, GII/3 Syd53, GIV Syd3, and GV NK24) showed that the shell domain contained more conserved residues than the predicted P domains (Figure 4). However, SaV GI/1 Mc114 and GI/5 Yokote1 shared more conserved continuous residues in the predicted P2 subdomain than other genogroups. The NoV P2 subdomain is thought to contain the determinants of strain specificity, cell binding, and antigenicity. For example, monoclonal antibodies that recognize regions in the P2 subdomain inhibit binding of NoV VLPs to cells (*28*,*29*). In a recent study, we analyzed cross-reactivities among 26 different NoV VLPs (6 GI and 12 GII genotypes) (*30*) and found

Table 2. Titers of antisera to viruslike particles of sapovirus strains in an antibody ELISA

Antisera	Mc114 (GI/1)	Yokote1 (GI/5)	Syd53 (GII/3)	Syd3 (GIV/1)	NK24 (GV/1)
Mc114 (GI/1)	512,000	64,000	32,000	1,000	<1,000
Yokote1 (GI/5)	32,000	512,000	32,000	2,000	4,000
Syd53 (GII/3)	4,000	8,000	512,000	4,000	<1,000
Syd3 (GIV/1)	2,000	16,000	8,000	2,056,000	1,000
NK24 (GV/1)	2,000	2,000	4,000	1,000	2,056,000

Table 3. Antigen ELISA absorbance values of viruslike particles of sapovirus strains

Antisera	Viruslike particles, P – N (P/N)*				
	Mc114 (GI/1)	Yokote1 (GI/5)	Syd53 (GII/3)	Syd3 (GIV/1)	NK24 (GV/1)
Mc114 (GI/1)	0.41 (9.19)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
Yokote1 (GI/5)	0.01 (1.13)	0.93 (19.59)	0.05 (1.97)	0 (1.00)	0 (1.00)
Syd53 (GII/3)	0 (1.00)	0.02 (1.42)	1.02 (21.33)	0.01 (1.14)	0.03 (1.50)
Syd3 (GIV/1)	0 (1.00)	0 (1.00)	0 (1.00)	1.44 (29.74)	0 (1.00)
NK24 (GV/1)	0 (1.00)	0 (1.00)	0 (1.00)	0.01 (1.11)	1.03 (21.58)

*Measured at 492 nm. P, hyperimmune serum; N, preimmune serum.

broad-range cross-reactivities for several NoV antisera. Our results suggested that these cross-reactivities were due to conserved amino acid residues located outside the P2 domain. Conversely, secondary structure predictions made by using PSIPRED secondary structural prediction software showed that helix structures could also influence the cross-reactivity among the NoV VLPs. In the current study, we determined the secondary structure of the 5 SaV VP1 amino acid sequences. Overall, SaV VP1 structures appear to be similar (online Appendix Figure, available from www.cdc.gov/EID/content/13/10/1519-appG.htm). The location of 3 helix structures in the shell domain and 1 helix structure in the C-terminal region were nearly identical for the 5 SaV VP1 sequences. Only SaV GV/1 NK24 was predicted to have a single helix structure in the P2 subdomain. These results suggested that the amino acid sequence, particularly the P2 subdomain, plays a major role in determining cross-reactivity among SaV strains. However, additional studies, including high-resolution VLP structural analysis, are needed.

Discussion

In this study, we analyzed genetic and antigenic relationships for 4 human SaV genogroups (GI, GII, GIV, and GV). We observed weak 2-way cross-reactivity with SaV GI/1 Mc114 and GI/5 Yokote1 antisera against the heterologous GI/5 Yokote1 and GI/1 Mc114 VLPs, respectively, by using an antibody ELISA. However, when we used an antigen ELISA, we found that GI/1 Mc114 and GI/5 Yokote1 VLPs were antigenically distinct. These weak cross-reactivities identified by using the antibody ELISA may have been influenced by several factors, including unfolded VLPs on the microtiter plates at the high pH (carbonate-bicarbonate buffer, pH 9.6) (*31*) or conserved continuous residues outside the predicted P2 domain. Therefore, these 2 SaV genotypes (GI/1 and GI/5) are for the most part antigenically distinct. Likewise, we found that the 4 human SaV genogroups were antigenically distinct in the antigen ELISA. To our knowledge, these new findings provide the first evidence that SaV antigenicity corresponded well with VP1 genogrouping and genotyping.

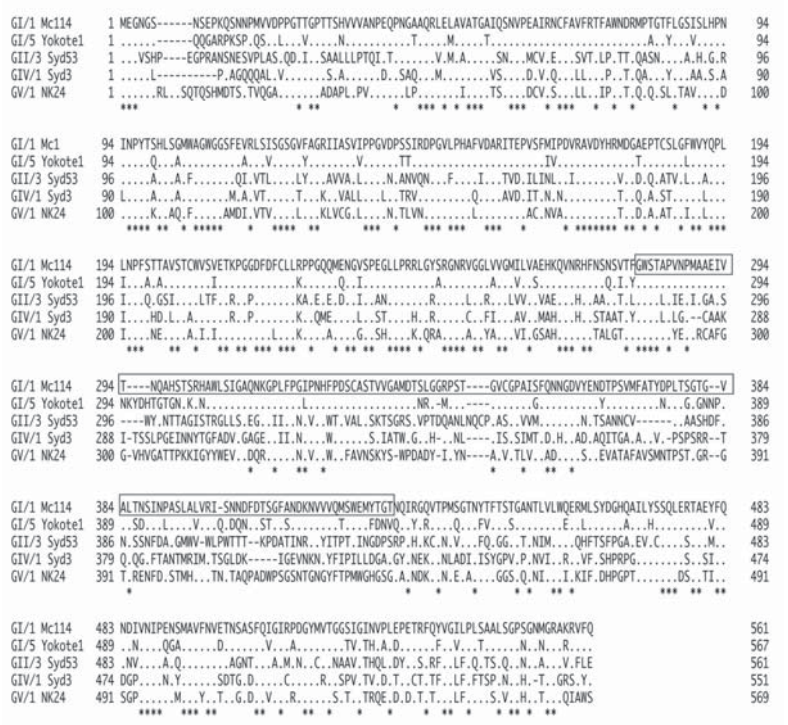

Figure 4. Amino acid alignment of capsid (VP1) sequences of sapoviruses GI/1 Mc114, GI/5 Yokote1, GII/3 Syd53, GIV/1 Syd3, and GV/1 NK24. Sequences in rectangular boxes represent predicted P2 domains (*13*). Asterisks indicate conserved amino acids among these 3 VP1 sequences.

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Evolutionary Relationships between Bat Coronaviruses and Their Hosts

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Recent studies have suggested that bats are the natural reservoir of a range of coronaviruses (CoVs), and that rhinolophid bats harbor viruses closely related to the severe acute respiratory syndrome (SARS) CoV, which caused an outbreak of respiratory illness in humans during 2002–2003. We examined the evolutionary relationships between bat CoVs and their hosts by using sequence data of the virus RNA-dependent RNA polymerase gene and the bat cytochrome b gene. Phylogenetic analyses showed multiple incongruent associations between the phylogenies of rhinolophid bats and their CoVs, which suggested that host shifts have occurred in the recent evolutionary history of this group. These shifts may be due to either virus biologic traits or host behavioral traits. This finding has implications for the emergence of SARS and for the potential future emergence of SARS-CoVs or related viruses.

Severe acute respiratory syndrome (SARS) emerged in November 2002 in southern People's Republic of China (*1*), and a SARS coronavirus (SARS-CoV) was identified as the etiologic agent (*2*). These events and the identification of SARS-CoV in animals associated with the wildlife trade in southern China (*3*) have led to a rapid resurgence of interest in CoVs of different origins. This resurgence led to discovery of 2 novel human CoVs (*4*,*5*); identification of SARS-like CoVs in horseshoe bats (*Rhinolophus macrotis*, *R. ferrumequinum*, *R. pearsoni*, and *R. sinicus*) (*6*,*7*); and identification of other CoVs in bat species (*R. sinicus*, *R. ferrumequinum*, *Miniopterus magnater* [*M. magnater* has been misidentified as *M. schreibersi* (*8*) in reports on SARS-like CoV], *Pipistrellus abramus*, *P. pipistrellus*, *Tylonycteris pachypus*, *Myotis ricketti*, and *Scotophilus kuhlii*) (*7*,*9*–*12*). However, evolutionary relationships among these CoVs and their bat hosts have not been examined.

Studies in species other than bats have examined host-virus phylogeny and identified coevolutionary relationships (*13*–*16*) or incongruous phylogenetic patterns (*17*). These findings suggest recent pathogen host shifts (defined as interspecies transmission followed by establishment and long-term persistence in the new host species [*18*]). Other studies have demonstrated that the relationship between viral phylogeny and geographic location and identification hosts (viral phylogeography [*19*]) can yield information on the origin of emerging zoonoses (*19*,*20*).

Knowing the high genetic diversity of bat CoVs, we carried out a systematic phylogenetic study of the viruses and their hosts to examine evolutionary relationships between bat CoVs and bats. The aim was to further investigate the origin of SARS-like CoVs and SARS. Our results suggest host-pathogen divergence and host shifts in the recent evolutionary history of these viruses and their hosts. We discuss host behavioral traits and viral traits that might have given rise to these patterns and comment on the implications of our findings for the emergence of SARS-CoV.

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Materials and Methods

CoV Sequences

Only CoVs from bats were included in this study. We used gene sequences that Tang et al. obtained from 10 bat species (*R. sinicus*, *R. ferrumequinum*, *R. macrotis*, *R. pearsoni*, *M. magnater*, *P. abramus*, *P. pipistrellus*, *T. pachypus*, *S. kuhlii*, and *Myotis ricketti*) (*10*). An additional 57 bat CoV sequences available in GenBank were also included in this analysis.

Bat Mitochondrial Cytochrome b (*cyt b*) Gene Sequences

Tissue samples were obtained from 3-mm wing membrane biopsy specimens from wild bats, which had been caught in 9 provinces of China, that had been preserved in 99% ethanol. Genomic DNA was extracted by using the DNeasy Tissue Kit (QIAGEN, Valencia, CA, USA) and stored at –20°C. We used complete *cyt b* sequences of *R. ferrumequinum*, *P. abramus*, and *P. pipistrellus*, which have recently been published and are available in Genbank. We generated *cyt b* sequences from *M. magnater* (n = 4), *T. pachypus* (n = 3), *R. macrotis* (n = 2), *R. pearsoni* (n = 2), *R. sinicus* (n = 2), *S. kuhlii* (n = 1), and *Myotis ricketti* (n = 1).

PCR mixtures were prepared in 50-μL volumes containing 25 μL 2× EXTaq DNA polymerase (TaKaRa, Kyoto, Japan). Two pairs of primers, Bat_Cytb_1 (5′-TAG AAT ATC AGC TTT GGG TG-3′) (*21*) with Bat_Cytb_2 (5′-AAA TCA CCG TTG TAC TTC AAC-3′) (*21*), and Bat_Cytb_2 with BAT15R (5′-TCA GCT TTG GGT GTT GAT GG-3′) (*22*), were used because of amplification specificity of certain primers in some species. Amplification was conducted at an initial denaturing temperature at 94°C for 30 s; 34 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 90s; and a final extension at 72°C for 10 min. The PCR samples were then stored at 4°C. The complete mitochondrial *cyt b* gene (1,140 bp) was amplified and sequenced. These sequences have been submitted to GenBank and accession numbers are shown in the Table.

Phylogenetic Analysis of CoV Sequences

For virus phylogeny studies, sequences from a 440-bp fragment of the RNA-dependent RNA polymerase (*RdRp*) gene, which is highly conserved among different CoVs, were obtained and analyzed (*10*). Multiple alignments of the 440-bp *RdRp* partial sequence of bat CoVs were conducted in ClustalX version 1.81 (*23*). Bayesian analyses were conducted with MrBayes version 3.1.2 (*24*).

Table. Bat host species analyzed and their typical roosting sites*

Host species	No. sampled (no. RT positive)	Roosting sites	GenBank accession no.†	Location
Miniopterus magnater	365 (56)	Caves	EF517305‡	Hubei, China
			EF517306‡	Yunnan, China
			EF517308‡	Hainan, China
			EF517307‡	Hong Kong, China
Myotis ricketti	76 (14)	Caves	EF517316‡	Beijing, China
			AB106608	Guangdong, China
			AJ504452	Laos
Pipistrellus abramus	55 (18)	Old buildings	AB085739	Japan
			AJ504448	Taiwan
P. pipistrellus	27 (6)	Old buildings	AJ504443	Taiwan
Scotophilus kuhlii	43 (5)	Under palm leaves	EF543860‡	Hainan
Tylonycteris pachypus	35 (6)	Internodes of bamboo	EF517315‡	Guangdong, China
			EF517313‡	Guangxi, China
			EF517314‡	Hong Kong, China
Rhinolophus ferrumequinum	49 (5)	Caves	DQ297575	Yunnan, China
			DQ351847	Jilin, China
			AB085725	Japan
R. macrotis	8 (1)	Caves	EF517311‡	Yunnan, China
			EF517312‡	Yunnan, China
R. pearsoni	78 (4)	Caves	EF517309‡	Guizhou, China
			EF517310‡	Yunnan, China
			DQ297587	Sichuan, China
R. sinicus	125 (24)	Caves	EF517303‡	Guizhou, China
			EF517304‡	Guizhou, China

*Bats roosting in caves usually have higher population densities and greater chances of physical contact. RT, reverse transcription.
†For mitochondrial cytochome b gene sequences.
‡These sequences were provided by our laboratory.

Neighbor-joining analyses (with the Jukes-Cantor model) were used to validate the Bayesian result in MEGA3 (*25*). A total of 67 unique CoV sequences (Figure 1) were analyzed with MrBayes version 3.1.2 in the generalized time reversible model of evolution as determined by the Akaike Information Criterion in MODELTEST version 3.7 (*26*).

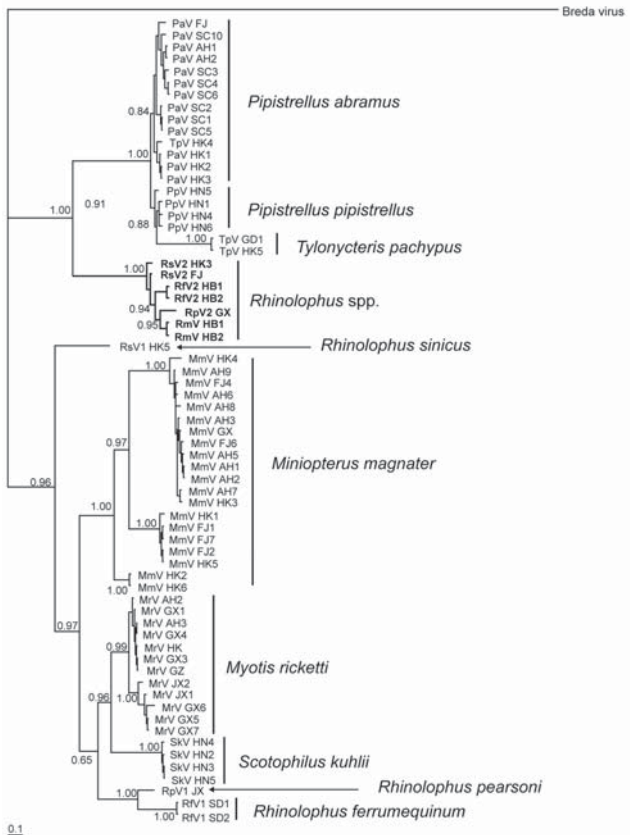

Figure 1. Phylogram of bat coronaviruses based on the 440-bp RNA-dependent RNA polymerase gene region. Methods used are described in the text. Values to the left of branches are Bayesian posterior probabilities. Scale bar at the lower left indicates 0.1 nucleotide substitutions per site. **Boldface** branches indicate severe acute respiratory syndrome–like coronaviruses, and species names to the right of lineages indicate putative reservoir host(s). Pa, *Pipistrellus abramus*; Tp, *Tylonycteris pachypus*; Pp, *P. pipistrellus*; Rs, *Rhinolophus sinicus*; Rf, *R. ferrumequinum*; Rp, *R. pearsoni*; Rm, *R. macrotis*; Mm, *Miniopterus magnater*; Mr, *Myotis ricketti*; Sk, *Scotophilus kuhlii*. Sequences obtained from GenBank were as follows: DQ412043 isolated from *R. macrotis* in Hubei Province (HB); DQ412042 isolated from *R. ferrumequinum* in HB; DQ071615 isolated from *R. pearsoni* in Guangxi Province (GX); DQ022305, DQ084199, DQ084200, DQ249213, and DQ249235 isolated from *R. sinicus* in Hong Kong (HK); DQ249214, DQ249215, DQ249216, DQ249217, and DQ074652 isolated from *T. pachypus* in HK; DQ249218, DQ249219, and DQ249221 isolated from *Pipistrellus abramus* in HK; DQ249224 isolated from *Myotis ricketti* in HK; and DQ249226, DQ666337, DQ666339, DQ666340, DQ249228, and DQ666338 isolated from *M. magnater* in HK. FJ, Fujian Province; SC, Sichuan Province; AH, Anhui Province; HN, Hainan Province; GD, Guandong Province; JX, Jiangxi Province; SD, Shandong Province.

Four consecutive Metropolis-coupled Markov chain Monte Carlo computations were run for 2 million generations, with trees sampled every 100 generations. Initial trees were random. On the basis of stabilization of preliminary runs, the first 3,000 trees were discarded before generation of the consensus tree. The Bayesian consensus tree was rooted to Breda virus (AY427798), a related CoV (Figure 1).

Phylogenetic Analyses of Bat *cyt b* Gene Sequences

For bat phylogeny, we used the complete mitochondrial *cyt b* gene to construct maximum likelihood (ML) and Bayesian phylograms. The *cyt b* sequence data were aligned by using ClustalX version 1.81 as above. ML analysis was performed by using PAUP* version 4.0b (*27*). The most appropriate substitution model (generalized time reversible + Γ + I) with the parameters matrix = 0.4835 × 9.6665 × 0.3815 × 0.2973 × 7.1418, base frequency = 0.3576 × 0.3420 × 0.0748, rates = gamma, shape = 0.6008, and proportion of invariable sites unable to accept substitutions = 0.4078 for ML and subsequent Bayesian analysis was calculated by using the program Modeltest 3.7 (*26*). We used heuristic searches (10 replicates, random addition of taxa, with tree bisection and reconnection branch swapping), followed by 100 bootstrap iterations for robustness of the ML tree. Bayesian analysis was also used to construct a tree with 4 simultaneous Markov chains for 1 million generations. Trees were sampled every 20 generations, and the first 5,000 trees were discarded before the consensus tree was made (on the basis of practical values of stabilizing likelihood).

Genetic Diversity among Bats and CoVs

We compared the genetic diversity of CoVs isolated from rhinolophids and vespertilionids and the corresponding diversity among bat taxa by using the index of nucleotide diversity (π) described by Nei (*28*) in Arlequin version 3.1 (*29*). Analyses were performed on uncorrected pairwise genetic distances between sequences.

Results

By combining information derived from the phylogram of bat CoVs, together with data on the geographic origin of viruses, we were able to describe the phylogeographic distributions for known CoVs from bats in China (Figures 1, 2; Table). Bat SARS-like CoVs formed a monophyletic clade. Species-specific host restriction was found for CoVs in 4 of 7 bats species (*Myotis ricketti*, *M. magnater*, *P. abramus*, and *T. pachypus*) sampled from >1 geographic location, and these clustered with high Bayesian posterior probability. Overall phylogenetic relationships between virus lineages were similar across our analyses, and well-supported genetic structure was observed within some CoV lineages. For example, CoVs isolated from *M.*

Figure 2. Distribution of coronaviruses isolated in the People's Republic of China. RsV, detected in *Rhinolophus sinicus*; PaV, detected in *Pipistrellus abramus*; TpV, detected in *Tylonycteris pachypus*; RfV, detected in *R. ferrumequinum*; RmV, detected in *R. macrotis*; PpV, detected in *P. pipistrellus*; SkV, detected in *Scotophilus kuhlii*; MrV, detected in *Myotis ricketti*; RpV, detected in *R. pearsoni*; MmV, detected in *Miniopterus magnater*; MpV, detected in *M. pusillus*. Abbreviations for provinces are shown in parentheses. SC, Sichuan Province; AH, Anhui Province; FJ, Fujian Province; HN, Hainan Province; GD, Guangdong Province; HB, Hubei Province; GX, Guangxi Province; SD, Shandong Province; JX, Jiangxi Province; HK, Hong Kong Special Administrative Region, People's Republic of China.

magnater were monophyletic but formed 3 well-supported clades with no evidence of geographic structure (Bayesian posterior probability [PP] = 1.0 for each). A similar pattern was apparent in CoVs from *Myotis ricketti*, which formed 2 geographically overlapping independent clades (PP = 0.99 and 1.0, respectively). One *T. pachypus* was infected by a virus that clustered with moderate statistical support (PP = 0.91) within the larger clade associated with *P. abramus*, which indicated a potential interspecies transmission event or recent evolutionary host shift (defined as interspecies transmission followed by establishment and long-term persistence in the new host species [*18*]) (Figure 1).

Phylograms of host sequences were also constructed and were essentially of the same topology with high support whether derived by using MrBayes version 3.1.2 or MEGA3 (data not shown). When we mapped host phylogram to virus, virus phylogeny did not always track host phylogeny (Figure 3). When separate host-virus phylograms were constructed for the 2 bat families (Verspertilionidae and Rhinolophidae), different corresponding relationships were evident. Verspertilionids and their CoVs showed phylogenetic congruence, and rhinolophids and their CoVs showed incongruous phylogenies (Figure 4).

We found evidence for evolutionarily divergent relationships for some vespertilionid viruses and their hosts when analyzed at the family scale (Figure 4, panel A). For example, divergence between viruses harbored by *P. pipistrellus* and *P. abramus* is congruent with their hosts. The divergence among other viruses was incongruent with divergence of host species, e.g., those from *S. kuhlii* and *Myotis ricketti*.

Rhinolophid bats and their viruses were analyzed at a different taxonomic scale (within genus). In this co-phylogeny, viral host shifts were the evident virus-host feature (Figure 4, panel B). Except for *R. macrotis*, all rhinolophidae bats had 2 distinct lineages of CoVs, and host shifts were found among viruses carried by *R. ferrumequinum*, *R. pearsoni*, and *R. sinicus*.

Genetic diversity of CoVs harbored by rhinolophids and vespertilionids was similar (vespertilionids $\pi = 0.27 \pm 0.13$; rhinolophids $\pi = 0.25 \pm 0.13$). In contrast, genetic diversity of *cyt b* sequences from bats was much higher among the vespertilonids ($\pi = 0.17 \pm 0.007$) than among the rhinolophids ($\pi = 0.09 \pm 0.006$).

Discussion

CoVs sequenced from different bats of the same species clustered together, even when bats were collected in locations 1,000–2,000 km apart. This pattern was found for CoVs from *P. abramus*, *T. pachypus*, *Myotis ricketti*, and *M. magnater*. Bats of the genus *Miniopterus* are known to migrate long distances (*30*), which explains why the phylogeny of viruses isolated from *M. magnater* sampled in distant places (Guangxi, Anhui, Fujian, and Hong Kong)

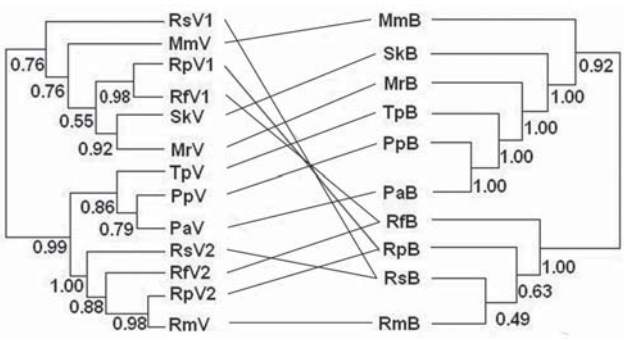

Figure 3. Phylogenetic relationships between coronaviruses (left) and their host bat species added for reference (right). Abbreviations on both sides denote viruses harbored by bats (marked as V on the left) and bats (marked as B on the right). Rs, *Rhinolophus sinicus*; Mm, *Miniopterus magnater*; Sk, *Scotophilus kuhlii*; Rp, *R. pearsoni*; Mr, *Myotis ricketti*; Rf, *R. ferrumequinum*; Tp, *Tylonycteris pachypus*; Pp, *Pipistrellus pipistrellus*; Pa, *P. abramus*; Rm, *R. macrotis*. Values below branches are Bayesian posterior probabilities. Although some of these values are low, our analysis demonstrated a pathway for future study (*28*). Lines between the 2 trees were added to help visualize virus and host sequence congruence or incongruence.

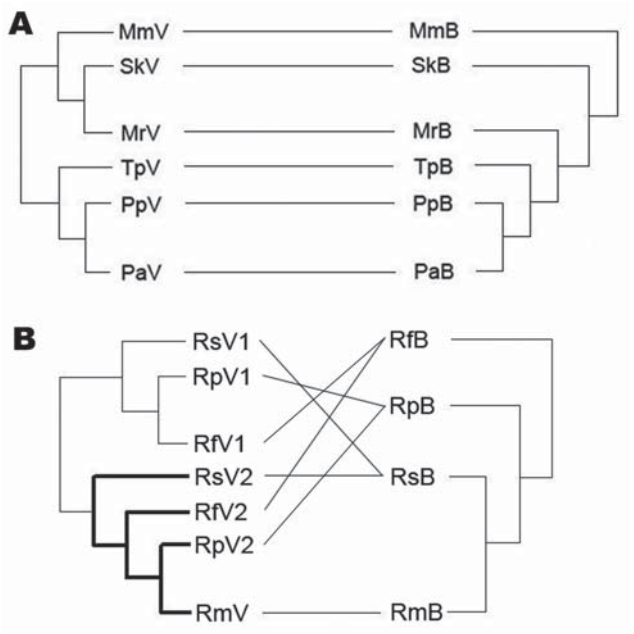

Figure 4. Phylogenetic relationships between coronaviruses (CoVs) (left) and bats (right) in the A) Vespertilionidae and B) Rhinolophidae. Abbreviations on both sides denote viruses harbored by bats (marked as V on the left) and bats (marked as B on the right). Mm, *Miniopterus magnater*; Sk, *Scotophilus kuhlii*; Mr, *Myotis ricketti*; Tp, *Tylonycteris pachypus*; Pp, *Pipistrellus pipistrellus*; Pa, *P. abramus*; Rs, *Rhinolophus sinicus*; Rf, *R. ferrumequinum*; Rp, *R. pearsoni*; Rm, *R. macrotis*. **Boldface** branches in panel B contain severe acute respiratory syndrome–like CoVs reported. Lines between bat and virus trees were added to help visualize congruence or incongruence. Although this figure implies differences in propensity for host shifts between these families, all but 1 of the vespertilionid CoVs are from different genera, whereas all rhinolophid CoVs are from the same genera, which make meaningful comparisons difficult. Overall mean genetic differences are much greater between vespertilionid species than between rhinolophid species.

lacks geographic structure. In nonmigrating species such as bats of the genera *Pipistrellus* and *Tylonycteris*, intimate physical contact of bats in same cave or the same bamboo roost site, as well as periodic exchange of bats among neighboring colonies, may facilitate virus transmission among populations.

Despite the co-roosting of many bats species, we found little evidence of host shifts for some viruses. For example, CoVs from *M. magnater* and *Myotis ricketti* sampled in the same cave in Guangxi were divergent, although sample size was limited. Although *Myotis ricketti* has a closer phylogenetic relationship with *T. pachypus*, *P. pipistrellus*, and *P. abramus* than with *M. magnater* and *S. kuhlii*, its behavior and habits are closer to those of the last group. For example, *Myotis ricketti* and *S. kuhlii* bats roosts in caves (although *S. kuhlii* also roosts under palm leaves), whereas *T. pachypus* roosts inside bamboo and *P. abramus* roosts almost entirely in old buildings. Thus, it seems plausible that the close phylogenetic relationship between viruses harbored by *Myotis ricketti* and *S. kuhlii* reflects the similar behavior and ecology of their hosts.

The phylogenetic and phylogeographic associations we found suggest that there may be a coevolutionary relationship between some bat CoVs and their hosts. For example, sister taxa within the genus *Pipistrellus* independently maintained 2 distinct viruses that share a most recent common ancestor. A similar relationship was apparent among the viruses of some closely related genera (e.g., *Pipistrellus* and *Tylonycteris*), whereby divergence of each genus was mirrored by divergence in viral phylogeny. However, viruses are usually thought to have evolved more recently than their hosts (*31*). Thus, apparent coevolutionary patterns may reflect either a high frequency of host shift among closely related bat species or simultaneous lineage splitting of hosts and viruses. Host shifts among related bats might be favored by a variety of mechanisms, including preadaptation to overcome immune defenses or greater rates of interspecific contact relative to distantly related bat species. Phylograms with better resolution would enable statistical comparison of phylogenetic congruence and estimation of divergence times.

In the vespertilionids, close phylogenetic concordance between host and virus suggests a close, possibly evolutionarily divergent relationship. However, there are different scales of comparison between the Vespertillionidae, in which all but 1 CoV came from separate genera, and the Rhinolophidae, in which we examined a co-phylogeny of multiple species within 1 genus. Genetic diversity in the vespertilionids sampled was nearly double that of the rhinolophids, which was probably due to the greater number of species sampled and their broader taxonomic range. Despite this greater genetic diversity among vespertilionid bat hosts, the genetic diversity of CoVs did not differ between vespertilionids and rhinopholids. This diversity suggests that vespertilionids may maintain undiscovered CoVs or that rhinolophids might harbor disproportionate CoV diversity relative to diversity of their genus. We propose that future work may identify more vespertilionid bat CoVs, which would enable an accurate comparison of propensity for host shifts within this group.

In the rhinolophids, the host phylogram demonstrated genetic divergence between *R. ferrumequinum* and other species, as shown by the division of *Rhinolophus* bats into 2 groups. Each of these groups harbors CoVs from 2 clusters (SARS-like CoVs and other CoVs), which suggests multiple introductions of CoVs into these species.

Lack of concordance between phylogenies of rhinolophid bats and their CoVs can be interpreted as evidence for host shifts between bats of the genus *Rhinolophus*. Different species of *Rhinolophus* are often observed roosting

inside the same cave, which facilitates virus transmission between species. However, the degree of host shifting of rhinolophid bat CoVs may not be particularly high relative to other genera of bats. This observation will be clarified when a greater diversity of CoVs from other bat genera is reported and the sequences are analyzed. These requirements support the need for further research on bat viruses (*32*,*33*).

Host-shifting within the genus *Rhinolophus* would likely be promoted if these bats shed CoVs in a way that makes them more available to other *Rhinolophus* spp.; had behavioral traits that lead to increased contact with other *Rhinolophus* spp.; or if CoVs harbored by these bats have structural, biologic, or other traits that make them more readily transmitted to other *Rhinolophus* spp. Two lines of evidence suggest that host traits are the most parsimonious explanation for host shifts within the genus *Rhinolophus*. First, SARS-like CoVs and other rhinolophid CoVs (RfV1 and RpV1) show evidence of interspecies transmission. Second, CoVs from other bat groups that are phylogenetically much closer to RfV_1 and RpV_1 than to the SARS-like CoVs do not show evidence of successful host shifts. Thus, the ability to jump hosts is unlikely to be a strictly viral trait.

The phylogeography of bat CoVs suggests that the bat SARS-like CoVs form a monophyletic clade that is both phylogenetically distinct from other bat CoVs and geographically isolated. Although we acknowledge that this interpretation may be limited by sample size, it may also indicate that rhinolophid bats, the hosts of a cluster of SARS-like CoVs within which human and civet SARS CoV nestle phylogenetically (*6*,*7*), are more likely to foster the host shifts of CoVs than are other bat species. The potential for close contact between bats, civets, and humans in the wildlife trade in southern China, coupled with a possible propensity of these bats to foster CoV host-shifts, could explain SARS-like CoVs as the source of SARS-CoV. This potential supports molecular results on bat CoVs that suggest a recent host shift from bats to civets or other animals and humans (*34*). Such host shifts may indicate a risk posed by other bat CoVs for novel disease emergence. Finally, the ability of SARS-like CoVs to be transmitted between and establish in new species (i.e., to undergo host shifts) is consistent with other CoVs. This has been shown for several CoVs of livestock species (*35*) and has been used to support their inclusion as 1 of the groups of viruses most likely to be responsible for emerging zoonoses, even before the emergence of SARS (*36*).

The total diversity of CoVs (including SARS-like CoVs) in bats has likely not been fully described. The genus *Rhinolophus* (*8*) contains 77 bat species distributed in Asia, Europe, and Africa. The recent discoveries of bat CoVs in the United States (*37*) and SARS-COVs in African bats (*38*) support the hypothesis that CoVs are diverse and widespread in bat species. Given the diversity of CoVs in this group, and their propensity for host shifts, further viral discovery in rhinolophids may assist in understanding and ultimately controlling the emergence of zoonotic viruses. Bats are increasingly recognized as reservoirs of many highly lethal zoonotic agents (*32*). Understanding their diversity, behavior, and mechanisms of virus transmission may play a key role in preventing future outbreaks of both known and unknown zoonotic diseases of bat origin.

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Rapid Increase of Genetically Diverse Methicillin-Resistant *Staphylococcus aureus*, Copenhagen, Denmark

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In Copenhagen, methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for <15 isolates per year during 1980–2002. However, since 2003 an epidemic increase has been observed, with 33 MRSA cases in 2003 and 110 in 2004. We analyzed these 143 cases epidemiologically and characterized isolates by pulsed-field gel electrophoresis, *Staphylococcus* protein A (*spa*) typing, multilocus sequence typing, staphylococcal chromosome cassette (SCC) *mec* typing, and detection of Panton-Valentine leukocidin (PVL) genes. Seventy-one percent of cases were community-onset MRSA (CO-MRSA); of these, 36% had no identified risk factors. We identified 29 *spa* types (t) and 16 sequence types (STs) belonging to 8 clonal complexes and 3 ST singletons. The most common clonal types were t024/ST8-IV, t019/ST30-IV, t044/ST80-IV, and t008/ST8-IV (USA300). A total of 86% of isolates harbored SCC*mec* IV, and 44% had PVL. Skin and soft tissue infections dominated. CO-MRSA with diverse genetic backgrounds is rapidly emerging in a low MRSA prevalence area.

For many years, methicillin-resistant *Staphylococcus aureus* (MRSA) has been a serious and common nosocomial pathogen in hospitals outside the Nordic countries and the Netherlands (*1*). Community-onset MRSA (CO-MRSA) was first reported in Western Australia in the early 1990s (*2*) and has, especially during the past 5 years, emerged as a global problem (*1*,*3*). CO-MRSA in the United States has mostly been caused by the Panton-Valentine leukocidin (PVL)–positive clones USA400 (sequence type [ST]1) and more recently by USA300 (ST8) (*1*,*4*–*7*). In Europe the increase in CO-MRSA has mostly been attributed to the PVL-positive ST80 clone (*3*,*8*). In Denmark, since 1980, MRSA has accounted for <100 MRSA isolates per year nationwide (www.danmap.org/pdffiles/danmap_2005.pdf). At our laboratory, MRSA has been isolated from <15 patients per year until 2002. Approximately half of the MRSA cases found in Denmark during these years were imported, i.e., through patients transferred from foreign hospitals (*9*).

Since 2003 the number of MRSA-positive persons increased both nationally and in Copenhagen. Nationally, the number increased from 50–105 new cases per year to 243 in 2003, 549 in 2004, and 864 in 2005. At Hvidovre Hospital, we found MRSA in 5 persons in 2001, 14 in 2002, 33 in 2003, 110 in 2004, and 170 in 2005. We describe the epidemiology of the emergence of MRSA in Copenhagen in 2003 and 2004 and characterize the genetic background of the isolates.

Materials and Methods

Setting

The Department of Clinical Microbiology, Hvidovre Hospital, services 4 of the 5 hospitals in Copenhagen and receives all microbiology samples from general practice in the Copenhagen and Frederiksberg Municipality (population 597,000). The total number of swabs received in the laboratory decreased slightly from 60,245 in 2000 to 55,223 in 2004. The principles for MRSA treatment, isolation of all hospitalized MRSA patients and hospital and household screening, were not changed before or during the study period. The only change was the introduction of new molecular typing methods.

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Patient Data

Upon identification of an MRSA isolate, the patient's attending physician was contacted to ensure correct antimicrobial chemotherapy and isolation procedures. The patient's physician was interviewed about the patient to define types of infections, identify risk factors, and establish epidemiologic links between patients. Most interviews were followed up by 1–5 more contacts. All interview data were registered prospectively in case-report forms and entered into the patient record files of our laboratory information system. The National Health Care Database (NHCD) was used to check for hospital admissions and ambulatory healthcare visits in Danish hospitals within the previous 2 years. These data enabled us to identify whether MRSA patients had been hospitalized in the same ward during the same period. The patients' home addresses, household members, and their general practitioners were identified in the NHCD. The patients were divided into 4 groups of acquisition mode of MRSA (*8*): 1) hospital-acquired (HA)–MRSA, MRSA isolated in a sample taken >48 h after admission or known exposure in a Danish hospital (domestic HA-MRSA) or a hospital abroad (imported HA-MRSA); 2) community-onset MRSA healthcare-associated (CO-HCA), MRSA isolated in a sample taken <48 hours after hospital admission or in general practice, with hospitalization and/or outpatient treatment in the previous year, having close contact to a person with HA-MRSA, residing in a nursing home, receiving in-home help or home nursing, being on dialysis, having cancer or diabetes, or being a drug abuser; 3) CO-MRSA community risk (CO-CR), MRSA isolated in a sample taken <48 hours after hospital admission or in general practice and having close contact to a person with CO-MRSA or having recently traveled to a country with high MRSA endemicity (no known contact with healthcare); and 4) CO-MRSA with no known risk factors (CO-NR), MRSA isolated in a sample taken <48 hours after hospital admission or in general practice, with no healthcare association, no known community risk, and no travel risk.

MRSA Isolates and Antimicrobial Susceptibility Testing

S. aureus isolates were identified by positive Staphaurex (Remel Europe Ltd., Dartford, UK) result and a positive coagulase test result. Only the first MRSA isolate from each patient was included in our study, for a total of 143 isolates in 2003 and 2004.

Susceptibility testing was performed on Isosensitest agar by the disk-diffusion method (antibiotic disks; Oxoid, Basingstoke, UK) according to recommendations of the Swedish Reference Group for Antibiotics (www.srga.org). Isolates were screened for resistance to methicillin by a cefoxitin 10-μg disk (*10*). Isolates were further tested against penicillin, cefuroxime, erythromycin, clindamycin, gentamicin, vancomycin, fucidic acid, rifampin, tetracycline, and moxifloxacin. Inducible clindamycin resistance was examined by using the double-disk method (*11*). All MRSA isolates were confirmed *mec*A positive by PCR (*12*). Multidrug-resistant MRSA was defined as an isolate resistant to ≥3 non–β-lactam antimicrobial agents.

Typing Methods

*Sma*I macrorestriction profiles were performed according to the HARMONY protocol (*13*) and analyzed with Bionumerics version 4.5 software (Applied Maths, Kortrijk, Belgium). Concatamerized phage λ DNA (New England Biolabs, Ipswich, MA, USA) was loaded in every sixth lane as a molecular weight standard to normalize the individual gels, and *Sma*I-digested *S. aureus* NCTC 8325 was used to normalize the gels to each other. Only DNA fragments in the range of the λ ladder (50–1,000 kb) were included in the analysis. Pulsed-field gel electrophoresis (PFGE) clusters were identified on an UPGMA (unweighted pair-group method with arithmetic mean) dendrogram based on Dice coefficients, where optimization and band position tolerance were set at 1% and 2%, respectively. A similarity coefficient of 80% was selected to define the clusters. Reference strains used for PFGE were USA100–1100 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and UK-MRSA 1, 3, 15, and 16 (HARMONY collection).

Sequencing of the staphylococcal protein A gene (*spa* typing) and multilocus sequence typing (MLST) were performed as previously described (*14*,*15*), except that PCR products were enzymatically purified with exonuclease I (New England Biolabs) and shrimp alkaline phosphatase (Amersham Biosciences, Piscataway, NJ, USA) before *spa* sequencing. Sequence reactions were performed on both DNA strands and analyzed on an ABI Prism 3100 (Applied Biosystems, Foster City, CA, USA). For PCR and sequencing of the *spa* gene, primers 1113F and 1496R were used. Designation of *spa* type was conducted by using the Ridom StaphType program (www.ridom.de) (*16*). MLST was performed on 57 (40%) of the 143 isolates, representing all *spa* types, and on every fifth randomly chosen isolate if the *spa* type was t024, t019, t044, or t008. STs and clonal complexes (CCs) were assigned through the MLST database (www.mlst.net).

Staphylococcal chromosome cassette (SCC) *mec* types were determined by an in-house multiplex PCR strategy in which types I–V were identified (*17*). Presence of PVL determinants was detected by PCR by using primers that overlap with previously published primer sequences (*18*,*19*). Primer sequences were forward 5′-TAG-GTA-AAA-TGT-CTG-GAC-ATG-3′ and reverse 5′-GCA-TCA-AST-GTA-TTG-GAT-AGC-3′.

Results

Patient and Infection Characteristics

In 2003 and 2004, 33 and 110 new cases of MRSA, respectively, were found. In 126 cases (88%) MRSA was from infection, and in 17 cases (12%) MRSA was found through screening of close family or hospital contacts to known MRSA patients.

Of the 143 MRSA cases, 42 (29%) were HA; 5 (3%) were imported, and 101 (71%) were CO. In 36 of the 101 CO-MRSA cases, the patient had a healthcare risk (CO-HCA), primarily due to hospitalization during the past year (21 cases) or residency in a nursing home (14 cases). Seven of the 36 patients were identified during the first 48 hours of hospitalization. For 30 patients a community risk (CO-CR) was identified, and for 35, no risk factors (CO-NR) were identified.

MRSA was found in samples from 50 patients during their hospitalization. Seventeen of these patients were in single-bed rooms; for those in shared rooms, we had no data on possible roommates for 5 case-patients, and the roommates of 3 case-patients were not screened. For 21 case-patients, MRSA screening of roommates was performed. Screening showed 4 MRSA-positive roommates with MRSA strains of the same *spa* type as that of the index patient (3 t024, 1 t015). In 1 intensive care unit case, transmission presumably occurred from staff to a patient in an adjoining room, as the *spa* type found (t003) was unique among our isolates.

Transmission among the 93 patients that were not hospitalized was more common. Of these, 11 resided in nursing homes; 5 were in the same nursing home. Another 14 patients lived alone. For 15 patients it was not possible retrospectively to determine whether anyone had lived with the patient (in most cases the index patient had died). In the households of 11 index patients, another 13 persons were found to be MRSA positive. The *spa* type most often found in family members was t019/ST30. In 11 families, all household members were MRSA negative. Household screening was not performed for 18 families.

Most of our MRSA cases were in the Amager region of Copenhagen. The 2-year incidence of CO-MRSA (HCA included) in Amager was 40:100,000 compared with 12:100,000 in the rest of Copenhagen.

Skin and soft tissue infections dominated (91 [64%] of 143 cases), followed by 17 urinary tract infections (UTIs, 12%), 9 deep-seated abscesses (6%), 5 lower respiratory tract infections (LRTIs, 3%), and 4 cases of septicemia (3%) (Table 1; Figure). Patients with HA-MRSA and CO-HCA MRSA had similar median ages: 78 (range 28–94 years) and 82 years (6–95 years), respectively. The patients with CO-NR MRSA and CO-CR MRSA had median ages of 32 (range 0–90 years) and 27 years (1–74 years), respectively (Table 1). The HA-MRSA and CO-HCA MRSA patients as well as the CO-NR MRSA and CO-CR MRSA patients were in many aspects similar and will be described as 1 group each.

Table 1. Demographics, infection types, and distribution of MRSA types in 143 cases of MRSA*

	Community onset, community risk	Community onset, no risk	Hospital acquired in Denmark	Community onset, healthcare associated	Imported
No. cases	30	35	37	36	
Male, %	67	46	57	42	
Median age, y (range)	27 (1–74)	32 (0–90)	80 (28–94)	82 (6–95)	
Carrier, no.	8	1	2	2	4†
Type of infection, no.					
SSTI	22	29	17	23	
Blood	0	0	2	2	
Deep-seated abscess, no.	0	1	5	3	
UTI	0	4	8	5	
LRTI	0	0	3	1	1†
Four most common CCs (87% of all isolates)					
CC 8, %	23	34	81	69	
t008, no.	3	7	0	2	
t024, no.	3	5	28	20	
CC 80, %	20	34	3	0	
CC 30, %	50	6	0	0	
CC 5, %	3	11	5	8	
Other MLST types,%	4	15	11	23	
PVL positive, %	83	80	8	17	
SCC*mec* IV, %	93	89	89	81	

*MRSA, methicillin-resistant *Staphylococcus aureus*; SSTI, skin and soft tissue infection; UTI, urinary tract infection; LRTI, lower respiratory tract infection; CC, clonal complex; MLST, multilocus sequence typing; PVL, Panton-Valentine leukocidin; SCC, staphylococcal chromosome cassette.
†A t037/ST239-III was imported from Greece, where this MRSA is common (*20*); ST239 is closely related to ST8 and is the well-known pandemic Brazilian/Hungarian clone (*21*); t041/ST111 was imported from an Italian hospital; t003/ST225-II (New York clone) was imported from Germany; t354/ST22-IV, a variant of EMRSA15, was imported from Lanzarote (Spain); and t067/ST125-I was imported from Spain.

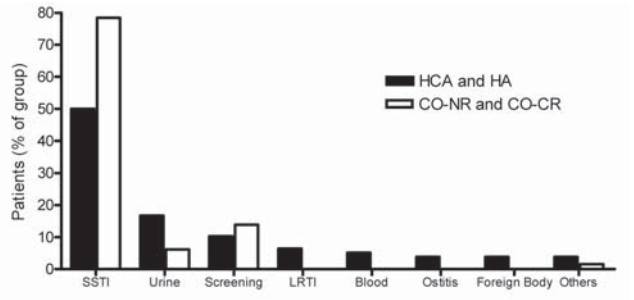

Figure. Distribution of infection types in community-onset methicillin-resistant *Staphylococcus aureus* (MRSA) and hospital-acquired (HA) MRSA. HCA, healthcare associated; CO-NR, community-onset MRSA with no identified risk factors; CO-CR, community-onset MRSA with community risk; SSTI, skin and soft tissue infection; LRTI, lower respiratory tract infection.

Susceptibility Testing of Isolates

Resistance to macrolides was detected in 85 (59%) of 143 isolates and in all isolates of *spa* type t024 and 7 of 12 t008 isolates. Constitutive macrolide resistance was found in 48% of erythromycin-resistant isolates. The resistance profiles of the 11 multiresistant isolates are described in the online Appendix Table (available from www.cdc.gov/EID/content/13/10/1533-appT.htm) (*3*,*8*).

HA and CO-HCA MRSA

Of 11 multidrug-resistant MRSA, 8 belonged to this group. Resistance to β-lactams only was found in 6 CO-HCA MRSA isolates (6 [8%] of 78) that belonged to 5 different CCs and contained either SCC*mec* IV or V.

CO-NR MRSA and CO-CR MRSA

Of 11 multidrug-resistant MRSA, 3 were in this group and 2 patients had no identified risk factors (t002/CC5 and t044/ST80). Of 65 isolates, 23 (35%) were resistant only to β-lactams, and all contained SCC*mec* IV. More data on resistance can be seen in the online Appendix Table.

PFGE, *spa* Typing, and MLST

There was a strong overall match between the clustering based on PFGE dendrogram, *spa* type, ST, or CC of the analyzed isolates (Table 2; online Appendix Figure, available from www.cdc.gov/EID/content/13/10/1533-appG.htm). The sequence-based typing methods showed 16 STs and 29 *spa* types (Table 2). MRSA isolates belonged to 8 CCs; CC8 was the most frequent (Table 2). The most common ST was ST8, represented by 72 isolates. The dominant *spa* type was t024/ST8, which accounted for 56 (39%) of 143 isolates. Other common MRSA were *spa* type t019/ST30 (17 isolates), *spa* type t044/ST80 (16 isolates), and *spa* type t008/ST8 (12 isolates). The 5 imported MRSA belonged to CC5 (2 isolates), CC 8 (t037/ST239-III), CC 22, and ST111. One CC1 strain was placed far from the other 2 CC1 isolates. The first isolate was ST748, t127, PVL negative, and SCC*mec* V; the other 2 were ST1, t175, PVL positive, and SCC*mec* IV (USA400-like). PFGE could not discriminate between MRSA t008, PVL-positive isolates, and t024 PVL-negative isolates but intermingled these isolates.

HA and CO-HCA MRSA

The 78 isolates (42 HA and 36 CO-HCA MRSA) represented 20 different *spa* types and 1 nontypeable isolate. A total of 48 (86%) of the 56 *spa* t024 were in this group. The patients were predominately elderly persons living in nursing homes or receiving home-care nursing. Most isolates belonged to CC8 (72%) (Table 2). Thirty-seven of the HA-MRSA isolates were considered Danish nosocomial isolates (domestic HA-MRSA), and 5 were from patients transferred from foreign hospitals (imported HA-MRSA).

CO-NR MRSA and CO-CR MRSA

The 65 isolates (35 CO-NR-MRSA and 30 CO-CR MRSA) represented 12 different *spa* types. All of the 17 t019/ST30, 15 of the 16 t044/ST80, and 10 of 12 of the t008/ST8 were found in this group. Only 8 patients with t024 were considered to have MRSA of CO-NR or CO-CR origin. Twenty-four cases were associated with household outbreaks, and transmission was suspected between children in 2 kindergarten classes.

Distribution of SCC*mec* Types and Prevalence of PVL Genes

SCC*mec* Type

SCC*mec* types could be determined for 140 (98%) of 143 isolates. SCC*mec* type IV was found in 122 (86%) of the isolates. Eighty-one percent of the HA and CO-HCA MRSA and 91% of the CO-NR/CO-CR isolates harbored SCC*mec* IV. The remaining isolates were represented by 5 type I (3.5%), 5 type II (3.5%), 2 type III (1.5%), 6 type V (4%), and 3 nontypeable (NT) (2%). Five CCs contained >1 SCC*mec* type: CC1 had IV and V; CC5 had I, II and IV; CC8 had III, IV, and NT; CC22 had IV and NT; and CC 45 had IV and V (Table 2).

PVL

Sixty-three (44%) MRSA isolates carried the PVL genes. Twenty-nine (97%) of 30 abscesses were caused by PVL-positive MRSA. PVL-positive isolates were also seen in 19 patients with chronic wounds, 8 MRSA carriers, 4 patients with UTI, 1 patient with LRTI, 1 with ear infection, and 1 with pus from a gallbladder. *spa* types and PVL were clearly correlated: all t019/ST30, t044/ST80, t008/ST8, t002/ST5, and t003/ST5 were positive for the PVL

Table 2. Genetic backgrounds and epidemiologic information on MRSA isolates*

CC	Multilocus ST	*Spa* types	Isolates, no. (total in CC)	No. PVL-positive isolates	SCC*mec* types (no. isolates)	HA/HCA MRSA or CR/NR MRSA % in the CC
CC1	ST1	t175	2	2	IV	33/67
	ST748†	t127	1 (3)	0	V	
CC5	ST5	t002	7	7	I (3), II (1), IV(3)	58/42
	ST125	t067	1	0	I	
	ST225	t003	4 (12)	4	II	
CC8	ST8	t024, t008, t430	56/12/2	12 (t008)	IV	74/26
		t064, t351	1/1	0	IV	
	ST239	t037	3 (75)	0	III (2), NT (1)	
CC15	ST15	t084	1	0	V	100/0
CC22	ST22	t005, t022, t354	2/1/1	0	IV, NT (t022)	50/50
		t541	1	0	IV	
	ST737†	t542	1 (6)	0	IV	
CC30	ST30	t019	17	17	IV	0/100
CC45	ST45	t015, t026, t065	2/1/1	0	IV, V (t026 and	100/0
		t116, t350	1/1 (6)	0	t116)	
CC97	ST97	t359	1	1	V	100/0
Singleton	ST80	t044, t376, t455	16/2/1 (19)	19	IV	5/95
Singleton	ST111	t041	1	0	I	100/0
Singleton	ST152	t355	1	1	V	0/100
Total			142	63		

*MRSA, methicillin-resistant *Staphylococcus aureus*; CC, clonal complex; ST, sequence type; PVL, Panton-Valentine leukocidin; SCC, staphylococcal chromosome cassette; HA/HCA/CR/NR, hospital acquired/healthcare associated/community risk/no risk. One isolate could only be typed by *spa* and multilocus sequence typing and is not included in the table.
†New ST.

genes. The remaining PVL-positive isolates were found in the following *spa* types: t175 (2/2), t355 (1/1), t359 (1/1), t376 (2/2), and t455 (1/1). None of the 56 *spa* t024 carried the PVL genes (Table 2).

HA and CO-HCA MRSA

Ten (13%) of the 78 isolates were PVL-positive. Three of them were part of a hospital outbreak with *spa* t003 imported in a patient from Germany.

CO-NR MRSA and CO-CR MRSA

Fifty-three (82%) PVL-positive isolates were found in the 65 strains in this group, 28 from patients with CO-NR MRSA and 25 from patients with CO-CR MRSA. PVL was present in 82% of CO-NR and CO-CR isolates, the isolates that we regard as truly community-acquired MRSA isolates.

Discussion

The high degree of genetic diversity (in both MLST background and SCC*mec* cassettes) found among MRSA isolates from a low-prevalence area is of global public health concern. The number of MRSA isolates doubled in <1 year in our area. Had the spread continued unhindered, MRSA would very rapidly have become a major source of illness as well as a healthcare financial concern for our hospitals and in the community. Although national guidelines to prevent MRSA transmission were first established in November 2006, an intervention program was introduced in Copenhagen in September 2005. We believe that our intervention program has had some effect: the number of cases in 2005 (170) increased to only 189 in 2006. While the current increase in CO-MRSA in the United States is predominantly by only 1 clone, USA300, we found 29 different *spa* types and 16 different STs in an area of <50 square miles. This finding suggests that the diversity has been caused not only by the spread of clones but also by the dissemination of the more mobile SCC*mec* elements IV and, to a lesser degree, V into methicillin-sensitive *S. aureus* (MSSA) (*22*). Typing MRSA with different methods gave additional information. The use of *spa* typing and PVL allowed the differentiation of MLST and PFGE identical isolates, and SCC*mec* typing could explain why MLST identical isolates had different PFGE patterns.

Our dominant clone, *spa* t024/ST8-IV, accounted for 39% of all isolates. The MRSA *spa* t024 clone is new in Denmark (*8*) and has been only sporadically reported, as MRSA or MSSA, to the SeqNet *spa* database (www.spaserver.ridom.de). The t024 clone was PVL negative but carried SCC*mec* IV, as did the typical PVL-positive community clones t044/ST80, t019/ST30, and t008/ST8. t024 belongs to CC8, as did the archaic clone that caused hospital outbreaks in Denmark in the late 1960s and early 1970s (*23*). The PFGE pattern of t024 is almost identical to that of t008 (USA300) but can be differentiated by being PVL negative and having a *spa* type that has lost 24 consecutive bases (1 repeat). The PVL phenotype of t024 CC8 strains was more like HA-MRSA around the globe. t024 was predominantly associated with nursing home outbreaks and home care nursing in the same area of Copenha-

gen as the nursing homes. Hospitalization of these patients led to small MRSA outbreaks. Our infection control team trained staff in the affected nursing homes in infection control. Isolation procedures in nursing homes were modified, compared with hospital regimes, to respect the fact that the patients were living in their own home. The nursing home staff were predominantly social workers with rudimentary infection control education. This, combined with the fact that many patients were not eligible for eradication treatment because of chronic wounds, could explain some of the difficulties in limiting the spread in nursing homes.

On the basis of *spa* typing, we have seen some evolution of the t024 clone. *spa* type t430 has evolved from t024 by the loss of 24 consecutive bases. The 2 patients with t430 were identified late in the t024 outbreak, and we find it more likely that a deletion occurred in t024 rather than that a new MRSA clone was introduced or that the SCC*mec* was transmitted to MSSA. In support of this view, samples from 1 patient taken the same day had t024 (axilla sample) and t430 (nose sample) (data not shown).

Some of the internationally well-known CO-MRSA clones were among our isolates (*3*,*24*). Most of the t008/ST8-IV could be identified as MRSA USA300–0114 genotype on the basis of PFGE typing (data not shown) (*25*). This finding is very troublesome because USA300 has evolved as the most rapidly spreading CO-MRSA in the United States and has become a dominant HA-MRSA in some hospitals (*24*). Special attention must be taken to control the spread of this clone. Sixteen (11%) isolates were t044/ST80-IV, which is a common CO-MRSA in Europe (*3*,*26*). This was a much more prevalent CO-MRSA clone in a national study of 81 Danish MRSA isolates from 2001, where 44% belonged to ST80-IV (*8*). We found that t019/ST30-IV was the most common CO-MRSA (17 patients, 12%). The t019/ST30-IV clone is known as the Southwest Pacific clone, and many of our cases had a geographic link to the Far East (data not shown). t019 was PVL positive, generally susceptible to all non–β-lactams, and caused severe abscesses. The PVL-positive MRSA clones were almost never associated with hospital outbreaks probably because these patients with skin and soft tissue infections were rapidly discharged.

Our findings of high prevalence of PVL-positive isolates (44%) are not surprising and are consistent with data from other studies (*27*–*29*) in which PVL is associated with skin and soft tissue infections, commonly described in CO-MRSA. Eighty-two percent of the CO-NR/CO-CR isolates contained the PVL genes, compared with only 13% of the HA/CO-HCA MRSA. We observed an age difference with a median age of 32 years (0–90 years) in the NR/CR group compared with 82 years (6–95 years) in the HCA group. These findings might indicate that the first group consists of patients with true community-onset MRSA and the second group is closely related to our HA-MRSA. Current definitions are becoming of decreasing usefulness as typical CO-MRSA strains invade hospitals.

A possible explanation for the increase in CO-MRSA could be a rise in outpatient antimicrobial drug use. Compared with other European countries (average 20 defined daily doses [DDD]/1,000 inhabitants/day) and the United States (26 DDD/1,000 inhabitants/day), Denmark had a low level of antimicrobial drug use in 2004 (14 DDD/1,000 inhabitants/day) (*30*). Although the use of systemic antimicrobial agents in Denmark has increased 14.7% from 2000 through 2004 (www.dfvf.dk/files/filer/zoonosecentret/publikationer/danmap/danmap_2004.pdf), it is unlikely that this is the only reason for the rise in MRSA.

Although we found a high number of CO-MRSA, our assessment of risk factors has some limitations. Having the data of all hospital admissions and outpatient hospital visits from the NHCD makes the division into the categories of HA-MRSA and CO-MRSA almost certain. However, because most of the patients were not directly interviewed about risk factors, some data might have been missed. Therefore, some of the CO-NR MRSA might actually be CO-CR or CO-HCA MRSA.

Previous guidelines used in Denmark for controlling MRSA in the hospitals by screening patients transferred from hospitals abroad are not sufficient to address today's CO-MRSA problems. In November 2006, the Danish National Health Board published national guidelines for preventing MRSA spread (www.sst.dk/publ/publ2006/cff/mrsa/vejl_mrsa.pdf). These guidelines introduce new approaches to limit the spread of MRSA in hospitals and in the community, where isolation of patients is not an option. In some hospitals we now have introduced admittance MRSA screening of all patients who have wounds or urinary catheters and we are isolating all residents from MRSA-endemic nursing homes. The hospital infection control teams see all patients with MRSA. In the community we have improved the screening of close contacts of MRSA patients and initiated a task force that visits patients at home and plans the eradication of MRSA carriage for all persons in the household. Our initial regime is 5 days' treatment with 4% chlorhexidine body wash once a day and 2% mupirocin nasal ointment 3× each day combined with washing of towels and bed linen and improved housekeeping. For some patients the treatment needs to be repeated (*31*), and patients with MRSA carriage that is difficult to eradicate are sometimes treated with systemic antimicrobial agents (*32*). Unfortunately, not all patients are decolonized by this procedure, and the long-term effect of eradication treatment, including the possible development of resistance to the antimicrobial agents used, is not well documented (*33*). Long-term studies on new and more effective methods to eradicate MRSA carriage are needed.

The rapid increase and genetic diversity of CO-MRSA in a country with low prevalence of HA-MRSA are of great concern, as these CO-MRSA are sporadically appearing in our hospitals. Especially worrisome is the discovery that MRSA USA300 genotype is in our community, as this MRSA has recently shown its epidemic potential in both the US community and US hospitals (*24*). MRSA isolation rates are increasing in other Nordic countries (*34*,*35*), but we need more data on CO-MRSA from countries in which classic HA-MRSA dominate. The current search-and-destroy policies need to be updated, refined, and intensified if Denmark hopes to remain a country with low MRSA prevalence.

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Dr Bartels is a PhD student working with MRSA. She plans to specialize in clinical microbiology.

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Personal Protective Equipment and Antiviral Drug Use during Hospitalization for Suspected Avian or Pandemic Influenza[1]

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For pandemic influenza planning, realistic estimates of personal protective equipment (PPE) and antiviral medication required for hospital healthcare workers (HCWs) are vital. In this simulation study, a patient with suspected avian or pandemic influenza (API) sought treatment at 9 Australian hospital emergency departments where patient–staff interactions during the first 6 hours of hospitalization were observed. Based on World Health Organization definitions and guidelines, the mean number of "close contacts" of the API patient was 12.3 (range 6–17; 85% HCWs); mean "exposures" were 19.3 (range 15–26). Overall, 20–25 PPE sets were required per patient, with variable HCW compliance for wearing these items (93% N95 masks, 77% gowns, 83% gloves, and 73% eye protection). Up to 41% of HCW close contacts would have qualified for postexposure antiviral prophylaxis. These data indicate that many current national stockpiles of PPE and antiviral medication are likely inadequate for a pandemic.

Although a new influenza pandemic may appear inevitable, critical parameters of transmissibility and attack rate are uncertain. Estimates based on extrapolations from the 3 influenza pandemics of the 20th century suggest that healthcare facilities in the United States alone may be required to cope with 314,000–734,000 additional hospitalizations and 18–42 million outpatient visits (*1*). During the early containment phase of a pandemic, patients with suspected infection are likely to be referred to hospitals for isolation, diagnosis, and treatment until the transmissibility and virulence of the pandemic strain are known. Although social distancing and school closures may reduce risk in the wider community (*2*), healthcare workers (HCWs) are likely to encounter repeated close exposures. If hospitals are to continue to function adequately, reliable access to effective personal protective equipment (PPE; gowns, N95 masks, gloves, and eye protection) and antiviral drug therapy will be necessary for an unpredictable period. With awareness of the recent severe acute respiratory syndrome (SARS) outbreak and with growing concern about human deaths from avian influenza (H5N1), governments worldwide have begun to stockpile PPE and antiviral medication.

Key strategies to control the speed and extent of viral spread within healthcare settings have been advocated by national government guidelines (*3*–*6*) and the World Health Organization (WHO) (*7*). These include rigorous infection control practices, prescriptive instructions for the use of PPE, and dissemination of antiviral medication. However, information regarding the required quantity and rate of use of these valuable resources in an outbreak situation is lacking, thereby limiting valid assessments of the adequacy

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of current stockpiles. This study aimed to estimate the resource needs that a hospital might face in the first few hours of management of a single patient who sought treatment with possible avian or pandemic influenza (API) or similar highly virulent respiratory infection.

Methods

In a prospective, multicenter, simulation exercise, we assessed the initial 6 hours of management of a patient (actor) who appeared for treatment at a hospital emergency department with a history consistent with API. Tertiary-level university teaching hospitals across eastern Australia were invited to participate. The inclusion criteria were willingness to join the simulation and possession of a formal local infection control protocol for the management of API that followed Australian (*3*) or WHO guidelines (*7*). The study was approved as a quality assurance project by the ethics committee at each participating site.

Conduction of Simulation

For each of the participating hospitals, the 6-hour simulation was conducted midweek, beginning between 8:30 and 9:30 AM, to avoid the busiest emergency department periods and to minimize the possibility that the care of actual patients might be compromised. The simulated patient was an actor unknown to the hospital staff, who appeared at the triage area of the emergency department and followed a prerehearsed script designed to trigger the hospital protocol for API. The standardized history included a 72-hour period of high fever, cough, shortness of breath, and severe malaise after a recent return from a Southeast Asian country. The patient reported handling unwell live poultry in a rural setting where human cases of avian influenza were known to have occurred. This standarized clinical scenario was chosen because guidelines for managing human cases of avian influenza (H5N1) form the current template for pandemic influenza case management (*4*,*5*,*7*). To heighten staff awareness of the appropriate management of an API case, each hospital organized education sessions on PPE use, infection control practices, and protocol familiarization in the 1–2 weeks before the simulation. Staff members were informed that the simulation would occur at some time during the allocated week (but not the exact day) and were instructed that hospital protocol should be followed as if it were an actual API case.

Each site had at least 3 trained infection control observers available who were familiar with using a modified version of a validated hand hygiene assessment data input tool (*8*) to accurately record potential API exposures in a standard manner. The observers were provided by the coordinating center or by the participating hospital. A principal investigator (A.S.) was present at each simulation to ensure standardization. The following 3 procedures were observed and assessed (Figure): 1) patient management through triage, emergency, radiology, and inpatient ward (including transfer between areas); 2) respiratory specimen collection, transport, and processing; and 3) cleaning of clinical areas after the suspected API patient had left the area or the simulation had been completed.

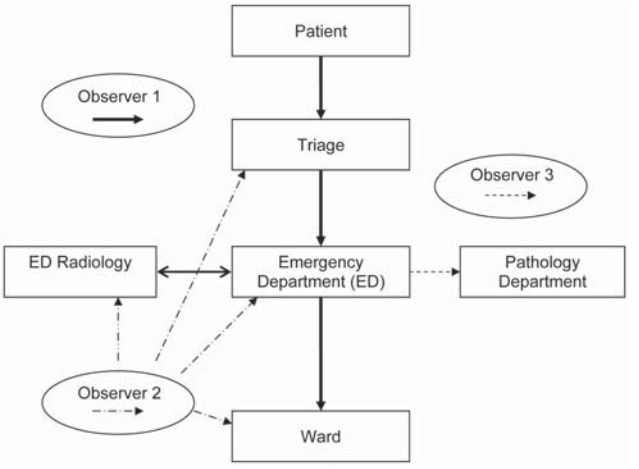

Figure. Study algorithm. Observer 1 follows the patient through all clinical areas, including transit between areas. Observer 2 monitors cleaning of clinical areas after use. Observer 3 monitors transport of clinical specimen to the pathology department and subsequent specimen processing.

Detailed observations were collated on infection control practice, clinical resources used, sequence of donning and removing PPE, time spent by the patient in each clinical area, and close contacts and exposures generated. The observation period could be stopped at any time if an actual patient's care was judged to be compromised by continuation of the simulation.

At the time of collecting blood, respiratory specimens, or chest radiographs, surrogate specimens (venipuncture tube containing water, water-moistened swabs, and archival chest x-ray, respectively) were substituted by the accompanying study observer. Surrogate blood and respiratory specimens were followed to the laboratory, where infection control practices were observed until specimens were sent to the reference laboratory for molecular testing.

Study Definitions

A HCW was defined as any person working within the healthcare facility. We used the WHO definition of a "close contact" as any person (including non-HCWs) coming within 1 m of an API patient within or outside of an isolation room or area (*7*). Close contacts were counted only once. An "exposure" was counted each time a close contact came within 1 m of the API patient. A "PPE item" included a disposable gown, pair of gloves, pair of protective eyewear, or N95 mask (or equivalent particulate respirator). A "PPE set" was defined as the appropriate combination of

Table 1. WHO Recommendations for HCW barrier precautions, dependent on type of exposure*†

HCW activity	Recommended PPE set
Close contact (<1 m) with potential API-infected patient within or outside of the isolation room or area	Gloves, gown, N95 mask (or equivalent particulate respirator), eye protection
Cleaning	Gloves, either gown or apron
Patient transport within healthcare facilities	Gown, gloves
Specimen transport and processing	Not defined except to use "safe handling practices"; interpreted as use of gloves (minimum) and gown if opening specimen bag.

*WHO, World Health Organization; HCW, healthcare worker; PPE, personal protective equipment; API, avian or pandemic influenza.
†Derived from (*7*).

PPE items recommended for HCW use in a particular clinical setting (*7*) (Table 1). "Opportunity for PPE item use" was defined as any instance of actual use of a PPE item during the study as well as any instance where the wearing of a PPE item was recommended by WHO guidelines (*7*), as objectively noted by accompanying study observers (Table 1). These items included PPE worn by HCWs involved in direct patient care (HCW close contacts) and ancillary HCWs who performed indirect clinical tasks associated with the API case-patient such as cleaning, ward support, and specimen transportation and processing. Environmental decontamination of clinical areas after use was considered adequate if cleaning and disinfection procedures were undertaken in a manner consistent with WHO recommendations (*7*). The time spent in each clinical area was recorded from when the API patient first entered an area to the time when the patient entered the next area.

For the purpose of identifying HCW close contacts who would be offered postexposure antiviral prophylaxis, HCW close contacts were stratified into either moderate- or low-risk groups derived from WHO criteria (*9*). High-risk close contacts, defined as "household or close family contacts of a strongly suspected or confirmed avian influenza (H5N1) patient" were not relevant to our study. The moderate-risk group included HCW close contacts wearing an insufficient or inappropriate PPE set during any of their exposures. The low-risk group included HCW close contacts wearing an appropriate PPE set for all exposures (*9*).

Outcome Measures

The study outcome measures were the following: 1) number of close contacts associated with the API patient during the initial 6 hours of patient management, including how many of these were HCW close contacts; 2) the total number of exposures experienced by close contacts; 3) overall quantity and type of PPE items (gowns, gloves, N95 masks, eyewear) actually used during the simulation by HCW close contacts and ancillary HCWs; 4) overall "opportunities for PPE item use" for HCW close contacts and ancillary HCWs (i.e., actual use plus missed opportunities for appropriate PPE use); and 5) stratification of HCW close contacts into medium- or low-risk groups for the purpose of recommending antiviral postexposure prophylaxis.

Results

Nine tertiary-level university teaching hospitals in 3 states of eastern Australia participated in the study (Table 2). The simulations occurred in the winter season, from May through August 2006. All sites conducted targeted staff education sessions 1–2 weeks before their exercise. Seven of the 9 simulations proceeded for the planned 6 hours of observation, and 2 were curtailed because of a critical need for the emergency department bed. Had these latter 2 sites continued, the patient would almost certainly have spent the entire study period isolated in the emergency department, as suitable ward beds were not available. The time spent in each clinical area for each site is summarized in Table 2. All sites performed radiography within the emergency department.

The number of close contacts and total exposures to the potential API patient are summarized in Table 3. The highest number occurred in the first hour of hospital care (triage and emergency department), which correlated with the initial intensive clinical and radiologic assessment and

Table 2. Participating institutions and time patient spent in each area*

	Hospital								
Characteristic	A	B	C	D	E	F	G	H	I
State	VIC	VIC	VIC	VIC	VIC	TAS	VIC	VIC	NSW
Urban/regional	Urban	Urban	Urban	Urban	Regional	Urban	Urban	Urban	Urban
Inpatient beds, no.	840	320	750	450	400	490	400	400	880
Annual admissions	67,700	40,000	79,500	47,200	61,200	52,300	45,300	93,100	71,600
Total simulation time, h	6	6	6	6	6	6	6	2.5	2.5
Triage time, h	0.3	0.3	0.1	0.1	0.1	0.1	0.1	0.2	0.1
ED time, h†	2	2.9	3	1.9	2.2	1.5	2.4	2.3‡	2.4‡
Ward time, h	3.7	2.8	3	3.9	3.7	4.4	3.5	–	–

*VIC, Victoria; TAS, Tasmania; NSW, New South Wales; ED, emergency department.
†Includes time spent in ED radiology unit.
‡Simulation of avian or pandemic influenza ended prematurely because beds were needed.

Table 3. Number of close contacts (CCs) and exposures to API patient*

Characteristic	No. CCs (no. exposures) per hospital									
	A	B	C	D	E	F	G	H	I	Mean
Total	17 (26)	15 (20)	6 (15)	11 (20)	14 (17)	12 (20)	11 (17)	10 (11)†	6 (8)†	12.3 (19.3)‡
By clinical area										
Triage	8 (8)	4 (4)	1 (1)	2 (2)	5 (5)	1 (1)	3 (3)	7 (7)	3 (3)	3.8 (3.8)
ED	5 (11)	7 (11)	3 (9)	7 (10)	6 (9)	7 (10)	6 (9)	3 (4)†	3 (5)†	5.9 (9.9)‡
Ward	4 (7)	4 (5)	2 (5)	2 (8)	3 (3)	4 (9)	2 (5)	–	–	3.0 (6.0)
By study period, h										
0–1	10 (12)	8 (8)	3 (4)	6 (8)	7 (7)	8 (8)	5 (5)	9 (10)	5 (6)	6.8 (7.6)
1–2	2 (3)	0 (1)	1 (2)	2 (2)	3 (5)	2 (6)	3 (3)	0 (0)	1 (2)	1.6 (2.7)
2–3	2 (4)	0 (2)	0 (4)	3 (5)	2 (3)	1 (3)	0 (2)	1 (1)†	0 (0)†	1.1 (3.3)‡
3–4	3 (5)	5 (6)	2 (3)	0 (2)	0 (0)	1 (1)	2 (3)	–	–	1.9 (2.9)
4–5	0 (1)	2 (2)	0 (1)	0 (1)	1 (1)	0 (2)	1 (2)	–	–	0.6 (1.4)
5–6	0 (0)	0 (1)	0 (1)	0 (2)	1 (1)	0 (0)	0 (2)	–	–	0.1 (1.0)
By HCW status										
Non-HCW	3 (3)	5 (5)	0 (0)	0 (0)	3 (3)	0 (0)	2 (2)	4 (4)†	0 (0)†	1.9 (1.9)‡
HCW	14 (23)	10 (15)	6 (15)	11 (20)	11 (14)	12 (20)	9 (15)	6 (7)†	6 (8)†	10.4 (17.4)‡
No. HCW CCs (%) who wore complete PPE set during each exposure§	2	3	5	9	8	8	8	2†	3†	6.1 (59)‡
No. HCW CCs (%) who wore N95 masks during each exposure§	12	7	6	10	11	12	9	5†	3†	9.6 (92)‡

*API, avian (H5N1) or pandemic influenza; ED, emergency department; HCW, healthcare worker; PPE, personal protective equipment.
†Incomplete data as simulation terminated after 2.5 h.
‡Excludes data from sites H and I.
§World Health Organization recommendations (Table 1).

specimen collection. Patient transfer between areas was another peak time for exposures. The average number of close contacts for each API patient during the study period was 12.3 (median 11, range 6–17), with 19.3 exposures (median 20, range 15–26). HCW close contacts constituted 85% of all close contacts; the remainder were patients or visitors who were generally exposed in the triage area.

All 9 sites processed the respiratory specimen, with an average of 2.9 HCWs (median 3, range 2–6) handling or transporting the specimen, predominantly in the pathology department. Two sites used a vacuum transport system to deliver specimens from the emergency department to the laboratory, contrary to WHO recommendations (*7*).

Environmental decontamination of clinical areas after departure of the suspected API patient was performed haphazardly at all sites. The triage area was appropriately cleaned in none of the 9 sites, whereas the emergency department and ward areas at sites that completed the full simulation were cleaned appropriately in 6 of 7, and 4 of 7 instances, respectively; 1–2 cleaners were required per clinical area to appropriately perform this task.

Large quantities of N95 masks, disposable gowns, gloves, and eye protection were used and indicated during the study period (Table 4). Adherence to appropriate use by HCWs (HCW close contacts and ancillary HCWs) was variable and depended on the particular PPE item, clinical area, and participating institution. Appropriate use of N95 masks by HCWs occurred in 93% of exposures (actual use/total opportunities for PPE use, 18/19.4), although the corresponding figures for disposable gowns, gloves, and eye protection were lower (77%, 83%, and 73%, respectively).

HCW close contacts were stratified into either moderate- or low-risk groups, depending on whether an appropriate PPE set was worn during every exposure. The proportions of HCW close contacts who appropriately wore a PPE set, rather than an N95 mask alone, for every exposure were 59% and 92%, respectively. Thus, depending on how rigorously WHO antiviral medication guidelines (*9*) were followed, from 8% to 41% of all HCW close contacts would be classified as having experienced a medium-risk exposure and therefore would potentially require postexposure antiviral prophylaxis. This amounts to an average of 0.8 to 4.3 courses of antiviral medication per suspected API patient during the initial 6 hours of management.

Discussion

To our knowledge, this is the first multicenter study to estimate the quantity of PPE and antiviral therapy that may be required to manage patients with suspected API admitted to hospitals. During the initial 6 hours of hospital assessment, the number of close contacts of a single suspected API patient was high (mean 12.3), with a mean number of exposures of 19.3. Not surprisingly, most (85%) close contacts were HCWs, and PPE use was at its most intense

Table 4. Actual and total opportunities for PPE item use by HCWs during the study period*

PPE item type	Actual PPE use (total opportunities for PPE item use) by hospital†										Compliance,¶ %
	A	B	C	D	E	F	G	H‡	I‡	Mean§	
N95 masks	20 (22)	11 (16)	18 (18)	20 (22)	16 (17)	23 (23)	18 (18)	6 (8)	8 (11)	18 (19.4)	93
Gowns	18 (29)	11 (18)	17 (21)	19 (24)	15 (17)	20 (25)	20 (21)	6 (11)	9 (12)	17.1 (22.1)	77
Gloves	27 (35)	12 (20)	18 (21)	21 (27)	19 (21)	23 (25)	26 (27)	8 (11)	10 (13)	20.9 (25.1)	83
Eye protection	4 (20)	4 (13)	14 (16)	18 (21)	14 (16)	21 (22)	17 (17)	3 (7)	4 (7)	13.1 (17.9)	73
Shoe protection#	–	4	2	–	9	–	–	–	1	2.1	
Hats#	–	1	13	–	14	–	–	–	5	4	

*PPE, personal protective equipment; HCWs, healthcare workers
†See Table 1 for definitions.
‡Incomplete data as hospitals H and I terminated simulation after 2.5 h.
§Excludes data from hospitals H and I.
¶Actual/total opportunities for PPE item use.
#Use of shoe protection and hats not proscribed by the World Health Organization for routine use; data recorded only if these items were used.

in the first hour of emergency department assessment. Our data suggest that in the initial 6 hours alone, HCWs managing suspected API case-patients would require ≈20–25 PPE sets (mean quantities: 19.4 N95 masks, 22.1 gowns, and 25.1 pairs of gloves). Although a high proportion of HCW close contacts (mean 92%) wore an N95 mask appropriately for all exposures, appropriate concomitant use of other PPE items was less (mean 59% of exposures). Even with the widespread availability of PPE, this observed inadequate utilization rate meant that from 8% to 41% of HCW close contacts were likely to require postexposure antiviral prophylaxis if current WHO recommendations were followed (*9*). If appropriate PPE, especially N95 masks, were not available, the number of HCWs who would experience moderate-risk API exposure requiring postexposure antiviral prophylaxis would increase substantially.

Notably, a substantial minority of close contacts (15%; ≈2 per API patient) were non-HCWs (e.g., hospital patients or visitors), generated primarily in the triage area. Although the duration of unprotected exposure was often short (<5 minutes) for these persons, they represent a potential risk for subsequent community and hospital spread of API. This highlights the importance, in triage and reception areas particularly, of using appropriate infection control measures and signage to assist in cohorting of potential API patients and minimizing exposure of unprotected bystanders.

The critical importance of effective PPE in hospital infection control was demonstrated during the outbreak of SARS in 2003 (*10–14*). Nosocomial transmission of SARS was a prominent feature of the epidemic (*15*) and played a large role in the initiation and maintenance of outbreaks. As reported in a case-control study by Seto et al. (*13*), staff who used masks (in particular), gowns, and performed hand hygiene were less likely to become SARS infected than those who did not. Similarly, Lau et al. (*14*) noted that inconsistent use of PPE by HCWs working on wards with SARS patients in Hong Kong was associated with a significantly higher risk for nosocomial disease transmission. Provision of adequate PPE stock is therefore likely to be important in controlling the spread of API.

Many countries are compiling extensive stockpiles of PPE and antiviral medications for use if a new pandemic occurs. Planning for sufficient numbers of resource items is complex and dependent on estimations of pandemic-related additional emergency presentations, hospitalizations, general practice, and outpatient visits. In Australia, official estimates of additional hospitalizations range from 57,900 to 148,000 (*4*). Our data suggest that management of this number of hospitalizations without regard for suspected influenza patients who are assessed but who are not sufficiently ill to require admission, would require from 1,123,260 to 3,714,800 PPE sets (depending on whether they were N95 masks, gowns, or gloves, or all 3 items). Although ascertaining (from these data) the number of courses of postexposure antiviral prophylaxis required is difficult, if stocks of readily available PPE were inadequate, the number of courses of antiviral medication required would likely increase dramatically, up to 12–13 courses per suspected API case during the initial 6-hour assessment. Thus, adequate stocks of PPE provide a means of protecting valuable antiviral drug stockpiles for use in ill or heavily exposed persons.

An important consideration when extrapolating our data to other healthcare systems is that recommendations regarding the optimal form of respiratory protection vary between countries. The WHO interim guidelines for management of human cases of avian influenza (AI) state, "HCWs working with AI-infected patients should select the highest level of respiratory protection available, preferably a particulate respirator… designed to protect the wearer from respiratory aerosols expelled by others" (*7*). This recommendation is reflected in the Australian pandemic

influenza guidelines (*3*) and explains the high use of N95 masks in our study. However, pandemic influenza plans in the United Kingdom (*5*), United States (*6*), and Canada (*16*) currently recommend the use of surgical masks for close patient care, unless the HCW is engaged in procedures in which aerosolization occurs. Thus the proportion of N95 masks to surgical masks required will vary between countries with different guidelines, which affects assessment of stockpile adequacy. Our study did not assess the relative efficacy of N95 masks compared with surgical masks for protection against API transmission.

This study has several limitations. First, the duration of the study was short (6 hours), much shorter than the likely in-hospital stay of days for a patient with severe influenza. Thus, total PPE and antiviral agent usage per admission is likely to be substantially higher. Second, the study was conducted at a less busy time of day for emergency departments and therefore may not reflect the greater number of persons who would likely be exposed in the triage and emergency department areas during busier periods. Third, the patient was not clinically unwell or hypoxic; thus, relatively few HCWs were required to assess, manage, or review the API patient's condition. Fourth, we observed the management of the index API case-patient alone, although we acknowledge that actual patients are likely to come to the hospital with other household members (high-risk close contacts). However, extending observation to include management of asymptomatic but potentially infectious accompanying persons in a standardized manner would have substantially increased the complexity of the exercise. Our findings, therefore, likely underestimate the true resources required and contacts exposed for the management of a genuine API patient. Finally, the presence of observers and the preceding education sessions may have artificially increased compliance with PPE use, although in the event of a true pandemic one might assume that HCW compliance rates would be high as they aim to minimize their personal risk. Also, this study was designed to quantify the use of PPE in an environment with raised awareness of infection control practice, mimicking that which might occur during a pandemic, and thus provide relevant data for health resource planners.

This study suggests that managing a single API patient is resource intensive and exposes a high number of persons to a potentially severe infection. These data represent the likely minimum clinical resources required during an API patient's initial hospital assessment using current WHO-derived infection control guidelines. Given our findings, if a global influenza pandemic occurs with attack rates even on the lower end of projected estimates, demand for PPE and antiviral medication in healthcare facilities will likely outstrip current supply in industrialized countries, let alone the supply in resource-poor settings. Further studies are needed to assess resource usage in other healthcare settings such as intensive care units, fever clinics, general practice, and the community.

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SurvNet Electronic Surveillance System for Infectious Disease Outbreaks, Germany

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In 2001, the Robert Koch Institute (RKI) implemented a new electronic surveillance system (SurvNet) for infectious disease outbreaks in Germany. SurvNet has captured 30,578 outbreak reports in 2001–2005. The size of the outbreaks ranged from 2 to 527 cases. For outbreaks reported in 2002–2005, the median duration from notification of the first case to the local health department until receipt of the outbreak report at RKI was 7 days. Median outbreak duration ranged from 1 day (caused by *Campylobacter*) up to 73 days (caused by *Mycobacterium tuberculosis*). The most common settings among the 10,008 entries for 9,946 outbreaks in 2004 and 2005 were households (5,262; 53%), nursing homes (1,218; 12%), and hospitals (1,248; 12%). SurvNet may be a useful tool for other outbreak surveillance systems because it minimizes the workload of local health departments and captures outbreaks even when causative pathogens have not yet been identified.

Surveillance of infectious disease outbreaks is important because outbreaks often require immediate intervention by the public health service. In addition, outbreaks may indicate deficiencies in infection control management and provide unique opportunities to investigate clinical and epidemiologic characteristics of the infectious agents, particularly in emerging infectious diseases. Timely and comprehensive outbreak reports need to be available not only at the affected administrative level but also at state, national, and international levels to detect and control multistate outbreaks (*1*–*4*). Electronic documentation and transmission of data are needed for rapid information exchange between institutions in charge of conducting, coordinating, or reporting control measures and should minimize additional work load for the public health service (*5*).

International regulations have resulted in increased requirements for outbreak reporting from the local to the international level (*6*,*7*). One of the major changes in the new International Health Regulations enacted in May 2005 is that infectious disease outbreaks of international concern must be reported to the World Health Organization, irrespective of the pathogens involved (*8*). Moreover, member states of the European Union are already obligated to report foodborne outbreaks to the relevant European Union institution according to the regulation on monitoring of zoonoses and zoonotic agents (*9*).

Outbreak surveillance for emerging infectious diseases is a particular challenge because small independent outbreaks may occur before they are recognized as part of a larger epidemiologic phenomenon. The complexity, the prolonged persistence of outbreaks, and the differing degree to which outbreaks are investigated locally make it much more difficult to ensure standardized and timely surveillance of outbreaks compared with surveillance of sporadic cases (*10*). To overcome these problems, the RKI (the federal institution responsible for infectious disease surveillance in Germany) developed the software and implemented an electronic outbreak reporting system (SurvNet) as part of its existing electronic surveillance system for notifiable diseases. SurvNet was fully implemented in January 2001 at all administrative levels of the German Public Health system and, in January 2006, at all levels of the German armed forces. The objective of the system is

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timely and easily retrievable epidemiologic information exchange on outbreaks at the local, state, and national levels. We describe the system, present epidemiologic aspects of reported outbreaks, and discuss the strengths and weaknesses after 5 years of practical use in Germany.

Material and Methods

Electronic Transmission of Data

All 431 local health departments in Germany verify locally identified notifiable diseases with reference to national case definitions and send case reports electronically through the 16 state health departments to the national surveillance unit at RKI. The SurvNet software organizes the electronic transmission of case-based datasets from peripheral databases in each local health department to databases of the respective state health department and finally to the RKI (*11*,*12*). The system transmits data to the RKI on all cases in Germany but without identifiable information on the persons involved. In contrast, a local health department has full data on all cases from their jurisdiction (*11*). The data collected in this system includes demographic characteristics, time, place, diagnostics, case definition criteria, exposure to risk factors, and associations with outbreaks as well as administrative data on where, when, and by whom the dataset is being installed and modified.

Outbreak Reporting

Single case records can be linked together in the SurvNet database by creating an outbreak report as a new database unit. Several outbreak reports at the local level can be further combined, which results in meta-outbreak reports (Figure). This so called "inverted tree" structure allows documentation of multicounty and multistate outbreaks (*13*). Outbreak reports can also be linked with outbreaks that initially were thought to be unlinked but are later identified as being part of the same epidemiologic event. Staff at local or state health departments, as well as at RKI, can electronically link outbreaks on the basis of epidemiologic evidence such as person, place, time, and pathogen; they can also manually enter descriptive categorizations based on the information provided by the outbreak reports that form part of this meta-outbreak (online Appendix Figure, available from www.cdc.gov/EID/content/13/10/1548-appG.htm).

Information Structure

The qualitative characteristics of the outbreak are covered by 7 sections: geographic setting, food consumption, bloodborne diseases, animal contact, waterborne diseases, person-to-person contact, and molecular fingerprinting. Each section includes a list of standardized items, of which >1 can be selected. Food consumption, for example, contains a selection of standardized food items defined by the first hierarchical order of the Eurocode 2 Food Coding System (*14*), a system developed to serve as a standard instrument for nutritional surveys in Europe.

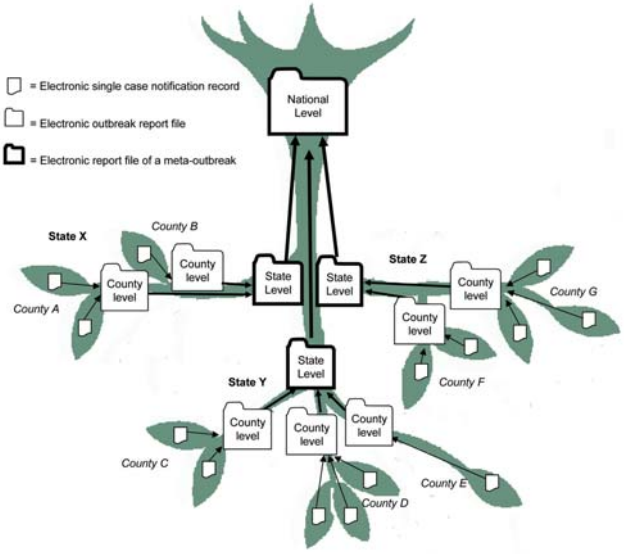

Figure. Inverted tree structure for organizing electronic outbreak reporting at different administrative levels.

Each selected exposure in the different sections is additionally categorized by using standardized evidence categories (Table 1). These range from "exposure confirmed by significant association in case-control or cohort study" to "majority of the cases of this outbreak had this particular exposure." For example, the category "breach of applicable standard recommendations supports epidemiologic link" is applicable if an outbreak investigation associated with meat consumption shows that consumed meat was not properly cooked. In addition to these standardized variables, results from molecular analysis of pathogens and complementary narrative information, such as anecdotal evidence, can also be included and categorized as "other information."

Outbreaks are generally linked to ≥1 pathogens identified as the causative agent(s) of the outbreak. For notifiable pathogens, the system provides a specific set of variables to allow validation against the respective case definition. Regarding pathogens for which sporadic cases are not notifiable, the agent can be selected from a list of 133 known human pathogens. If a pathogen cannot be detected, the cases can still be transmitted as part of an outbreak. The system automatically generates an outbreak profile consisting of general tables and graphs on the descriptive epidemiology of the outbreak, including epidemic curves, categorizations by age and sex, and geographic distribution of cases. Statistical overviews of reported outbreaks are published in the annual epidemiologic report on infectious diseases in April or May after the reporting year (*15*). Outbreaks

Table 1. Levels of evidence supporting associations with named exposures, reported infectious disease outbreaks, 2004 and 2005, Germany

Level of evidence	No. exposures (%)						
	Geographic setting	Person-to-person	Food	Blood	Water	Animal	All
Pathogen (in linked person or environmental sample) indicates epidemiologic link	98 (1.4)	3,544 (64.7)	65 (3.8)	4 (1.3)	3 (3.5)	8 (13.6)	3,722 (25.2)
Significant association by epidemiologic study (e.g., case control or cohort study)	763 (10.7)	369 (6.7)	139 (8.2)	148 (47.3)	5 (5.9)	2 (3.4)	1,426 (9.7)
Most cases had same exposure	6,063 (85.2)	1,361 (24.8)	1,342 (79.4)	60 (19.2)	44 (51.8)	32 (54.2)	8,902 (60.4)
Breach of applicable standard recommendations supports epidemiologic link	N/A	N/A	57 (3.4)	28 (8.9)	3 (3.5)	2 (3.4)	90 (0.6)
Other reasons	191 (2.7)	207 (3.8)	88 (5.2)	73 (23.3)	30 (35.3)	15 (25.4)	604 (4.1)
All entries	7,115 (100)	5,481 (100)	1,691 (100)	313 (100)	85 (100)	59 (100)	14,744 (100)
All outbreaks	7,074	5,400	1,637	311	85	59	14,566

of special interest are highlighted in short profiles in the weekly Epidemiological Bulletin, which may be followed by full outbreak reports in the same bulletin or other scientific journals (*16*).

Outbreaks and Statistical Analyses

Data presented in this article cover all outbreaks reported to the RKI from 2001 through December 2005 as of July 31, 2006. After the German infectious disease control law was passed, an outbreak was defined as ≥2 cases with an epidemiologic link (*12*). A case was considered epidemiologically confirmed if the clinical picture and an epidemiologic link to at least 1 laboratory-confirmed case was present as specified by the national case definition for the respective disease (*17*), e.g., a person with diarrhea and no laboratory diagnosis who had ingested the same implicated food item as ≥1 patients with laboratory confirmed salmonellosis. Outbreaks, which were part of a meta-outbreak, were not counted separately because the case data were already included in the respective meta-outbreak (Figure).

Unless otherwise specified, analyses were limited to outbreaks caused by notifiable pathogens defined by the national surveillance case definitions (*18*). Duration of an outbreak was defined as the interval between the onset of the first and the last case of the outbreak. Date of diagnosis was used if date of onset was missing. Reporting delay between the different public health levels was based on the electronic time stamps for entering respective data into the database and arrival at the RKI. Reporting delay was computed for the years 2002 through 2005, as technical constraints did not allow these analyses for the 2001 data. Chi-square testing was used to compare the proportion of outbreaks with food as a source for various pathogens.

The system for documenting qualitative descriptions of the outbreaks has undergone major revisions over the years. Therefore, data on these details are presented only for the years 2004 and 2005, to ensure a consistent and comparable data structure. Microsoft SQL Server 2005 (Microsoft Corp., Redmond, WA, USA) was used for database management. For the descriptive statistics, we used Statistical Package for the Social Sciences (SPSS) 15.0 for Windows, Version 15.0/1 (SPSS Inc., Chicago, IL, USA).

Results

From January 2001 through December 2005, a total of 30,578 outbreaks associated with notifiable pathogens were reported to RKI. Of 1,340,487 cases of notifiable diseases reported to RKI during this period, 253,720 cases (19%) were part of a reported outbreak; the rest were reported as sporadic cases (Table 2). Of these outbreaks, 90% were caused by pathogens of the intestinal tract (e.g., *Salmonella*, norovirus, rotavirus, hepatitis A virus, enteropathogenic *Escherichia coli*, and *Campylobacter*), and 10% (3,201) were caused by influenza virus (713), *Mycobacterium tuberculosis* (637), measles virus (501), and others (1,350, by 47 notifiable pathogens) (*12*). The size of the outbreaks ranged from 2 to 527 cases. Table 3 shows the number and duration of outbreaks by size and pathogens and indicates that duration increases with the size of the outbreak. The longest median durations were observed in outbreaks caused by hepatitis A virus (22 days) and by *M. tuberculosis* (73 days).

In addition to the 30,578 outbreaks associated with notifiable pathogens, 772 outbreaks were reported but not associated with any specific pathogen; 155 outbreaks were associated with pathogens that are not notifiable as single cases. Among these 155 outbreaks, 25 (16.8%) were associated with varicella-zoster virus, 26 (16.1%) *Staphylococcus* spp., 24 (15.5%) *Sarcoptes scabiei*, 16 (10.3%) coxsackie virus, 15 (9.7%) adenovirus (nonconjunctivitis), 11 (7.1%) *Streptococcus* spp., 10 (6.5%) astrovirus, and 28 (18.1%) outbreaks with 1 of 16 other pathogens. The distribution of these pathogens did not show any significant change over the years. The size, duration, and

Table 2. Total number of cases and outbreaks of notifiable disease, 2001–2005, Germany

Data point	2001	2002	2003	2004	2005	All
Total no. reported cases	245,133	284,425	252,119	267,130	291,680	1,340,487
No. (%) cases as part of reported outbreaks	22,146 (9.0)	67,498 (23.7)	48,855 (19.4)	58,204 (21.8)	57,017 (19.5)	253,720 (18.9)
No. outbreaks (any size)	3,981	6,914	6,261	6,340	7,082	30,578
No. (%) outbreaks with <5 cases	3,118 (78.3)	4,573 (66.1)	4,524 (72.3)	4,007 (63.2)	4,945 (69.8)	21,167 (69.2)
No. (%) outbreaks with ≥5 cases	863 (21.7)	2,341 (33.9)	1,737 (27.7)	2,333 (36.8)	2,137 (30.2)	9,411 (30.8)

reporting delay for these different kinds of outbreaks are compared in Table 4.

A location setting was reported for 9,946 outbreaks (33%). Of 10,008 listed items, the most frequently named categories were households (5,262; 53%), nursing homes (1,218; 12%), hospitals (1,248; 12%), and kindergartens (783; 8%) (Table 5).

In the 13,422 outbreaks reported in 2004 and 2005, at least 1 exposure associated with the outbreak was reported in 10,205 (76%) outbreaks, which added up to a total of 22,001 field entries (average 2.2 entries per outbreak). For 15,978 (66%) of these 24,208 field entries, an evidence category was provided by the reporting local health departments. The distribution of these categories is shown in Table 1. In 954 (9.3 %) of all 10,205 outbreaks linked to a specific exposure, the evidence of this linkage was based on a statistically significant association in a case-control or cohort study. For the 2,195 outbreaks with ≥10 cases, this type of evidence was reported in 248 (11.3 %) outbreaks, compared with 706 (8.8%) of the 7,998 outbreaks with <10 cases relative risk (RR) = 1.3, $\chi^2 = 12.5$, $p<0.001$.

For 1,637 (64%) of the 2,554 outbreaks in 2004 and 2005 that were linked to food, information was available about the evidence on which the association was based. For 204 (12%) of these, the linkage was supported by either statistically significant association or by detection of the causative pathogen in a food sample. In these 12% of outbreaks in which the exposure linkage was supported by the 2 latter methods, the proportion of outbreaks linked to food varied between causative pathogens. In 999 of such outbreaks caused by *S. enteritidis* spp., 14% (141) were associated with food either by a statistically significant association or by detection of the causative pathogen in a food sample; this association was found for 8% (28 of 359) *Campylobacter* outbreaks, 1% (16 of 1,239) norovirus outbreaks, and 0.2% (2 of 940) rotavirus outbreaks ($\chi^2 = 215.6$, $p<0.001$).

The median delay from receipt of the first case notification until electronic filing of an outbreak report at the local health department was 4 days in 2002, 1 day in 2003, and 0 (i.e., same day) in 2004 and 2005. The median reporting delay from electronic filing of the outbreak report at the local health department to arrival of the electronic report at RKI was 1 day in 2002, 2 days in 2003, and 3 days in 2004 and 2005. The overall median delay from receipt of the first case notification by the local health department until arrival of the electronic outbreak report at RKI remained stable at 7 days from 2002 through 2005.

Discussion

Effective surveillance of emerging infectious diseases requires a system able to transmit locally detected outbreak reports at an early stage, for example, when an epidemiologic investigation is still under way. The SurvNet outbreak surveillance system ensures continuous updating of the outbreak reports as more cases are identified or linked to the outbreak, long before an outbreak investigation has been finalized in a written report. This system also facilitates rapid

Table 3. Size and median duration* of outbreaks by pathogen, 2001–2005, Germany

Outbreak type, no. cases/median duration	Size of outbreak								
	2	3	4	5	6–9	10–49	50–99	≥100	All
Salmonella	5,855/2	2,134/2	1,006/2	504/2	721/3	636/6	34/16	10/22.5	10,900/2
Norovirus	1,169/2	641/3	469/3	342/4	984/6	3,694/9	636/17	140/26	8,075/7
Rotavirus	2,570/3	941/4	425/6	176/7	396/8	532/13	28/29	2/49	5,070/4
Campylobacter	2,032/1	390/2	123/3	44/3	45/4	34/8	1/8	0	2,669/1
Mycobacterium tuberculosis	454/62.5	102/105	44/103	16/229.5	19/282	2/253.5	0	0	637/73
Influenza	420/2	163/3	67/3	19/6	17/5	25/15	2/38.5	0	713/3
Hepatitis A virus	227/18	102/22	42/23	33/44.5	32/51.5	7/106	1/77	1/106	445/22
Giardia	192/2.5	52/12.5	27/13	4/12	3/74	4/27	0	0	282/4
Salmonella paratyphi	13/2	3/20	0	0	0	1/40	0	0	17/4
Other	1,052/2	318/6	134/8	61/14	88/17	102/40	8/83.5	7/123	1,770/6
All pathogens	13,984/2	4,846/3	2,337/4	1,199/4	2,305/6	5,037/9	710/18	160/27	30,578/3

*Duration and median duration are given in full days.

Table 4. Comparison among outbreaks, 2001–2005, Germany*

Characteristic	Linked to notifiable pathogens	Linked to nonnotifiable pathogens	No link to any specific pathogen
No. outbreaks	30,578	155	772
Median duration, d	3	9	4
Median no. cases per outbreak (minimum, maximum)	3 (2,527)	8 (2,153)	10 (2,110)
Median duration from report of first case until outbreak report filed at local health department, d	26,597	154	772
Median duration between filing of outbreak report at local health department until arrival of report at RKI, d	1	0	0
Median duration from report of first case until outbreak report arrives at RKI, d	2	2	3

*RKI, Robert Koch Institute.

electronic linkage of apparently independent outbreaks, for example, in different states, enabling subsequent analysis of the entire meta-outbreak. Although legally not considered an outbreak, single case notifications of rare diseases with strong public health implications (e.g., anthrax) will of course be captured through the SurvNet system as single case records and will result in immediate investigation and action by local authorities.

During the past 5 years, the SurvNet outbreak surveillance system has managed standardized collection, transmission, and reporting of complex information generated by outbreak investigations of all 431 local health departments in Germany. As shown in this report, the system also covers diseases for which the causative pathogen is either not identifiable or identified but not notifiable when occurring sporadically. Local health departments become aware of such incidents because outbreaks or infections with new or unknown pathogens that are potentially dangerous to the public are also notifiable under German law. This ability makes SurvNet particularly useful for the surveillance of emerging infectious diseases for which laboratory diagnosis may often be delayed or not yet possible. SurvNet has the advantage of managing epidemiologic information that laboratory-based systems or syndromic surveillance systems alone cannot easily provide. Essential epidemiologic evidence can be retrieved only through local outbreak investigations that are usually conducted by local health departments (*19*), which constitute the most critical component of outbreak detection and investigation. (*3*,*5*).

The SurvNet system appears to capture far more outbreaks per population than published collections of outbreak reports in other countries. For example, the Electronic Foodborne Outbreak Reporting System managed by the Centers for Disease Control and Prevention (CDC) has listed 1,319 foodborne outbreaks in the year 2004 within the United States (estimated incidence rate of 0.4 outbreaks per 100,000) compared with 1,263 foodborne outbreaks captured in SurvNet in that same year in Germany (incidence rate 1.5/100,000) (*20*). A similar difference is seen when comparing data from the SurvNet system in Germany with surveillance data on foodborne outbreaks in England and Wales or to the number of *Salmonella* outbreaks collected by different surveillance systems in France (*21*–*23*). These differences could be due to different case definitions, true difference in incidence caused by significantly poorer food safety in Germany, or other reasons. However,

Table 5. Locations of outbreaks by pathogen, 2004 and 2005, Germany*

	No. (%)						
Location	Norovirus (n = 3,141)	*Salmonella* spp. (n = 2,703)	Rotavirus (n = 1,985)	*Campylobacter* spp. (n = 1,005)	Hepatitis A (n = 139)	Others (n = 973)	All (n = 9,946)
Household	395 (13)	1,993 (73)	1,338 (67)	758 (75)	102 (72)	676 (69)	5,262 (53)
Nursing home	1,040 (33)	24 (1)	136 (7)	5 (0)	0 (0)	13 (1)	1,218 (12)
Kindergarten	368 (12)	61 (2)	290 (15)	7 (1)	9 (6)	48 (5)	783 (8)
Hospital, laboratory	1,035 (33)	20 (1)	175 (9)	5 (0)	1 (1)	12 (1)	1,248 (12)
Hotel, cruise ship	58 (2)	169 (6)	16 (1)	120 (12)	12 (8)	93 (9)	468 (5)
Restaurant	72 (2)	258 (9)	1 (0)	48 (5)	3 (2)	10 (1)	392 (4)
Other location	34 (1)	80 (3)	28 (1)	36 (4)	7 (5)	61 (6)	246 (2)
School, university	34 (1)	18 (1)	0	7 (1)	8 (6)	20 (2)	87 (1)
Special event, festival, etc.	24 (1)	55 (2)	4 (0)	9 (1)	0	6 (1)	98 (1)
Work place	37 (1)	21 (1)	0	10 (1)	0	17 (2)	85 (1)
Dormitory, military casern	56 (2)	9 (0)	9 (0)	1 (0)	0	11 (1)	86 (1)
Bus/ train, etc.	2 (0)	5 (0)	0	4 (0)	0	2 (0)	13 (0)
Prison	2 (0)	4 (0)	3 (0)	0	0	3 (0)	12 (0)
Refugee camp	0	2 (0)	0	0	0	8 (1)	10 (0)
Total number of listed items	3,157 (100)	2,719 (100)	2,000 (100)	1,010 (100)	142 (100)	980 (100)	10,008 (100)

*Outbreaks may be reported in >1 location.

the higher outbreak incidence rate in the SurvNet system is likely the result of its higher sensitivity, at least in part. Technically, the system is an integral part of the routine surveillance for notifiable diseases, which means that local health departments are required to enter and administer only outbreak-related data, because most of the information from the database of notifiable disease cases is being used in both systems. This synergism is likely to encourage local health departments to use the system and thus improve its sensitivity. Because all of the outbreaks identified in this system are events identified and investigated by local public health staff, the positive predictive value of detecting a true outbreak is likely to remain high. This is one of the major advantages of SurvNet compared with outbreak detection systems based on statistical algorithms of case reports. Data from SurvNet may, in fact, serve as the standard to validate statistical outbreak detection algorithms (*24*). SurvNet may also provide data to identify prognostic criteria that would help in forecasting the natural development of a specific outbreak (*25*–*28*). However, details of outbreak reports have not yet been systematically validated, so careful interpretation of the information is essential. Compared with CDC's outbreak reports analyzed by Ashford and colleagues, the SurvNet system appears to be much timelier, although a direct comparison is not possible because the types of investigated outbreaks and the definitions of the reporting delays are not directly comparable (*5*).

The European Food and Safety Authority is currently building a reporting system for foodborne outbreaks in the European Union using the methods developed in SurvNet (pers. comm., P.Mäkelä, European Food Safety Authority). The Eurocode 2 System used here to categorize food appears to be user friendly and is available on the Internet with instructions on how to categorize food items that appear difficult to assign to 1 category (*14*). Most outbreaks registered in this system were caused by pathogens of the gastrointestinal tract, yet only for a minor portion was reliable evidence available linking these outbreaks to food. This reminds us that outbreaks caused by gastroenteric pathogens, particularly those caused by norovirus, should not be overinterpreted as foodborne outbreaks.

Our data suggest that only 11.3% of reported exposures in outbreaks with ≥10 cases were statistically significant and associated with the outbreak through case-control or cohort studies. In 37% of the reported foodborne outbreaks in SurvNet, the reporting local health departments were able to associate a meal but not a specific food item with the outbreak. Similarly, Jones et al. have observed that most foodborne outbreak investigations in the United States did not identify a specific food item (*10*). Local health departments must be motivated to improve outbreak investigations to increase the validity of the information received through this system (*29*). In addition to intensifying training programs for the local public health service, RKI is currently developing support tools, such as predesigned electronic line lists of cases and decision-supporting algorithms, to be included in the SurvNet system. Additional training and support tools will assist local health department personnel in the use of epidemiologic methods for outbreak investigations. A new information technology structure will facilitate these additions and further improve the timeliness of the system.

SurvNet has the advantage of being able to document complex multistate outbreaks of any cause. For example, SurvNet was able to capture an outbreak of 1,024 cases of epidemic conjunctivitis, which started within the German armed forces and spread to the civilian population throughout the country (*30*). Comparatively few of the reported outbreaks (3%) were linked to nonnotifiable pathogens or could not be linked to specific pathogens at all. However, this demonstrates that SurvNet is able to cover outbreaks caused by unknown or emerging infectious diseases.

Outbreak surveillance of SurvNet has already provided valuable information for topics of public health relevance. By confirming and quantifying the increase of hospital-based norovirus outbreaks in recent years, SurvNet has contributed to the development of specific recommendations on how to prevent and control norovirus outbreaks in hospitals and nursing homes (*31*–*33*). In 2006, a sharp increase of norovirus outbreak reports was noted at RKI from reporting weeks 43 through 47. This led to a countrywide alert in the national weekly epidemiologic bulletin in week 48 and was subsequently echoed by an alert throughout Europe in the Eurosurveillance Weekly Journal 2 weeks later (*34*–*35*).

Although our report cannot replace a surveillance system evaluation, some system attributes can be addressed. Over a period of 5 years, SurvNet has demonstrated the ability to collect and analyze a large number of outbreak reports in a federal administrative environment of 431 local health departments and 16 federal states in Germany with a total population of 82 million inhabitants. This fact already indicates that requirements of simplicity, acceptability, and stability appear to have been met. SurvNet also seems to compare favorably to other systems in timeliness and sensitivity. The ability of SurvNet to capture outbreaks with unidentified or new pathogens in a systematic way indicates its suitability for outbreak surveillance of emerging infectious diseases. Given the federal structure in Germany and its reflection in the SurvNet design, this system might also be a blueprint for other large national or international outbreak surveillance systems, particularly in the context of the new international health regulations.

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Borrelia burgdorferi Infection and Cutaneous Lyme Disease, Mexico

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Four patients who had received tick bites while visiting forests in Mexico had skin lesions that met the case definition of erythema migrans, or borrelial lymphocytoma. Clinical diagnosis was supported with histologic, serologic, and molecular tests. This study suggests that *Borrelia burgdorferi* infection is in Mexico.

Lyme disease is the most frequently reported vector-borne infectious disease in the United States and Europe (*1*,*2*). Studies have suggested that *Borrelia burgdorferi* infection might be endemic to Mexico (*3*,*4*). We searched for histologic, immunologic, and molecular evidence of *B. burgdorferi* infection in patients with cutaneous manifestations suggestive of Lyme disease in Mexico.

The Study

From June 1999 to October 2000, 4 patients in Mexico City had clinical manifestations suggestive of Lyme disease (*5*,*6*). Two (36 and 54 years of age) had erythema migrans lesions, and 2 (9 and 34 years of age) had borrelial lymphocytoma lesions. Two reported having been bitten by a hard tick; the other 2, by a nonflying insect. Bites occurred while camping in forests: 3 near Mexico City (National Park La Marquesa) and 1 in Quintana Roo, a southern state in Mexico. All patients lived in Mexico City and had never traveled outside Mexico.

Two patients were treated for acute skin lesions (consistent with erythema migrans), malaise, and arthralgia. The skin lesion was an erythematous macula with regular, reddish edges and a pink center. One patient had a 5-cm lesion on the left forearm; the other had a 6-cm lesion on the left thigh. For the 2 other patients, a nodular erythematous cutaneous lesion (consistent with lymphocytoma), 0.5–2 cm in diameter with regular edges, developed 2 months after the bite. One patient's lesion was on the earlobe; the other's, on the left cheek.

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Serum from each patient was tested for immunoglobulin M (IgM) and IgG against *B. burgdorferi* sensu lato by using a commercial ELISA (cutoff optical density 0.200 and indeterminate 0.200–0.400) (Enzygnost Borreliosis, Dade Behring, Marburg, Germany) (*7*). A *Treponema pallidum* ELISA (Abbott Murex, Wiesbaden, Germany) was performed to rule out cross-reaction with *T. pallidum* infection. Serum samples positive for *B. burgdorferi* by ELISA were further tested by Western blot (WB) by using the Marxblot assay (MarDx Diagnostics, Carlsbad, CA, USA) and Centers for Disease Control and Prevention (CDC) criteria (*5*).

Serum samples from the 2 lymphocytoma patients were positive for *B. burgdorferi* by ELISA and WB (Figure 1, panel A; Table). For the 2 erythema migrans patients, serum samples taken 2 weeks after the tick bite were negative for *B. burgdorferi* IgM and IgG; but 2 months later, 1 patient became seropositive, confirmed by WB (Figure 1, panel B; Table).

Histologic examination of skin biopsy specimens from each erythema migrans lesion showed a mononuclear cell infiltrate in the superficial and deep dermis; infiltrate included lymphocytes and plasma cells around the perivascular zones. Biopsy samples of lymphocytoma lesions showed dense nodular lymphocytic infiltrates in the reticular dermis with well-delineated lymphoid follicles, no atypical mitosis, B-lymphocytes (anti-CD20, DAKO, Carpentería, CA, USA) in the germinal center (Figure 2, panel A), T-lymphocytes (anti-CD45 RO+) in the follicular zone (Figure 2, panel B), and no CD3+ cells.

DNA was extracted from the biopsy samples (Repli-g Mini Kit, QIAGEN, Valencia, CA, USA) and used for PCR amplification of a fragment of *fla* gene specific for *B. burgdorferi* sensu lato species as well as for a fragment of *ospA* gene, as described (*8*–*10*). DNA from a skin biopsy of a patient with systemic lupus erythematous was used as negative control, and DNA (10 pg/μL) from *B. burgdorferi* sensu stricto B31 served as positive control. All procedures from DNA extraction to amplification were performed twice for each sample. Amplified products were further tested by Southern blot (SB) hybridization with probes

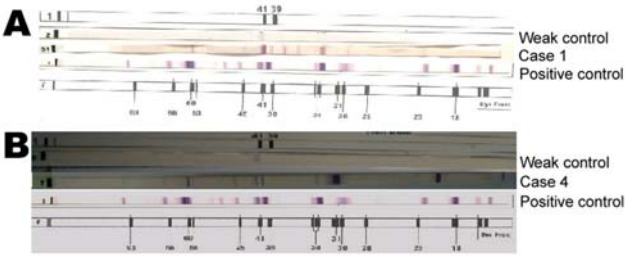

Figure 1. Western Blot (WB) immunoglobulin G results from cutaneous cases. A) WB with serum from patient 1, showing erythema migrans. B) WB with serum from patient 4, showing lymphocytoma; a strong positive and a weak positive control were included.

Table. Serologic IgG and molecular test results for patients with cutaneous lesions suggestive of Lyme borreliosis*

Diagnosis	ELISA OD	WB, molecular weight, kDa†	PCR for *fla* gene	SB‡
Erythema migrans	0.469	23,30,39,41,45,58	+	*Borrelia burgdorferi* sensu stricto
Erythema migrans	0.146	NT	+	*B. burgdorferi* sensu stricto
Lymphocytoma	0.472	23,28,39,41,58,66	+	*B. burgdorferi* sensu stricto
Lymphocytoma	0.574	23,39,41,45,66,93	+	*B. burgdorferi* sensu stricto

*Ig, immunoglobulin; OD, optical density; WB, Western blot; SB, Southern blot; NT, not tested.
†WB assay with *B. burgdorferi* sensu stricto antigen (Marxblot test), protein size of the bands recognized by the patient`s serum.
‡SB assay was done by using the probe specific for *B. burgdorferi* sensu stricto.

specific for *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii*, as described (*9*). DNA from the 4 biopsy samples was positive for *B. burgdorferi* sensu lato *fla* gene by PCR and confirmed as *B. burgdorferi* sensu stricto by SB (online Appendix Figure, available from www.cdc.gov/EID/content/13/10/1556-appG.htm; Table). All DNA biopsy samples were negative by SB with the probes specific for *B. garinii* and *B. afzelii*. We were able to amplify the *OspA* gene for only 1 case of erythema migrans, by using PCR and SB tests (data not shown).

The PCR products of the *fla* gene from 3 patients and of the *ospA* gene from 1 patient were sequenced by using a commercial kit (GenomeLab DTCS-Quick Start Kit, Beckman Coulter, Inc., Fullerton, CA, USA) with the sequencer from Beckman Coulter, Inc., according to manufacturer's instructions. We used the DNAMAN program (Lynnon Corporation, Vaudreuil-Dorion, Quebec, Canada) to align the sequences with the reported sequences of the *B. burgdorferi* sensu stricto B31 strain (online Appendix Figure). For the 2 erythema migrans cases, we found 3 base substitutions (online Appendix Figure, panel C), 1 of which was not conserved, leading to a change in amino acid (G for R in the 75 aa); these 2 sequences had 99% homology with the sequence of *B. burgdorferi fla* gene of isolate B31 (BLAST program) (*11*). For the lymphocytoma case, we found 2 base substitutions, the same as those of the erythema migrans cases, including the nonconserved base substitution (online Appendix Figure, panel C).

Regarding the *ospA* gene in the erythema migrans case, the sequence showed 1 base substitution that was not conserved, leading to a change in the amino acid 5 (L for I). The sequence of this case had 99% homology with the plasmid Ip54 gene of B31strain sequence (*11*) (online Appendix Figure, panel D).

The 3 adult patients received doxycycline 200 mg/day for 3 weeks; the child received amoxicillin 50 mg/kg a day for 3 weeks. For all patients, lesions were gone at the end of the treatment and had not recurred 3 years later.

Conclusions

Erythema migrans is the diagnostic marker for Lyme disease associated with *B. burgdorferi* infection (*5*,*6*). Histologic data from our 2 erythema migrans cases agreed with data reported for other erythema migrans cases (*5*). Moreover, the 2 erythema migrans cases were positive for *B. burgdorferi* sensu stricto *fla* gene and 1 for *ospA* gene; the 3 cases had a high degree of homology to the sequences of strain B31. In addition, 1 case met CDC criteria for seropositivity to *B. burgdorferi* infection (*5*).

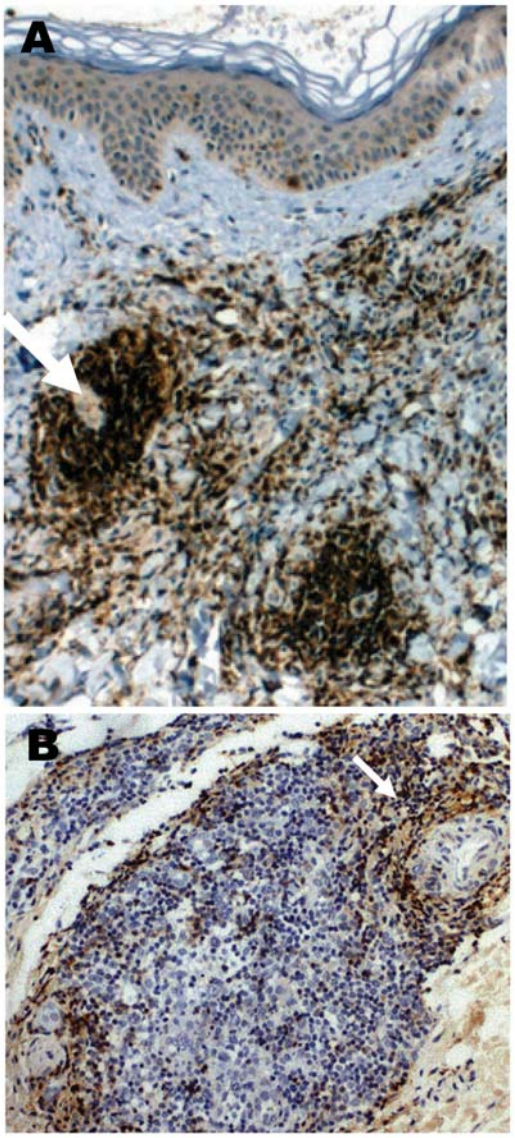

Figure 2. A case of lymphocytoma. A) Immunohistochemical image with anti-CD20 antibody showing a nodule with a dense B-lymphocytes infiltrate in the dermis (arrow); magnification ×100. B) Immunohistochemical image with anti-CD45 Ro antibody showing T-lymphocytes at the periphery of a nodule (arrow); magnification ×250.

Borrelial lymphocytoma is a rare clinical entity reported mostly in Europe (*12–14*) and sporadically in the United States (*15*). In this study, histologic and immunohistochemical data from the 2 lymphocytoma cases agreed with data from previous cases. These results were not specific enough to be considered diagnostic; however, germinal centers are present in 80% of borrelial lymphocytoma cases (*12*). Serum samples from 2 patients were positive by WB, which fulfills CDC criteria (*5*). In 1 case, *fla* gene was amplified and sequenced, showing high homology with the *fla* gene from *B. burgdorferi* sensu stricto strain B31 (*11*). Few reports describe genotyping of *B. burgdorferi* species in borrelial lymphocytoma. In Slovenia, *B. afzelii* and *B. bissettii* were identified (*13*); in Germany, *B. garinii* was identified (*14*). In our lymphocytoma patients, we identified *B. burgdorferi* sensu stricto. That the 2 borrelial lymphocytoma cases occurred in patients who had visited the same national park suggests that *B. burgdorferi* is endemic to that area.

This study documents *B. burgdorferi* infection in Mexican patients. Relevant epidemiologic data are 1) cases occurred after visiting forest areas, 2) patients reported having been bitten by a nonflying insect, 3) cases occurred during the summer-fall season, 4) no patient reported having traveled to another country, and 5) all skin lesions resolved after treatment with an antimicrobial drug. Our results suggest that *B. burgdorferi* infection occurs in Mexico and that continuous surveillance for Lyme disease in Mexico should be mandatory.

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Using Death Certificate Reports to Find Severe Leptospirosis Cases, Brazil

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Severe leptospirosis with pulmonary hemorrhage is emerging globally. Measures to control leptospirosis through sanitation depend on accurate case finding and reporting. Rapid death certificate reporting, plus necropsy of persons who died of leptospirosis, facilitates public health intervention and could provide an important tool in assessing the global burden of leptospirosis.

Although pulmonary involvement has been well recognized as a major component of leptospirosis (*1*), worldwide attention to severe pulmonary hemorrhagic syndrome (SPHS) emerged after an outbreak in rural Nicaragua in 1995 in which most cases lacked jaundice and renal failure, the classic manifestations of severe leptospirosis (*2*). A report from Peru, where leptospirosis is endemic, pointed out that SPHS can complicate human leptospirosis without jaundice or renal failure (a condition known as Weil syndrome) (*3*). Although not well recognized globally in general clinical practice, SPHS is considered a major clinical problem in some leptospirosis-endemic regions, ranging from the Andaman and Nicobar Islands (*4*) to urban coastal Brazil (*5*).

Epidemic leptospirosis most commonly occurs after flooding in densely populated centers in developing countries, and it continues to be an important clinical problem in urban coastal Brazil. São Paulo is the most populous Brazilian state (population ≈40 million). The annual reported incidence of leptospirosis from 1969 through 1996 was 0.53–1.13 cases per 100,000 inhabitants (*6*). In the São Paulo metropolitan area (population 10.5 million), which accounts for 68% of all reported cases, the annual incidence of leptospirosis was 1.9–3.7 cases per 100,000 inhabitants in the period 1998–2004. During the same period, case-fatality rates varied from 11.0% to 18.0% (Health Municipality Secretariat of São Paulo, unpub. data). In the São Paulo urban setting, SPHS is a common feature of leptospirosis. A case series of São Paulo patients with leptospiral SPHS found that 23 (55%) of 42 patients died from this disease from 1994 through 1997 (*7*).

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The Study

In 2004, PRO-AIM (Program for Improvement of Death Cause Information in São Paulo, abbreviated from the Portuguese name) was initiated in the São Paulo metropolitan area to provide better reporting of causes of death. Leptospirosis is a major infectious disease in São Paulo, and we started active surveillance for cases of acute renal failure and jaundice, pulmonary hemorrhage, or a combination of both. Detailed questionnaires on clinical and epidemiologic features were completed, and necropsies were performed to improve identification of fatal leptospirosis cases in São Paulo.

The central component of this program is a death certificate system. Fatal cases of leptospirosis are a component of this registry as a major category. Death certificates with any clinical features suggestive of leptospirosis or isolated SPHS are referred to the Municipal Health Secretariat of São Paulo. The Secretariat analyzes and attempts to confirm such cases. Confirmation protocols include laboratory analysis, clinical and epidemiologic assessment, and verification by necropsy of case-patients who had pathologic features characteristic of leptospirosis in lungs, kidneys, and liver. If fatal leptospirosis is confirmed within 72 hours, public health authorities can be alerted. In a setting of high leptospirosis transmission, this step may forestall expansion of an epidemic. Furthermore, the Brazilian experience, as well as that of other countries (*1*), includes a high rate of misdiagnosis of leptospirosis and other clinically indistinguishable acute febrile illnesses such as dengue or scrub typhus fever. Thus, rapid identification and confirmation of fatal leptospirosis are important tools for surveillance, public health interventions, and alerting clinicians.

We describe our initial experience of actively seeking, identifying, and reporting fatal cases of leptospirosis. Using data from January 1, 2004, through August 31, 2006, we used a cross-sectional approach to analyze death certificate data from the São Paulo metropolitan area. Fatal cases were confirmed in the laboratory by serologic or immunohistochemical examination, supplemented by epidemiologic and clinicopathologic evidence. Pathologic criteria were an important adjunct to confirm cases because ≈30% of deaths of patients hospitalized for leptospirosis occur within 24 hours, which may preclude serologic diagnosis because of delayed antibody responses. Pathologic examination can identify constellations of known complications as well as unexpected features. In fulminant infection, histopatho-

logic findings confirm involvement of typical targeted organs such as acute tubular necrosis or interstitial nephritis, acute loss of cohesion of hepatocytes, and pulmonary hemorrhage, which is important because, in leptospirosis-endemic regions, potential leptospirosis patients may have had previous infection and thus preexisting antileptospiral antibodies (*8*). We emphasize, however, that pathologic criteria are only confirmatory if clinical and epidemiologic criteria for infection are fulfilled. In the experience reported here, 5 cases (which were included in the laboratory confirmation group) were confirmed by immunohistochemical examination. Leptospiral antigen detection in postmortem samples is important for confirming the diagnosis (*9*) but is limited by tissue deterioration if there is a prolonged period between death and necropsy.

In 2004, 42 (15%) of 285 cases of reported leptospirosis cases were fatal; in 2005, 28 (11%) of 262 cases were fatal; and from January 1 through June 2006, 31 (19%) of 167 cases were fatal. The Table shows the distribution of fatal cases from January 2004 through August 2006. Of 101 fatal cases, 62 were confirmed by a combination of serologic and immunohistochemical testing; 15 cases were suggested on the basis of clinical, pathologic, and epidemiologic findings; and 24 were suspected on the basis of strong circumstantial clinical and epidemiologic evidence.

Conclusions

Isolation of leptospires in culture from clinical specimens is the standard for diagnosis but faces logistical obstacles in real-world settings. In our study, only 6 blood samples were collected for culture, and they were uninterpretable because of contamination. Although not definitive for identifying infecting leptospiral serovars, microscopic agglutination test titers suggested that the most frequently reacting serogroups in São Paulo are Icterohemorrhagiae (72%) and Autumnalis (14%).

Of the 101 fatal cases, necropsies were performed for 42. Of these 42 necropsies, 27 cases were confirmed by a combination of necropsy and positive serologic test results. Fifteen fatal cases were suggested by a combination of necropsy findings plus clinical and epidemiologic evidence.

Of all fatal cases confirmed by necropsy, Weil syndrome with concomitant pulmonary hemorrhage was documented in 86% of cases in 2004, in 67% in 2005, and in 69% in 2006. Less common manifestations included Weil syndrome without pulmonary hemorrhage, and isolated pulmonary hemorrhage (Table).

The frequency of clinical manifestations of severe pulmonary disease (with or without hemorrhages) was 76% among fatal cases and 26% among nonfatal cases. Importantly, 46% of all patients with severe pulmonary symptoms died, a finding that is consistent with the literature (*1*,*8*).

The system of active death notification we describe can be an important tool to evaluate the emerging complications of leptospirosis and the global extent of disease due to leptospirosis. Nonetheless, this, as well as any system of active death certification notification, has several limitations. A major problem is increasing the number of necropsies, which may be limited by logistics or cultural norms. Increasing the necropsy rate can be aided by prompt response through the death certificate notification system and involvement of public health authorities. Another problem is the lack of serologic and cultural confirmation. Molecular diagnosis would be ideal and could readily be introduced into this system.

The incidence of SPHS seems to have changed elsewhere, e.g., in Salvador, another urban area of Brazil, where the Copenhageni serovar predominates. Pulmonary hemorrhage seems to have newly emerged since the year 2000 (A. I. Ko, pers. comm.). An active surveillance, death certificate–based reporting system represents an important tool that should provide further insights into the natural history and changing clinical manifestations of leptospirosis infection in diverse geographic regions in which the disease is endemic and epidemic. Systematic reporting focused on the most severe cases of leptospirosis has the potential for providing a data-driven basis for ministry-level policy development, institution of public health control measures, and quantitative assessment of the global severity of leptospirosis.

Table. Data from death notification reports in the São Paulo, Brazil, metropolitan area, January 2004–August 2006

Data from reports	2004	2005	2006
Total no. reported leptospirosis cases	285	262	167
Annual incidence per 100,000 inhabitants	2.7	2.4	1.7
Fatality rate, %	15	11	18
Total no. deaths	42	28	31
Fatal cases			
Laboratory confirmed, no. (%)	28 (67)	17 (60)	17 (55)
Clinical and epidemiologically assessed, no. (%)	14 (33)	11 (40)	14 (45)
Total no. necropsies (%)	14/42 (33)	15/28 (53)	13/31 (41)
Frequency of concurrent Weil syndrome and pulmonary hemorrhage, no. (%)	12/14 (86)	10/15 (67)	9/13 (69)
Frequency of Weil syndrome without pulmonary hemorrhage, no. (%)	1/14 (7)	4/15 (27)	3/13 (23)
Frequency of pulmonary hemorrhage, no. (%)	1/14 (7)	1/15 (6)	1/13 (8)

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Duration of Antibody Responses after Severe Acute Respiratory Syndrome

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Among 176 patients who had had severe acute respiratory syndrome (SARS), SARS-specific antibodies were maintained for an average of 2 years, and significant reduction of immunoglobulin G–positive percentage and titers occurred in the third year. Thus, SARS patients might be susceptible to reinfection ≥3 years after initial exposure.

Severe acute respiratory syndrome (SARS) represents the first pandemic transmissible disease to emerge in this century. It was caused by a previously unknown coronavirus, the SARS-associated coronavirus (SARS-CoV) (*1*). SARS-CoV spreads from animals to humans by a rapid adaptation and evolution process (*2*,*3*). A large number of closely related viruses are present in wildlife reservoir populations (*4*–*6*). Therefore, due to cross-species transmission of the same or a similar coronavirus, SARS could recur. Immune protection against infection with other human coronaviruses, such as OC43 and 229E, is short-lived (*7*). To assess SARS patients' risk for future reinfection, we conducted a longitudinal study of immunity in convalescent patients.

The Study

Shanxi Province in China was 1 of the SARS epicenters during the 2002–03 outbreaks. For our study, serum samples were taken from patients in 7 designated SARS hospitals in the province during March–August 2003. Follow-up serum samples were taken at 6 months, 1, 2, and 3 years after the onset of symptoms. A total of 176 cases that met the World Health Organization (WHO) SARS case definition (*8*) and had known transmission history were included in this study. The study was conducted as part of a national SARS control and prevention program; use of serum from human participants was approved by the Committee for SARS Control and Prevention, Department of Science and Technology, the People's Republic of China.

Titers of serum antibodies to SARS-CoV were determined by using a commercially available ELISA kit (BJI-GBI Biotechnology, Beijing, China). The ELISA was based on an inactivated preparation of whole-virus lysate. The kit was the first commercial kit approved by the Chinese Food and Drug Administration for specific detection of SARS-CoV antibodies and has been widely used in several studies (*9*–*11*). Manufacturer's instructions were followed without modification. Briefly, for every ELISA plate, 1 blank, 1 positive, and 2 negative controls were included. For detection of immunoglobulin G (IgG), a 1:10 dilution of testing serum (100 μL) was added to antigen-coated wells, and the plate was incubated at 37°C for 30 min. Horseradish peroxide (HRP)–conjugated antihuman IgG (100 μL) was then added for detection of bound antibodies. For detection of IgM, the incubation of primary antibodies was extended to 60 min, followed by detection with HRP-conjugated antihuman IgM. Optical density (OD) readings were deemed valid only when the negative control OD was ≤0.10 and the positive control was ≥0.50 on the same ELISA plate. The cutoff for IgG and IgM determination was defined as 0.13 and 0.11, respectively, plus OD of the negative control. When the OD of the negative control was <0.05, 0.05 was used for the calculation. In this study, the OD readings of negative controls from different testing were consistently <0.05, so the cutoff ODs for IgG and IgM were 0.18 and 0.16, respectively. Serum samples that had an OD greater than or equal to the cutoff value were considered positive. Weak positive samples (i.e., OD<2× cutoff value) were retested in duplicate on the same day; only reproducible positive results were included in the final analysis. All data were processed by using Excel version 7.0 (Microsoft Corp., Redmond, WA, USA) and SPSS software version 10 for Windows (SPSS Inc., Chicago, IL, USA).

Among the cohort, 163 (92.61%) of 176 ($\chi^2 = 200.11$, $p = 0.000002$) were IgG positive, which indicated that most patients who met the WHO case definition were indeed infected with SARS-CoV. As shown in the Table, at ≈7 days after the onset of symptoms, the percentage who were IgG positive was ≈11.80%. This percentage continued to increase, reached 100% at 90 days, and remained largely unchanged up to 200 days. Furthermore, after 1 and 2 years 93.88% and 89.58% of patients, respectively, were IgG positive, which suggests that the immune responses were maintained in ≥90% of patients for 2 years. However, 3 years later, ≈50% of the convalescent population had no SARS-CoV–specific IgG. The OD changes correlated with the changes to the IgG-positive percentage, although the

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Table. Cumulative rates of SARS-CoV antibodies among 176 SARS patients with known transmission histories*

Time after symptom onset, d	IgG			IgM†		
	No. samples tested	No. positive samples (%)	Average OD	No. samples tested	No. positive samples (%)	Average OD
0–7	17	2 (11.76)	0.046	14	3 (21.43)	0.136
8–14	26	10 (38.46)	0.190	22	14 (63.64)	0.312
15–20	22	17 (77.27)	0.351	19	12 (63.16)	0.477
21–30	36	33 (91.67)	0.493	21	16 (76.19)	0.560
31–60	72	67 (93.06)	0.627	22	14 (63.64)	0.320
61–90	35	33 (94.29)	0.745	15	5 (33.33)	0.167
91–120	11	11 (100.00)	0.965	ND	ND	ND
121–210	23	23 (100.00)	0.932	ND	ND	ND
211–365	49	46 (93.88)	0.734	ND	ND	ND
366–763	96	86 (89.58)	0.535	ND	ND	ND
764–1,265	28	15 (53.57)	0.250	ND	ND	ND

*SARS-CoV, severe acute respiratory syndrome–associated coronavirus; Ig, immunoglobulin; OD, optical density; ND, not determined because for most samples the IgM readings already reached background level on day 90.
†For some patients, we did not have enough serum to test for IgM after testing for IgG; hence, a smaller number of serum samples were tested for IgM than for IgG.

rate of change varied. Both the OD readings (0.93) and positive percentages peaked at 90–120 days; however, the rate of reduction of the average OD readings was much faster, dropping by 22% (0.73) and 40% (0.54) at 1 and 2 years, respectively, after symptom onset (Figure 1).

A similar observation was obtained for IgM trends in this same cohort. The percentage of patients who were IgM positive within the first 7 days was 21.4% and peaked at 76.2% after 21–30 days (Table). The patterns of IgM-positive percentage and average OD readings were similar; both peaked at 21–30 days. After 60 days, the average OD readings dropped to 0.167, close to the cutoff value of 0.160.

Among the cohort of patients with known transmission histories, we were able to obtain a complete collection of serum samples from 18 patients at 6 months, 1, 2, and 3 years. The IgG levels of these 18 patients were analyzed separately to obtain an IgG trend that more accurately represented convalescent SARS patients (Figure 2). All 18 patients had positive IgG at 6 months and at 1 year (i.e., 100% positive); only 1 patient became IgG negative at 2 years. However, at 3 years, the positive percentage dropped to 55.56%. The reduction of OD values mimicked that of the positive percentage, again at a faster rate. The average OD readings dropped from 0.94 at 6 months to 0.64 at 1 year, which represents a reduction of 33.33%. The OD further dropped to 0.52 (45.83% reduction) by 2 years and to 0.25 by 3 years.

Conclusions

To our knowledge, the 3-year follow-up conducted in this study is the longest longitudinal study ever reported. With a large number of patients who had confirmed transmission history (176) and a complete dataset for 18, the level of confidence is high that the results obtained in this study are representative for convalescent SARS patients. Similar results have been reported from longitudinal studies of SARS patients with smaller cohort size (18–98 patients) and shorter follow-up period (240 days to 2 years) (*9*–*14*). The general trend of IgM peaking at ≈1 month after symptom onset and IgG peaking at 2–4 months was consistent among different studies.

Our results provide strong evidence that SARS-CoV antibodies are reduced ≥3 years after the symptom onset. Because antibodies play an important role in protective

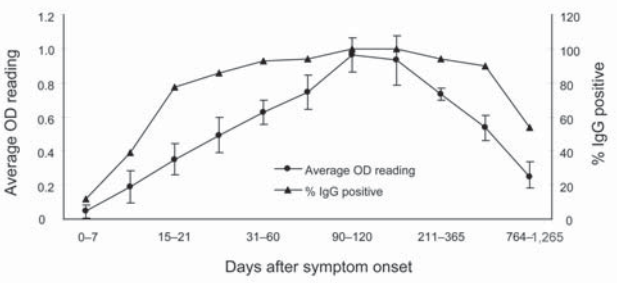

Figure 1. Change of immunoglobulin G (IgG) patterns among 176 convalescent severe acute respiratory syndrome patients with known transmission history. See the Table for number of samples used for the calculation at each time point. OD, optical density.

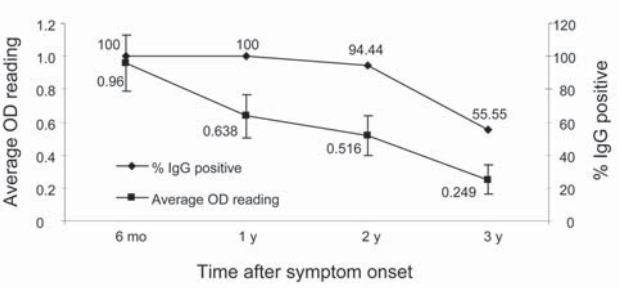

Figure 2. Change of immunoglobulin G (IgG) patterns among 18 convalescent severe acute respiratory syndrome patients with a complete collection of sequential serum samples at the time points shown. The 18 patients were selected from the cohort of 176 patients for whom transmission history was known. OD, optical density.

immunity against SARS-CoV (*15*), the findings from this study will have important implications with regard to assessing risk for reinfection among previously exposed populations (e.g., hospital staff) and evaluating the duration of antibody-mediated immunity that any candidate vaccine could provide.

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Isolation of *Bartonella* sp. from Sheep Blood

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A *Bartonella* sp. was isolated from sheep blood. Bacterial identification was conducted by using electron microscopy and DNA sequencing of the 16S rRNA, citrate synthase, riboflavin synthase, and RNAase P genes. To our knowledge, this is the first report of ovine *Bartonella* infection.

Bartonella spp. are potential zoonotic pathogens that frequently cause bacteremia without overt disease in reservoir hosts (*1*). Several new *Bartonella* spp. were found in wild and domestic ruminants from Europe and North America (*2–4*), but previous blood cultures performed on 150 domestic sheep and 84 bighorn sheep failed to isolate *Bartonella* spp. (*2*,*5*).

The Study

We isolated a *Bartonella* sp. from 2 successive lots of commercial defibrinated sheep blood received from mid-February to mid-March 2007, from a US supplier. Bottles (10 from lot 1 and 6 from lot 2), each containing 100 mL, were newly opened on the day of receipt. Approximately 0.2 mL was aseptically removed and spotted onto the surface of a Columbia agar (BBL; Becton Dickinson, Sparks, MD, USA) plate containing 5% defibrinated sheep blood. The remaining blood was stored at 4°C. Plates were incubated at 35°C in an atmosphere of 7% CO_2 and examined daily for bacterial growth. Blood appeared sterile after 7 days; however, by 14–21 days, pinpoint bacterial colonies were recognized in the blood film. After 3–4 weeks, mature colonies (Figure 1, panel A) were rough, off-white, difficult to disperse but nonadherent to the agar surface, and ≈1 mm in diameter. Monomorphic colonies grew in all samples within each lot. Estimated concentrations in the starting pools of blood from lots 1 and 2 were 750 CFU/mL and 25 CFU/mL, respectively.

The cells were small, gram-negative rods, 0.47–0.60 μm in diameter, and 0.8–1.9 μm in length (Figure 1, panel B). Flagella were not observed. Growth was not detected after transfer of colonies to Columbia blood agar and several other available diagnostic media that contained blood products (e.g., chocolate agar, Centers for Disease Control and Prevention anaerobe agar, Bordet-Gengou agar, Mycoplasma agar, and hemin-supplemented thioglycolate medium). Colony transfer to Columbia blood agar plates that were first overlaid with sheep blood (from a presumed uninfected lot) resulted in only 2 or 3 colonies. Repeat cultures from lot 1 showed a 97% reduction in colony numbers after 37 days of storage and no growth in samples after 72 days of storage.

PCR was performed as described (*6–8*) by using template DNA obtained from a representative colony from each lot. PCR products from the lot 1 isolate (SB1) and lot 2 isolate (SB 2) had identical DNA sequences (sequencing performed at the University of Tennessee Core DNA Sequencing Facility). Phylogenetic trees of *Bartonella* spp. based on individual 16S rRNA, citrate synthase (*gltC*) and riboflavin synthase (*ribC*) sequence alignments showed greatest similarity to *Bartonella melophagi* (Figure 2).

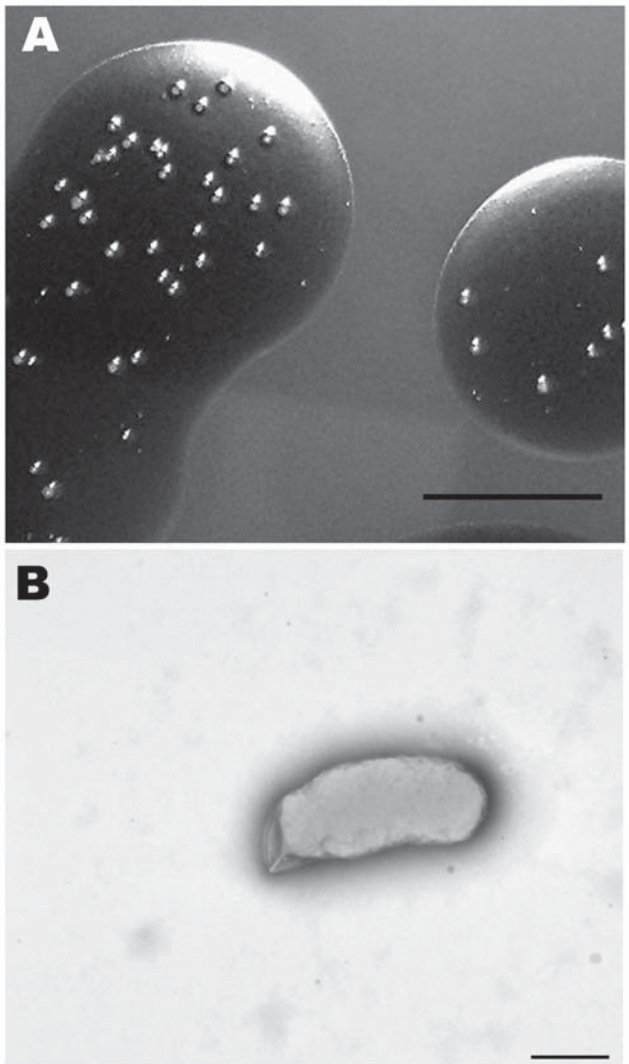

Figure 1. Morphologic analysis of a *Bartonella sp*. isolated from sheep blood. A) Colonies growing in sheep blood surface biofilm seen in reflected light after 25 days. Scale bar = 10 mm. B) Transmission electron micrograph of a representative cell that was dispersed from a 25-day-old colony and negatively stained with 0.5% potassium phosphotungstic acid. Scale bar = 500 nm.

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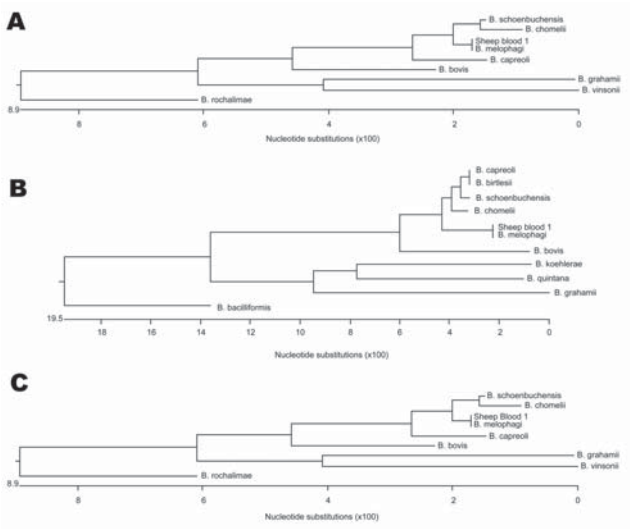

Figure 2. Phylogenetic trees (DNAstar ClustalW Slow and Accurate; DNASTAR Inc., Madison, WI, USA) of 3 *Bartonella* genes. A) Citrate synthase. B) Riboflavin synthase. C) 16S rRNA. Sheep blood 1 is compared with species showing the highest homology for each gene.

Initial comparison of the DNA sequence with double-strand agreement from the 16S rRNA gene most closely matched (1,283/1,286 bp [99%]) that of *Wolbachia melophagi* (GenBank accession no. X89110). However, a shorter sequence (1,212 bp) from the SB1 isolate aligned closely (99.8%) with both *W. melophagi* and *B. melophagi* (GenBank accession nos. X89110 and AY724770). The *gltC* gene sequences were consistent with those of *B. melophagi* (GenBank accession nos. AY692475, AY724769, and AY724768). The matches of both DNA strands were 275/275 bp (100%) with each strain. The DNA sequence with double-strand agreement from the *ribC* gene also matched (473/473bp [100%]) that of *B. melophagi* (GenBank accession no. EF605287). The RNAase P gene (*rnpB*) sequence had more distant matching (95.6%) with sequences of *B. weissi* (GenBank accession no. AF376050) and *B. sp*. Deer 159/660/1 (95.7%) (GenBank accession no. AF376051). DNA sequences from the *B. melophagi rnpB* gene were not available in GenBank for comparison. The DNA sequences determined in this study have been assigned the following GenBank accession nos.: 16S rRNA (EF689897), citrate synthase (EU020109), riboflavin synthase (EU020110), and RNAase P (EU020111).

Conclusions

The source of *Bartonella* sp. was likely intrinsic contamination from bacteremia in donor sheep. Blood was obtained from multiple live sheep with sterile, closed blood collection systems and from venipuncture sites that were prepared by shearing and treatment with antiseptics. Each 5-L lot (1 L/sheep) was pooled and prepared for sale in a separate, clean, well-equipped laboratory facility. Histories of sheep were not determined. Young age (*9*) and contact with wildlife (*2*) or cross-species vectors (*5*) may increase the risk for *Bartonella* infection in sheep.

Arthropod vectors often transmit *Bartonella* infections. *Melophagus ovinus*, commonly called a sheep ked, is a hemophagous ectoparasite of sheep (*5*). The organism from which DNA sequence of a 16S rRNA gene was isolated was an uncultured bacterial endosymbiont of sheep keds initially called *W. melophagi*. However, taxonomists now agree that the organism from which the original sequence came should be removed from the genus *Wolbachia* and placed in the genus *Bartonella* (*5*). On the basis of DNA sequence data, candidate status was proposed for the new species *B. melophagi* (M. Vayssier-Taussat, L. Halos, H.-J. Bouluis, unpub. data, available from www.ncbi.nlm.gov/taxonomy/browser/wwwtaxcgi?id = 291176). An organism with DNA sequence matching that of *B. melophagi* was recently isolated from a sheep ked (M.Y. Kosoy, K.W. Sheff, A.I. Irkhin, unpub. data, available from www.ncbi.nlm.gov/taxonomy/browser/wwwtax.cgi?id = 291176).

Sheep blood is often used in the laboratory with the expectation that it is free of bacteria. However, routine animal health surveillance and quality control procedures may fail to detect *Bartonella* spp. Optimal growth conditions for this organism are unknown. In this study, growth was only observed in fresh sheep blood. We were unable to obtain sufficient growth after in vitro passage for further phenotypic characterization. A novel liquid culture medium that supported growth of *Bartonella* spp. also used fresh, defibrinated sheep blood as a growth supplement (*10*). High-level *Bartonella* bacteremia may be transient, and the sensitivity of PCR for detection in pooled blood or individual sheep has not been established. PCR assays performed on lots 1 and 2 after 1 month of storage did not detect *Bartonella* spp. PCR was not performed at the time of blood collection. Risks associated with *Bartonella* infection in sheep are unknown. Precautions to reduce potential transmission of *Bartonella* are advised when handling sheep blood.

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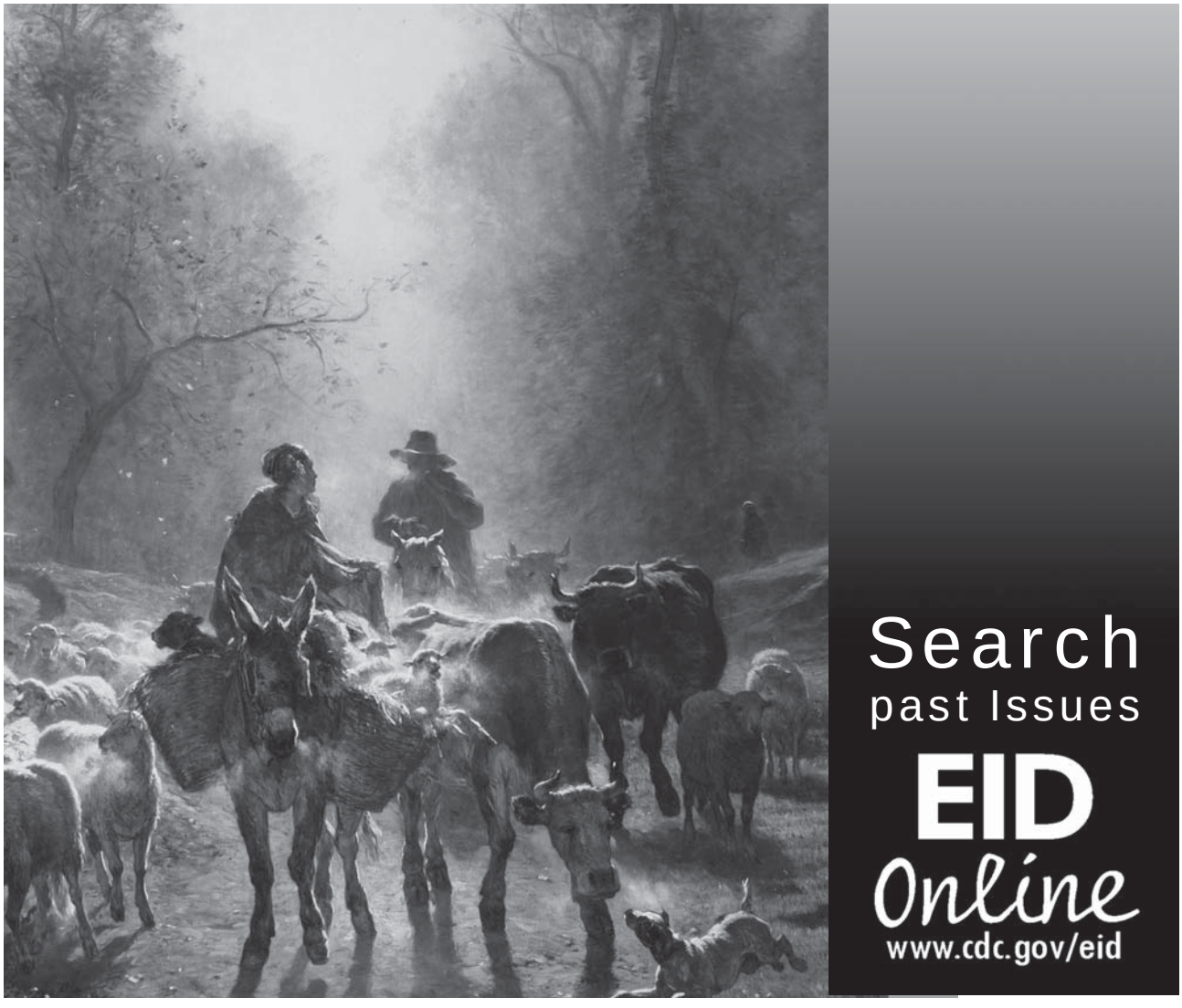

Chlorine Inactivation of Highly Pathogenic Avian Influenza Virus (H5N1)

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To determine resistance of highly pathogenic avian influenza (H5N1) virus to chlorination, we exposed allantoic fluid containing 2 virus strains to chlorinated buffer at pH 7 and 8, at 5°C. Free chlorine concentrations typically used in drinking water treatment are sufficient to inactivate the virus by >3 orders of magnitude.

Growing concerns about the public health threat posed by highly pathogenic avian influenza (HPAI) subtype H5N1 has prompted interest in evaluating environmental control measures for this virus. The World Health Organization has noted that more information is needed on the effectiveness of inactivation of subtype H5N1 in water (*1*). Since 2002, HPAI (H5N1) has been reportedly isolated from >50 different wild avian species, mainly aquatic birds in the order Anseriformes (*2*). Experimentally infected waterfowl shed moderate to large quantities of the virus in their feces and respiratory secretions (*3*,*4*). HPAI viruses can persist in simulated water environments, although generally for shorter periods than low pathogenic avian influenza viruses (*5*,*6*). Open bodies of water, including drinking water reservoirs, can become contaminated by birds that are actively shedding virus or by waterfowl carcasses. Surface runoff also represents a potential source of contamination for groundwater. In terms of avian health, drinking water has been implicated in the transmission of avian influenza among domestic poultry (*6*–*8*).

Chlorination represents the most common form of disinfection used in water treatment. Most published reports on virus inactivation in water have dealt with enteric viruses, and government guidelines for water treatment have focused on this group. Despite general acceptance that the outer lipid envelope associated with influenza viruses would make them susceptible to chlorination, no published reports specifically address the effect of chlorine on the H5N1 subtype of avian influenza.

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The Study

Two clade 2 strains of HPAI (H5N1) virus were used in this study (*9*): 1 isolated from domestic poultry, A/chicken/Hong Kong/D-0947/2006 (courtesy of K. Dytring; Agriculture, Fisheries and Conservation Department, Hong Kong Special Administrative Region of China) (*10*), and 1 from a wild swan, A/WhooperSwan/Mongolia/244/2005 (*3*). The infectious virus was propagated in embryonated eggs of specific pathogen-free (SPF) leghorn chickens (*11*), and infective amnioallantoic fluid was harvested 96 h after inoculation.

Inactivation experiments were conducted as previously described (*12*). The initial chlorine level was chosen to achieve a chlorine residual that would be typical of drinking water after satisfying the initial chlorine demand of the amnioallantoic fluid. Briefly, virus-infected allantoic fluid was diluted (1:1,000) into continuously stirred, chlorinated, chlorine demand–free phosphate buffer (0.05 M, pH 7.0 and 8.0). Chlorine measurements were made immediately before the chlorine was neutralized by the addition of 0.1 mL of sodium thiosulfate (10% w/v). Separate reaction vessels were used for each exposure time. Reaction vessels containing only the virus and buffer without chlorine served as controls for determination of virus titers in the absence of chlorine and were assayed at the end of the longest exposure time period (60 s). Negative buffer controls without virus or chlorine were also included. All test and control samples were treated in the same manner. Preliminary investigations indicated that the virus can be readily inactivated at room temperature (data not shown). To slow the rate of inactivation, experiments were conducted at 5°C.

The infectivity of the samples was quantified by using microtiter endpoint titration (*6*), and virus titers were expressed as median 50% tissue culture infectious dose $(TCID)_{50}$/mL (*13*). Primary cultures of chicken embryo fibroblasts prepared from 9- to 11-day-old SPF chicken embryos were used in these assays. Virus-infected cells were incubated at 37°C under 5% CO_2 for 96 h and examined by light microscopy for cytopathic effect (CPE). Culture plates were stained with 1% (w/v) crystal violet in 10% (v/v) neutral-buffered formalin for further examination. Failure to produce CPE indicated that the virus was not capable of infecting the cells. The neutralized buffer control without virus did not cause CPE. All experiments were conducted in duplicate under Biosafety Level 3 agricultural conditions.

Inactivation levels were determined by comparing the $\log_{10}$ transformed $TCID_{50}$/mL virus titers in the control samples with the titers in the chlorine-exposed samples. The lowest detectable virus titer was 2.17 $\log_{10}$ $TCID_{50}$/mL, independent of pH or virus strain. *Ct* values (the chlorine concentration, *C* [mg/L], multiplied by the exposure time,

Table 1. *Ct* values (mg-min/L) for inactivation of HPAI (H5N1) virus by free chlorine at 5ºC*

Strain	pH	Log_{10} inactivation		
		1.0	2.0	3.0
Hong Kong†	7	0.14	0.27	0.41
	8	0.26	0.53	0.79
Mongolia‡	7	0.13	0.26	0.39
	8	0.23	0.46	0.68

*Ct, $0.5(C_0 + C_{0.5})/2 + C_{0.5}$ [1–exp (–k (t–0.5))]/k, where C_0 = chlorine concentration at time zero (mg/L); $C_{0.5}$, chlorine concentration at 0.5 min (mg/L); k, exponential chlorine decay rate; t = time (min). HPAI, highly pathogenic avian influenza.
†A/chicken/Hong Kong/D-0947/2006.
‡A/WhooperSwan/Mongolia/244/2005.

t [min]) were used to determine the rate of inactivation for the 2 pH levels. *Ct* values are commonly used to make disinfection recommendations for water treatment and provide a means for comparing biocidal activity for various microorganisms (*14*). *Ct* values were plotted against log_{10} virus titers to determine *Ct* values for a given level of inactivation (Table 1).

The results of the chlorination experiments (Table 2) represent the means of duplicate experiments differing by <0.10 mg/L of free available chlorine. Initial titers of all virus preparations yielded $log_{10}TCID_{50}$/mL values ≥5.26, which enabled *Ct* calculations for inactivation over several orders of magnitude. The A/chicken/Hong Kong/D-0947/2006 strain preparations exhibited a slightly higher chlorine demand, ≈1.5 mg/L after 1 min, compared with 1.0 mg/L for the A/WhooperSwan/Mongolia/244/2005 strain during the same time interval. As anticipated, inactivation was slower at pH 8.0 than at pH 7.0. Table 1 lists the mean *Ct* values (mg-min/L) required to achieve 1, 2, and 3 orders of magnitude inactivation for both strains at the 2 pH levels. Covariance analysis of the decay coefficients indicated no significant difference in the inactivation of the 2 virus strains at pH 8.0 ($p = 0.10$). Rapid inactivation at pH 7.0 did not allow for statistical evaluation.

Table 2. Inactivation of HPAI (H5N1) virus by free chlorine at 5°C*

Strain	pH	Time, s	Free chlorine, mg/L	Virus titer log_{10} $TCID_{50}$/mL	Log_{10} reduction
Hong Kong†	7	0	2.08	5.32	NA
		15	ND	<2.17	>3.15
		30	0.65	<2.17	>3.15
		60	0.52	<2.17	>3.15
	8	0	2.08	5.70	NA
		15	ND	3.88	1.82
		30	0.76	2.67	3.03
		60	0.59	<2.17	>3.53
Mongolia‡	7	0	1.86	5.26	NA
		15	ND	<2.17	>3.09
		30	0.85	<2.17	>3.09
		60	0.77	<2.17	>3.09
	8	0	2.04	5.53	NA
		15	ND	3.39	2.14
		30	1.10	<2.17	>3.36
		60	1.08	<2.17	>3.36

*HPAI, highly pathogenic avian influenza; $TCID_{50}$, median 50% tissue culture infectious dose; NA, not applicable; ND, not determined.
†A/chicken/Hong Kong/D-0947/2006.
‡A/WhooperSwan/Mongolia/244/2005.

Conclusions

The results of this study confirm that avian influenza (H5N1) is readily inactivated by chlorination. Although the viral inoculum exerted a considerable initial chlorine demand, the maintenance of a free chlorine residual (0.52–1.08 mg/L) was sufficient to inactivate the virus by >3 orders of magnitude within an exposure time of 1 minute. Chlorine demand would also be anticipated when the virus is associated with fecal material. These findings indicate that the ability to compensate for an initial chlorine demand followed by exposure to a relatively low level of free chlorine for a short time is sufficient to inactivate the virus by chlorination. For drinking water disinfection at conditions similar to those used in this study, the US Environmental Protection Agency specifies free chlorine *Ct* values of 6 and 8 mg-min/L to achieve enteric virus inactivation of 3 and 4 orders of magnitude, respectively (*14*). According to our results, these *Ct* values would be more than sufficient to inactivate HPAI (H5N1) in the water environment. The information on chlorine disinfection presented here should be helpful for developing risk management procedures regarding the role of water in the transmission of the virus to humans and poultry.

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Predominance of Rotavirus P[4]G2 in a Vaccinated Population, Brazil

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We identified 21 rotaviruses in 129 patients with diarrhea in a Brazilian city with high rotavirus vaccine coverage. All rotaviruses were genotype P[4]G2 with 1 mixed infection with P[NT]G9. Although virus predominance could have occurred randomly, the vaccine may be less protective against P[4]G2. Prospective surveillance is urgently needed.

Rotavirus causes severe diarrhea, illness, and death worldwide (*1*). Infection rates with rotavirus remain high despite improved sanitation, and vaccination is likely to be the best control strategy (*2*,*3*). Several candidate vaccines are being developed, 2 are already licensed (*4*,*5*) and at least 5 are being evaluated (*2*,*6*). The 2 licensed rotavirus vaccines are designed to provide protection against rotavirus gastroenteritis caused by the most common worldwide circulating rotavirus serotypes (*2*). These include G types G1, G2, G3, and G4 and P types P[4] and P[8] (on the basis of variability in the outer capsid proteins VP7 and VP4, respectively).

One of the currently licensed vaccines (Rotarix; GlaxoSmithKline, Rixensart, Belgium), a live, attenuated, human monovalent rotavirus P[8]G1 vaccine, was highly efficacious for preventing severe rotavirus gastroenteritis in phase III efficacy studies in Latin America and Europe. These studies included Brazil, where the main circulating rotavirus genotypes were P[8]G1 and P[8]G9 (*5*). Brazil therefore took the unprecedented step of introducing this vaccine into its national Expanded Program for Immunization in March 2006 and provided 2 free doses to all children <3 months of age. Vaccination coverage among eligible age cohorts has increased and reached 51% in Sergipe (www.datasus.gov.br) in northeastern Brazil. However, this vaccine appears less effective in preventing severe rotavirus gastroenteritis caused by P[4]G2 strains (*5*), and immunologic pressure exerted by the vaccine may cause emergence of rotavirus genotypes that are not controlled by the vaccine. This possibility could change the pattern and distribution of the most prevalent rotavirus strains in the vaccinated population.

Because this is an unprecedented epidemiologic situation, we monitored the effect the vaccine might have on predominant genotypes. We describe rotavirus genotypes recovered from children with acute diarrhea in Aracaju, Sergipe, Brazil, after the widespread introduction of the vaccine.

The Study

Children with acute diarrhea who came to 2 public hospitals (Joao Alves Filho and Municipal da Zona Norte) and 3 health centers that provided health services to a population (Santa Maria) in Aracaju, Brazil, were enrolled from November 2006 to February 2007. Children who came to the hospitals were enrolled consecutively on specific days of the week by study health workers, and children who came to the health centers were visited at home after we checked the daily attendance lists of the centers. Acute diarrhea was defined as any episode <14 days duration with ≥3 watery stools per day. Background and clinical information were collected after obtaining parental consent, and stool samples were stored frozen in duplicate at –80°C until analyzed in Liverpool, UK. Information on rotavirus vaccination was obtained from parents and cross-checked against vaccination record cards. A child was considered vaccinated if 2 doses of the vaccine had been recorded on the vaccination card. Rotavirus detection, genotyping, electropherotyping, isolation of strains in cell culture, and sequencing were performed as described (*7*). Severity of diarrhea episodes was classified according to a modified Vesikari score (*8*). Data were analyzed by using descriptive statistics in Epi-Info 2002 (Centers for Disease Control and Prevention, Atlanta, GA, USA). The study protocol was reviewed and approved by the Ethics Committees of the Liverpool School of Tropical Medicine and the Federal University of Sergipe.

A total of 129 patients with a median age of 12 months (range 1 month–12 years) were enrolled. Of these, 63 (49%) were <1 year of age, 39 (30%) were 1–2 years of age, and 27 (21%) were >2 years of age. A total of 21 children (16%) were positive for rotavirus by ELISA. Of these children, 20 were identified among 89 children enrolled in the hospital and 1 was identified among 40 children enrolled in the health centers ($p = 0.002$). Forty-eight children (37%) had received the rotavirus vaccine. The frequency of rotavirus infection by vaccination status and age is shown in the Table. Among children <1 year of age, 3 (7%) of the 44 vaccinated children were infected with rotavirus compared with 5 (26%) of 19 children who did not receive the

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Table. Frequency of rotavirus infection by vaccination status and age, Aracaju, Sergipe, Brazil*

Age, y	Rotavirus	Vaccinated, no. (%)	Not vaccinated, no. (%)	p value
<1	Positive	3 (7)	5 (26)	0.05
	Negative	41 (93)	14 (74)	
1–2	Positive	1 (25)	8 (23)	NS
	Negative	3 (75)	27(77)	
>2	Positive	NA	4 (15)	
	Negative	NA	23 (85)	

*Children were considered vaccinated if they had received 2 doses of vaccine. NS, not significant; NA, not applicable.

vaccine ($p<0.05$). Among children 1–2 years of age, 4 had received the vaccine and 1 (25%) of them was infected with rotavirus compared with 8 (23%) of the 35 children who did not receive the vaccine (p not significant).

The median (range) diarrhea severity scores of children with and without rotavirus infection were 12.9 (10–15.8) and 9.4 (5.3–13.5), respectively ($p<0.001$). Although numbers are small, vaccinated children had a median (range) diarrhea severity scores of 12.5 (7–15) if they were infected with rotavirus and 7 (3–17) if they were not infected. Similarly, the median (range) severity scores for unvaccinated children were 13 (8–15) and 11 (4–14) for children with and without rotavirus infections, respectively (p not significant).

All 21 rotavirus infections were with genotype P[4]G2. One child had a mixed infection with P[4]G2 and P[NT]G9. Nineteen specimens had short electropherotype strains, 1 was positive but with an undefined pattern, and 1 had insufficient RNA to produce a pattern.

Conclusions

Sergipe has achieved relatively high rotavirus vaccine coverage (54%) since introduction of the vaccine in 2006, with 48,165 doses provided in Aracaju. The vaccine was well received by the local population, and as new eligible children continue to be vaccinated, it is likely that vaccination levels will reach the high coverage currently attained for oral polio vaccine (100%) (http://tabnet.datasus.gov.br/cgi/tabcgi.exe?idb2005/f13.def).

To our knowledge, this is the first report from Brazil of 1 rotavirus genotype predominating in a population after introduction of a vaccine. The P[4]G2 strain is a genotype for which effectiveness of the vaccine appears to be lower. This genotype has been previously reported in Brazil but represents only 6.1% of all the genotypes published since 2000. The proportions of strains with P[4]G2 has ranged from 0% to 27% in various studies, and no study reported that this was the predominant strain. Our finding of 100% prevalence of this genotype is unusual. Limited evidence of the effectiveness of Rotarix vaccine against the P[4]G2 strain has been reported (*9*,*10*) because the VP4 and VP7 proteins are not found in the P[8]G1 strain that is included in this vaccine.

Although our numbers are small, a lower proportion of vaccinated children had rotavirus-associated diarrhea, which likely reflects the protective effect of the vaccine. Four children were infected despite having been vaccinated and their infections were as severe as those in children who had not received the vaccine. This finding confirms that the vaccine does not afford complete protection against infection. Although predominance of the P[4]G2 strain in this population could be due to random preponderance of this genotype and is unrelated to vaccine use, this epidemiologic finding highlights the need for postlicensure surveillance of the vaccinated population.

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Novel Variant of Tickborne Encephalitis Virus, Russia

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We isolated a novel strain of tickborne encephalitis virus (TBEV), Glubinnoe/2004, from a patient with a fatal case in Russia. We sequenced the strain, whose landmark features included 57 amino acid substitutions and 5 modified cleavage sites. Phylogenetically, Glubinnoe/2004 is a novel variant that belongs to the Eastern type of TBEV.

Tickborne encephalitis virus (TBEV) was originally isolated in the Far Eastern region of Russia in 1937 (*1*). TBEV is defined as a species within the *Mammalian tickborne virus* group (genus *Flavivirus,* family *Flaviviridae*) (2). The TBEV species includes 3 subtypes, Far Eastern (previously RSSE), Siberian (previously West-Siberian), and Western European (previously Central European encephalitis [CEE]) viruses. Recently, taxonomic improvements were proposed, and TBEV were divided into 4 types: Western, Eastern, Turkish sheep, and Louping ill (*3*). TBEV has been found in nearly 30 countries in Europe and Asia (*4*), and ≈700 million persons live in areas (excluding the People's Republic of China) where TBEV infection is endemic. The annual incidence of TBEV infection is estimated to be as many as 14,000 cases (*5*). Eleven thousand TBE cases occur annually in Russia, but only ≈150 cases are registered in Primorsky District, Russia (*6*). The Far Eastern subtype is considered to be the most pathogenic for humans, with a mortality rate of ≥20%. The Western European subtype is less virulent and lethal (*7,8*). The TBEV genome consists of a single-stranded, positive-sense RNA of ≈11,000 nt that encodes 3 structural and 7 nonstructural proteins (*9*). The differences in nucleotide sequences encoding protein E between subtypes of TBEV may reach 18%–19%; amino acid sequences are considerably more conserved (*10*). In 2004, a total of 76 confirmed TBE cases occurred in the spring-summer season in Primorsky District; 10 were fatal. We describe 1 case caused by a novel variant of TBEV.

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The Patient

A 15-year-old boy received 2 tick bites near the village of Glubinnoe, in the northern part of Primorsky District, on May 30 and June 1, 2004, respectively. He had not had any vaccinations against TBEV infection. The first prodromal symptoms developed on June 8. A high fever with strong headache and paresis of cervical muscles developed in the next 2 days. On June 11, the patient was transported by emergency airplane from a local hospital to the Regional Clinical Hospital No. 1 in Vladivostok, where high fever with pronounced meningeal symptoms and complete disorientation in place and time were observed. Coma and acute respiratory dysfunction due to paralysis of respiratory muscles developed the next day, and the patient was put on an automatic respirator. He died of acute cardiovascular insufficiency and heart failure on June 17.

Virus strain Glubinnoe/2004 was isolated from a brain sample from the patient by using pig kidney embryo (PKE) cells. One hundred microliters homogenized brain diluted 1:100 was applied onto PKE cells in minimal essential medium supplemented with 2% fetal calf serum. The cells were incubated at 37°C for 4 days, and cell culture supernatants were used for second passage on PKE cells. The virus for sequencing and immunologic experiments was purified from infected PKE cells on third passage by centrifugation on a sucrose gradient (*11*). The protocol of study was approved by the Institutional Review Board of the Institute of Epidemiology and Microbiology, Vladivostok. Handling of the infectious material was performed under Biosafety Level 3–4 conditions.

A panel of monoclonal antibodies (MAbs) to TBEV was used for ELISA as described earlier (*12*). Viral RNA was extracted by using a RIBO-sorb kit (InterLabService Inc., Moscow, Russia); then RNA was transcribed to cDNA and amplified by PCR. The purified cDNA fragment was used for sequencing in a Beckman CEQ2000XL sequencer (Beckman Coulter, Inc., Fullerton, CA, USA). Fifty primers were designed on the basis of the TBEV sequence (GenBank DQ989336), allowing ≈150-bp overlap between adjacent PCR fragments. Each PCR fragment was independently amplified and sequenced 3×. Sequences were aligned with ClustalX (*13*). Molecular data were statistically processed by using the program MEGA (*14*). The program PHYLIP version 3.57 (University of Washington, Seattle, WA, USA) or PUZZLE version 4.0.2 (University of Munich, Munich, Germany) was used for constructing a phylogenetic tree.

The nucleotide sequences of the viral isolates were compared with published complete polyprotein sequences of TBEV (Table 1). The complete coding sequence of Glubinnoe/2004 was 10,886 nt. We found profound differences in the nucleotide sequences between listed TBEV strains and the Glubinnoe/2004 strain. The identity with 2

Table 1. Tickborne encephalitis virus (TBEV) strains used in the study and comparison of identify for full-length polyprotein sequences with strains Glubinnoe/2004 of TBEV*

Virus, subtype, strain	Place of origin	Year of isolation	Glubinnoe/2004	
			% Identity of nt sequence	% Identity of aa sequence
TBEV, Eastern type				
Glubinnoe/2004	Glubinnoe, Primorsky district, Russia	2004	100.0	100.0
205	Khabarovsk district, Russia	1973	95.2	98.4
Sofjin-HO	Khabarovsk district, Russia	1937	94.9	98.3
Oshima 5–10	Oshima, Japan	1995	94.7	98.2
Senzhang	China	1953	94.8	98.4
MDJ-01	China	2003†	94.6	98.0
Vasilchenko	Novosibirsk, Russia	1969	85.9	94.9
Zausaev	Tomsk, Russia	1985‡	85.9	94.9
EK-328	Estonia	1972	85.7	94.9
TBEV, Western type				
Neudoerfl	Neudoerfl, Austria	1971	84.3	93.4
263	Temelin, Czech Republic	1987	84.5	93.3
Hypr	Brno, Czech Republic	1953	84.1	93.0
TBEV, Turkish sheep type				
Turkish sheep encephalitis	Turkey	1969	82.9	91.0
TBEV, Louping ill type				
Louping ill, strain 369/T2	Selkirkshire, Scotland	1929	83.2	91.0
Spanish sheep encephalitis	Spain	1987	82.7	91.0

*TBEV type classification described previously (*3*). nt, nucleotide; aa, amino acid.
†Time of submission of the sequence to GenBank.
‡Virus was isolated from a patient with chronic form of TBEV infection; a patient was infected in 1973 in Tomsk; the virus was isolated in Moscow in 1985 from brain.

typical Far Eastern strains, 205 and Sofjin-HO, was 95.2% and 94.9%, respectively (Table 1). The complete Glubinnoe/2004 nucleotide sequence was compared with other TBEV sequences available in GenBank. The conservation of the strain's nucleotide sequences ranged from 82.7% to 95.2%, whereas amino acid sequences' conservation ranged from 91.0% to 98.4%, depending on the type of TBEV (Table 1). To further delineate the genetic variation, we analyzed polyprotein sequences of 37 other flaviviruses in comparison with Glubinnoe/2004 (Table 2). Fifty-three and 57 amino acid (aa) substitutions were found when Glubinnoe/2004 was compared to strains 205 and Sofjin-HO, respectively, and 14 of these were unique substitutions for all studied flaviviruses. Most substitutions were located in the C-terminal hydrophobic domain (CTHD) of proteins C, NS3, and NS5. The CTHD had 5 substitutions in 20 aa fragment; NS3, 10 substitutions, and NS5,16 substitutions. We also found that 5 putative cleavage sites of polyprotein were modified; the changes were located in viral C/CTHD and anchored C/prM sites. No substitutions were found in well-known features of protein E, such as the 12 cysteine residues, potential N-glycosylation sites, fusion peptide, and DEXH core motif of the NS3 helicase. A cysteine residue in position 4 of NS1 protein was replaced with a glycine; this mutation was described previously only for Sofjin-HO (BAB72162).

We also performed phylogenetic analysis of the fully sequenced TBEV strains and Glubinnoe/2004 (Figure 1) and analyzed 100 different protein E gene sequences available in GenBank (data not shown). Both phylogenetic trees clearly demonstrated that Glubinnoe/2004 belonged to the Eastern type and formed a separate clade (branch or group) within the type. The time of divergence of Glubinnoe/2004 from the Oshima-Sofjin and Senzhang groups was calculated by using the average substitution rate analysis (*15*). We estimate that Glubinnoe/2004 and Senzhang group diverged 300–470 years ago and Glubinnoe/2004 and Oshima-Sofjin group, 320–490 years ago. Our findings suggest that parallel evolution of different genetic groups of TBEV occurred in the relatively small Far Eastern region of Russia.

The growth curves for Glubinnoe/2004 and 205 viruses in PKE cells at 37°C are shown in Figure 2. The virus yield rapidly increased 9–18 h after infection and then stabilized. The maximum difference in virus yields (in 50% tissue culture infective doses [$TCID_{50}$]/mL) between Glubinnoe/2004 and 205 viruses was 2.1 $TCID_{50}$/mL at 12 h postinfection and nearly 10× from 15 to 36 h. By 72 h postinfection, PKE cells infected by Glubinnoe/2004 and 205 were completely lysed. We also evaluated the replication of viruses by directly measuring viral E protein levels by using 2 MAbs. E protein level rapidly increased 15–24 h postinfection and was similar for both strains. This finding correlated with a production rate of infectious virions with delay close to 6 h, which demonstrates that the Glubinnoe/2004 strain may produce more infectious virions

Table 2. Individual amino acid substitutions of Glubinnoe/2004 and 205/Sofjin-HO viruses and unique substitutions in comparison with 37 flaviviruses, determined by polyprotein alignment*†

Gene	Amino acid substitutions Glubinnoe/2004 → 205 or Sofjin-HO		Unique substitutions	Changed putative cleavage sites	Summary of substitutions 205/Sofjin-HO
	205	Sofjin-HO			
Viral C	No	M_{43} → L; A_{54} → V; N_{64} → K	No	No	0/3
CTHD	A_{99}‡ → V; I_{108} → V; M_{111} → I; M_{113}‡ → V; F_{115}‡ → L	A_{99}‡ → V; I_{108} → V; M_{111} → L; M_{113}‡ → V; F_{115}‡ → L	No	Viral C/CTHD (RGKRR/SAA†DW) anchored C/prM (GM‡TF‡A/ATVRK)	5/5
prM	No	No	No	No	0/0
M	R_{210}‡ → P	R_{210}‡→ P; I_{246} → M; I_{266}→ V	R_{210}*	prM/M (SRTRR/SVLIR‡)	1/3
E	K_{508} → R; I_{597} → T; T_{646} → N; V_{743} → A	A_{433} → V; T_{646} → N; M_{740} → V; G_{759} → S	No	No	4/4
NS1	G_{780}‡ → C; K_{883} → R; S_{951} → P; I_{1053} → T	I_{1053} → T; V_{1122} → I	No	E/NS1 (LGVGA/DVGG‡A)	4/2
NS2A	R_{1180} → K; R_{1227} → S; G_{1250} → S; G_{1277} → E; C_{1311} → Y	R_{1180} → K; R_{1227} → S; G_{1250} → S; G_{1277} → E; I_{1296} → T; A_{1297} → V	No	No	5/6
NS2B	V_{1423} → M	V_{1423} → M	No	No	1/1
NS3	E_{1563} → D; G_{1650} → E; I_{1673} → S; I_{1707} → T; T_{1828} → S; A_{1861} → V; P_{1948} → Q; V_{1975} → G; D_{1988} → N; A_{2062} → T	D_{1491}‡ → G; S_{1551} → Y; E_{1563} → D; G_{1650} → E; I_{1673} → T; N_{1731} → S; T_{1828} → S; T_{1869} → A; P_{1948} → Q; V_{1975} → G; D_{1988} → N; A_{2062} → T	E_{1563}; G_{1650}; P_{1948}; V_{1975}; D_{1988}	NS2B/NS3 (RTARR/SD‡LVF)	10/12
NS4A	D_{2143} → E ;V_{2165} → A	D_{2143} → E; A_{2173} → G	D_{2143}	No	2/2
NS4B	M_{2283} → L; I_{2314} → M; A_{2331} → V; V_{2349} → I; A_{2472} → V	M_{2283} → V; F_{2347} → L; S_{2457} → A	M_{2283}	No	5/3
NS5	K_{2625} → R; A_{2757} → G; G_{2758} → D; E_{3013} → G; S_{3014} → F; G_{3033} → E; K_{3074} → R; V_{3080} → I; S_{3187} → G; P_{3251} → R; V_{3260} → I; I_{3297} → V; V_{3342} → I; K_{3389} → E; Y_{3402} → D; N_{3406} → E	K_{2526} → R; M_{2641} → V; A_{2757} → G; G_{2758} → D; E_{3013} → G; Y_{3030} → H; V_{3080} → I; R_{3188} → G; P_{3251} → R; I_{3297} → V; V_{3342} → I; V_{3343} → A; R_{3384} → K; K_{3389} → E; Y_{3402} → D; N_{3406} → E	A_{2757}; G_{2758}; P_{3251}; K_{3389}; Y_{3402}; N_{3406}	No	16/16
Total			14	5	53/57

*Position number corresponds to the polyprotein sequence Glubinnoe/2004 of tickborne encephalitis virus (TBEV). CTHD, C-terminal hydrophobic domain.
†The following polyprotein sequences were used for polyprotein alignment: DQ862460 (TBEV, Glubinnoe/2004); ABJ74160 AAN73266 BAB71943 BAB72162 AF 069066 AAD34205 ABF46836 AAO43537 NP_043135 Neudoerfl AAZ80455 AAA86739 (TBEV, 263); Q01299 Hypr ABB90676 BB90675 AAQ91606 Bogolubovka NP 878909 Bogoluvovska AAR98531 Kubrin ABB90677 NP_044677 NP_620108 AAF75260E5 P29837 (AAQ91607 NP_722551 NP_620099 AAL32169 ABB90669 ABB90671 YP_224133 ABE73208 ABB90673 ABB90670 ABB90674 ABB90668 ABB90672 NP_689391).
‡For substitution in cleavage sites.

in the early stage of infection of a PKE cell. The discovered mutations in 4 of 5 cleavage sites in viral polyprotein are required for cleavage of structural viral proteins. This finding may explain the more robust formation of infectious virions in the early stages of infection.

Serologic data demonstrate that strain Glubinnoe/2004 has epitopes well recognized by 14 anti-TBEV MAbs (data not shown). Based on these data, we concluded that all protein E epitopes of strain 205 virus are also present on Glubinnoe/2004 virions and are not affected by 4 aa substitutions found in protein E. This would suggest that modern vaccines against TBE and diagnostic immunologic kits will be effective against this novel variant.

Conclusions

We have genetically characterized TBEV isolate Glubinnoe/2004 by determining its complete coding nucleotide sequence and comparing it with most of the available TBEV sequences. We found 53 and 57 aa substitutions in comparison with strains 205 and Sofjin-HO, respectively, and 14 of these were unique for 37 flaviviruses. Most substitutions were located in the CTHD of proteins C, NS3, and NS5. Phylogenetic analysis showed that Glubinnoe/2004 appears to be a separate lineage within the Eastern type of TBEV. We estimate that strain Glubinnoe/2004 diverged from Eastern TBEV in Senzhang group ≈300–470 years ago and from viruses in the Oshima-Sofjin group ≈320–490 years ago.

Five putative cleavage sites of the viral polyprotein were changed, including 4 sites responsible for processing of structural proteins. Strain Glubinnoe/2004 replicated more effectively in PKE cells than did strain 205. These data suggest that Glubinnoe/2004 could be used for production of vaccines, instead of strains 205 and Sofjin, and for development of diagnostic kits. This conclusion is supported by immunologic data with anti-TBEV MAbs, which demonstrated that viral glycoprotein E has a conserved antigenic structure typical of the Eastern type of TBEV.

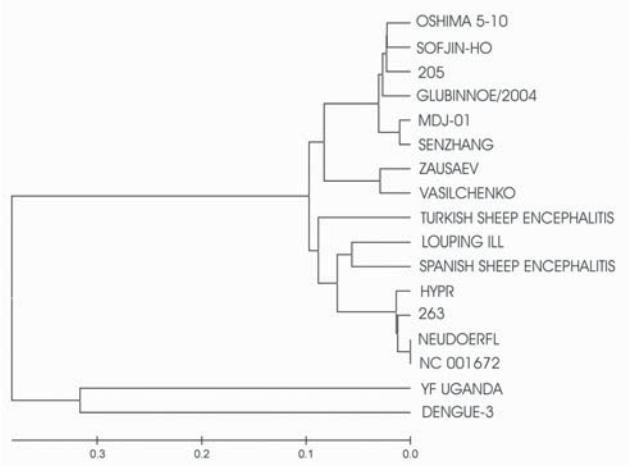

Figure 1. Phylogenetic tree, based on complete polyprotein sequences of tickborne encephalitis virus (TBEV). Original names of TBEV are presented. The multiple sequence alignments were obtained with ClustalX, and the tree was constructed by the neighbor-joining method. The following sequences were used for the phylogenetic tree: DQ862460 (TBEV, Glubinnoe/2004) ABJ74160 AAN73266 BAB71943 BAB72162 AF069066 AAO43537 NP_043135Neudoerfl AAA86739 (TBEV, 263); Q01299 Hypr ABB90676 BB90675 NP_044677 NC_001672 (TBEV); AY217093 (TBEV, MDJ-01); AY968065 (yellow fever virus, Uganda); DQ675533 (dengue 3).

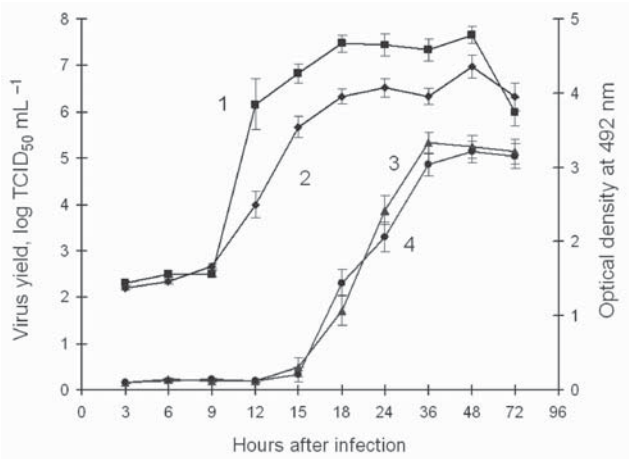

Figure 2. Growth curves and E protein synthesis of Glubinnoe/2004 and 205 in pig kidney embryo (PKE) cells: monolayer of PKE cells were infected with Glubinnoe/2004 and 205 of tickborne encephalitis virus (TBEV). The cells were frozen and thawed 3× to release the virus, and infectious titers were determined by the viral cytopathic effect (in 50% tissue culture infective doses [$TCID_{50}$]/mL^{-1}) assay in PKE cells. ELISA using 10H10 and biotin-labeled EB1 anti–protein E monoclonal antibodies was used to determine the E protein levels in virus-infected cells. 1, Glubinnoe/2004, virus yield; 2, 205, virus yield; 3, Glubinnoe/2004, E protein synthesis; 4, 205, E protein synthesis. Bars represent mean ± SD.

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Exposure to Wild Primates among HIV-infected Persons

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HIV-1 is an immunosuppressive pathogen. Our behavioral data for 191 HIV-1–infected rural Cameroonians show frequent exposure to nonhuman primates through activities such as hunting and butchering. Immunosuppression among persons exposed to body fluids of wild nonhuman primates could favor the process of adaptation and subsequent emergence of zoonotic pathogens.

Worldwide, ≈1% of the population is immunodeficient. Although immunodeficiency has numerous causes, such as malnutrition or iatrogenic medical therapies for cancer and organ transplantation, the most significant factor globally is HIV-1 infection (*1*). In 2006, ≈40 million persons were infected with HIV-1, and >50% were in sub-Saharan Africa, where AIDS caused 2.1 million deaths (*2*).

Immunodeficiency resulting from HIV-1 infection renders the host susceptible to infections usually controlled by cellular immunity through unrelenting loss of CD4+ T-helper lymphocytes. This susceptibility predisposes affected persons to common disease-causing pathogens such as *Mycobacterium tuberculosis*, *Salmonella* spp., *Coccidioides* spp., and *Histoplasma* spp. Other pathogens that are rarely pathogenic for immunocompetent persons, such as *Cytomegalovirus*, human herpesvirus-8, *Pneumocystis* spp., *Cryptococcus* spp., and *M. avium* complex, also become common causes of disease.

HIV-1–induced immunosuppression has also been proposed as a factor affecting the global emergence and reemergence of diseases (*1*,*3*). Among emerging infectious diseases in humans, ≈75% are caused by zoonotic pathogens (*4*), highlighting the potentially important risk for zoonotic exposures for HIV-1–infected populations. Central African forests, where hunting and butchering nonhuman primates are common practices, provide a ripe environment for zoonotic transmission (*5*). These areas have fostered human acquisition of Ebola (*6*,*7*), monkeypox (*8*), simian immunodeficiency viruses (*9*), simian foamy viruses (*10*), and primate T-lymphotropic viruses (*11*). Because HIV-1 infection is epidemic in Africa, persons involved in hunting and butchering of wild animals (including nonhuman primates) are possibly HIV-1–infected and thus at risk for successful infection with novel zoonotic viral infections. Additionally, HIV-1–induced immunosuppression in the wider community poses an additional risk for secondary transmission that could facilitate early viral adaptation to humans (*12*).

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The Study

As part of a community-based HIV-1 prevention campaign, February 2001–January 2003, we collected oral questionnaire data about basic demographics and behavior associated with exposure to the blood or body fluids of wild animals. In addition, blood samples were collected and transported to a central laboratory for HIV testing. We present behavioral data pertaining to animal exposures of HIV-1–infected persons in 17 rural villages in Cameroon (*5*). These are key sites for the emergence of nonhuman primate retroviruses because of the high levels of human contact with wild nonhuman primates (*5*) and cross-species transmission of simian foamy virus (*10*) and primate T-lymphotropic viruses (*11*).

Study participation was voluntary and performed under a protocol approved by the Johns Hopkins Committee for Human Research, the Cameroon National Ethical Review Board and the HIV Tri-Services Secondary Review Board. A single project assurance was obtained from the Cameroonian Ministry of Health and accepted by the National Institutes of Health Office for Protection from Research Risks.

HIV testing was performed by using an ELISA/Western blot algorithm. The ORTHO HIV1/2 (ORTHO Clinical Diagnostics Gmbh, Neckargemünd, Germany) ELISA was used as the screening test, and the HIV Blot 2.2 (Genelabs Diagnostics, Singapore) Western blot assay was used for confirmation.

Complete questionnaire data and plasma samples were collected from 3,955 persons, of whom 46.3% were female and 53.7% were male. Age range was 16–97 years (42% 16–30, 27% 31–45, 21% 46–60, and 10% >60 years). Screening for HIV-1 infection found 191 seropositive persons (prevalence 4.8% overall, 1.9%–16.3% from the 17 sites), of whom 60.2% were female and 39.8% were male. No persons were HIV-2 seropositive.

The HIV-1–infected persons were examined in greater detail. Within the younger age group (16–30 years), wom-

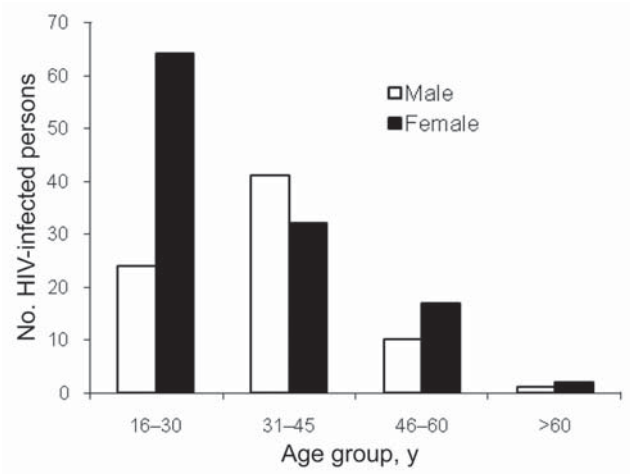

Figure 1. Age distribution of HIV-positive persons in 17 rural villages in Cameroon.

en were overrepresented; among persons >30 years of age, the number of infected men and women was similar (Figure 1). Of the HIV-1–infected persons, 89.0% reported having lived in a major city or another country, compared with 82.8% of the HIV-negative study population). Agricultural activities were reported as daily activities by 46.6% of the HIV-1–positive persons; household activities, by 22.0%; and fishing, by 13.6%. Hunting was reported as a daily activity by 12.6%, and contact with wild animals was reported by an even higher proportion. Among HIV-1–positive persons, 79.6% reported butchering wild animals (as many as 20× per month), 26.2% reported hunting wild animals (also as many as 20× per month), 12.6% reported having kept a wild animal as a pet, and 95.8% reported eating wild animals (Figure 2).

HIV-1–infected persons had significant contact with nonhuman primates; hunting of these species was reported by 11.0%. Monkeys were hunted up to 10× per month (median 3× per month); chimpanzees and gorillas were hunted less frequently (always <1× per month). In terms of butchering, 55.5% reported butchering nonhuman primates: monkeys ≤10× per month (median 1× per month), chimpanzees 2× per month (median <1× per month), and gorillas ≤1× per month. Furthermore, 8.4% of HIV-1–infected persons reported keeping nonhuman primates as pets, and 83.8% reported eating nonhuman primates.

Other direct animal exposures were reported by HIV-1–infected persons (Table); bites or scratches from wild animals were reported by 12.0% and from nonhuman primates by 2.6%. Although 4.7% of persons reported having received injuries during hunting and butchering, none reported having received injuries during hunting or butchering of nonhuman primates. However, 1.7% of the rural population in this area reports such injuries (*5*).

Conclusions

These data demonstrate an overlap of areas where HIV-1 is epidemic and areas where human-nonhuman primate contact is common. This overlap is cause for concern because humans and nonhuman primates share susceptibility to a range of pathogens, and the potential for successful cross-species transmission from nonhuman primates to humans is considered great (*5*). Access to treatment for HIV-1 infection is improving but is limited in remote central African communities; progressive disease and immunosuppression develop in most persons in these areas. Exposure of immunocompromised persons to nonhuman primates poses ongoing opportunities for zoonotic viruses to leap to humans, and the high concentration of other immunocompromised hosts offers an increased risk for secondary transmission and adaptation to humans. The emergence of HIV-1 is an example of such a process; data suggest several abortive nonhuman primate-to-human transmission events before eventual establishment of the HIV pandemic. This foothold gained by HIV-1 may now offer a boost for other pathogens to enter the human population. Moreover, the prevalence of HIV-1 in rural areas is lower than that in adjacent urban communities in Cameroon (*13*) and may increase. Such circumstances are not limited to central Africa; recent reports from Asia have demonstrated the risk for zoonotic infections with nonhuman primate viruses (*14*,*15*). And although nonhuman primates may present particular risks for disease emergence, HIV-associated immunosuppression likely increases the risk for acquisition, adaptation, and emergence of zoonoses infecting other animals that are hunted extensively in these communities (Figure 2), such as monkeypox and hantaviruses in rodents and Lyssavirus in bats.

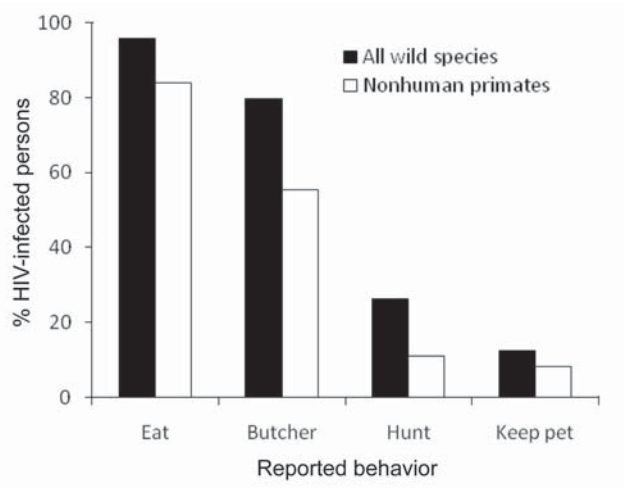

Figure 2. Percentage of HIV-positive persons in 17 rural villages in Cameroon who reported different types of contact with all wild animal species and with nonhuman primates.

Table. Injuries from wild animals received by HIV-positive persons in 17 rural villages in Cameroon, February 2001–January 2003

Participant code	Age, y	Sex	Injury	Animal
CAM2476LE	40	M	Bite or scratch	Chimpanzee
CAM4401KO	30	M	Bite or scratch	Monkey
CAM1231NG	37	M	Bite or scratch	Monkey, snake
CAM2177SA	32	F	Bite or scratch	Monkey, snake
CAM0989MO	16	F	Bite or scratch	Gorilla
CAM1188NG	19	M	Injury on finger	Not recorded
CAM2602ND	46	F	Many injuries on finger	Not recorded
CAM0212MA	45	M	Machete injury on finger during butchering	Antelope
CAM1669LE	44	M	Injury on hand during butchering	Antelope
CAM2888HA	26	F	Injuries on hand during butchering	Antelope
CAM2931HA	17	F	Injured during butchering	Antelope
CAM2162SA	30	M	Bite or scratch	Crocodile
CAM0074NY	48	M	Machete injury during butchering	Pangolin
CAM2418LE	40	M	Bite or scratch	Pangolin
CAM1788LE	24	F	Bite or scratch	Pangolin
			Injured on finger during butchering	Snake
CAM1172NG	59	F	Bite or scratch	Rodent
CAM2387LE	33	F	Bite or scratch	Rodent
CAM3503MB	40	M	Injuries on leg during butchering	Rodent
CAM3569MB	25	M	Bite or scratch	Rodent
CAM4434KO	41	M	Bite or scratch	Rodent
CAM4225YI	38	M	Bite or scratch	Insects
CAM4233YI	22	F	Bite or scratch	Snails
CAM0908MO	39	M	Bite or scratch	Snake
CAM1590LE	31	M	Bite or scratch	Snake
CAM1970LE	28	F	Bite or scratch	Snake
CAM2190SA	65	F	Bite or scratch	Snake
CAM2345LE	30	F	Bite or scratch	Snake
CAM2378LE	52	F	Bite or scratch	Snake
CAM2973HA	40	F	Bite or scratch	Snake
CAM3674SO	32	M	Bite or scratch	Snake
CAM4020MU	32	M	Bite or scratch	Snake

The risk for emergence of novel zoonotic infections in rural hunting communities should be considered in healthcare policy. Community health education and HIV/AIDS counseling should account for the fact that many persons in these communities rely on wild animals for food and household income. Targeted interventions could include culturally appropriate suggestions for avoiding handling or butchering of wild animals, such as developing alternative food sources, or taking precautions if such activities are necessary. Reducing the prevalence of HIV-1–induced immunosuppression through prevention and treatment and minimizing zoonotic exposures will be crucial for preventing future outbreaks of novel viral pathogens in humans.

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Multidrug-Resistant *Salmonella* Typhimurium, Pacific Northwest, United States

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We compared human and bovine isolates of *Salmonella enterica* using antimicrobial-drug resistance profiles and pulsed-field gel electrophoresis. From 2000 through 2006, we observed an increase in a novel multidrug-resistant clone of *S.* Typhimurium with no recognized phage type. This clone may represent an emerging epidemic strain in the Pacific Northwest.

Nontyphoidal salmonellosis has been characterized by a pattern of dissemination of clonal *Salmonella enterica*. The most well-known example of this was *S. enterica* serovar Typhimurium definitive phage type 104 (DT104), a clone that emerged and disseminated globally in the 1990s (*1*). Other examples of epidemic clones include *Salmonella* serovar Wien, which spread from North Africa through Europe in the 1970s; *S.* Typhimurium phage type 10 (DT10), which disseminated across Canada in the 1970s; and other phage types of *S.* Typhimurium associated with cattle that disseminated widely in Europe during the 1980s (*2*). More recently, multidrug-resistant (MDR) *S.* Agona was disseminated in poultry in Belgium; MDR *S.* Paratyphi B dT+ in Germany, Belgium, and the Netherlands; and MDR *S.* Newport that harbors the plasmid-mediated AmpC resistance in the United States (*3*). Epidemic clones may remain largely restricted to the animal reservoir for years before their incidence rises among human infections: *S.* Typhimurium phage type 204c disseminated throughout the United Kingdom in cattle from 1980 through 1984, but its proportion among human *S.* Typhimurium infections was not high during that period (*4*). Some epidemic clones, for example DT10 and DT104, expanded in the absence of a specific resistance advantage (*1*). The mechanisms underlying emergence, dissemination, and subsequent decline of novel *Salmonella* clones are unknown, although some researchers have hypothesized that fitness-associated genetic factors may allow more efficient dissemination in specific hosts and environments (*5*) and that acquisition of these fitness genes may be phage mediated (*6*).

One approach to understanding clonal replacement events is to conduct prospective surveillance of *Salmonella* infections in animal and human hosts. We initiated such a study to compare human- and animal-origin *Salmonella* strains by serovar, antimicrobial drug resistance pattern, and pulsed-field gel electrophoresis (PFGE).

The Study

In Washington, all clinical laboratories and healthcare providers are required to submit *Salmonella* isolates to the Washington State Department of Health Public Health Laboratory (PHL) for serotyping and surveillance (Washington Administrative Code 246–101–201). The PHL is a participant in PulseNet, a national surveillance system established in 1996 that allows state and local public health epidemiologists to compare PFGE profiles of *Salmonella* strains from all US regions (*7*). All human isolates are compared at the PHL by using a standard PFGE PulseNet protocol (*8*). Beginning in January 2004, all human-origin isolates were obtained from the PHL by the Washington State University Zoonosis Research Unit in Pullman. Animal-origin isolates were obtained from the Washington Animal Disease Diagnostic Laboratory in Pullman, the Washington Avian Health Laboratory in Puyallup, and a private veterinary diagnostic laboratory. In addition to isolates originating from clinical veterinary submissions, we obtained isolates from a research study targeting *Salmonella* occurrence on dairy farms in Washington (D. Hancock et al., unpub. data). All isolates were tested for resistance to a panel of antimicrobial drugs with a standard disk-diffusion method (*9*) according to Clinical and Laboratory Standards Institute guidelines (*10*) (Table 1). Isolates collected from January 1, 2000, through April 2005 were tested for susceptibility to a panel of antimicrobial drugs (Table 1). Animal-origin isolates that matched human-origin isolates by serotype and resistance pattern were further compared by using a standard PFGE protocol (*8*). Cluster analysis using the unweighted pair group method with arithmetic mean analysis (UPGMA) of Dice similarity coefficients based on PFGE banding patterns was computed in Bionumerics (Applied Maths, Sint-Martens-Latem, Belgium). To limit the effect of nonindependence among isolates, only the first in each serotype and resistance pattern group within each herd and calendar year was included in the analysis. Statistical significance

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Table 1. Trends in resistance of bovine-origin *Salmonella* Typhimurium PFGE types TYP035 and TYP187*

Resistance characteristics†	2001	2002	2003	2004	2005	2006
TYP035						
ASu			1	1	1	
ACaz						1
AKSuT		1			1	
AKSSuT	7	7	1	4	1	4
ACKSSuT	1	1				
AKSSuTCaz				3		
AKSxtSSuT				1		
ACKSSuTCaz			2	2		
ACKSxtSSuT					1	
AKSuTCaz					1	
AKSSuTCaz					1	1
ACKSSuTCaz					2	1
ACKSxtSSuTCaz		1	1	6	4	
AGKSxtSSuTCaz						1
Total	8	10	5	17	12	8
TYP187						
ACSSuTCaz				1		
AKSSuTCaz					1	12
AKSuTCaz					1	
Total				1	2	12

*PFGE, pulsed-field gel electrophoresis.
†A, ampicillin; Su, triple-sulfa; Caz, ceftazidime; K, kanamycin; T, tetracycline; S, streptomycin; C, chloramphenicol; Sxt, trimethoprim-sulfamethoxazole; G, gentamicin. In April 2005 nitrofurantoin, trimethoprim, and ciprofloxacin were dropped from the panel, and amoxicillin-clavulanic acid, nalidixic acid, and ceftazidime-clavulanic acid were added.

testing of the change in proportions was conducted in EpiInfo version 6.0 with χ^2 for linear trend (*11*).

We observed an increase in bovine-origin *S.* Typhimurium isolates that were represented by 2 highly similar PFGE patterns, identified as Washington State PulseNet types TYP035 and TYP187 (Table 2). They were clearly distinguishable from phage type DT104 strains isolated during the same time frame (online Appendix Figure, available from www.cdc.gov/EID/content/13/10/1583-appG.htm). To determine whether these *Xba*I PFGE types were clonal, we characterized 60 *S.* Typhimurium isolates, including 32 TYP035-TYP187 isolates from bovines and 19 TYP035-TYP187 isolates from humans, by PFGE following digestion of DNA by *Spe*I. *Spe*I patterns within each *Xba*I type showed only minor banding variations. Thirteen isolates that were *Xba*I-TYP035 had *Spe*I patterns indistinguishable from those of *Xba*I-TYP187 isolates. In the cluster analysis of *Spe*I patterns, the TYP035 and TYP187 isolates' *Xba*I patterns were at least 90.1% similar compared to an overall 83.7% similarity for all *S.* Typhimurium isolates in the analysis. Characterization of isolates within the *Xba*I PFGE type TYP035 also included plasmid profiles of 38 isolates with published plasmid-profiling methods (*12*). Isolates were selected for plasmid profiling to maximize the diversity of resistance patterns, geographic origin, and year of isolation. Plasmid sizes were estimated relative to a BAC-Tracker Supercoiled DNA ladder (EPICENTRE Biotechnologies, Madison, WI, USA). Fifteen of the 38 tested isolates harbored a single ≈120-kb plasmid. The remainder had variable plasmid profiles, but the plasmid profiling data did not correlate with resistance phenotype or *Spe*I-banding variations (data not shown). Similar results were obtained with phage typing of a convenience sample of 31 isolates by Rafiq Ahmed at the National Microbiology Laboratory, Winnipeg, Manitoba, Canada. All 31 TYP35 isolates were phage-type untypable with the standard Colindale panel of phages, although an additional set of phages were able to discriminate among some of them (R. Ahmed, pers. commun.). The resulting categories of untypable phages (UT2, UT5, UT7, and UT8) were also not consistent with either plasmid profile or resistance phenotypes (data not shown).

The proportion of all bovine independent *S.* Typhimurium isolates in the TYP035–TYP187 clade increased significantly from 2000 to 2006 in Washington (χ^2 for linear trend, $p<0.01$). The proportion also increased significantly among human *S.* Typhimurium isolates during the same period (χ^2 for linear trend, $p<0.01$) (Table 2). Expansion of TYP035 apparently occurred in the bovine population of the Pacific Northwest before its increase among human isolates because in 2000 it represented over one quarter of all bovine *S.* Typhimurium, but it was uncommon among human-source *S.* Typhimurium before 2002 to 2003 (Table 1). Similarly, TYP187 was detected in bovine isolates 2 years before its detection in human isolates. Resistance characteristics varied among TYP035 and TYP187 isolates. Before 2003, the predominant resistance pattern among TYP035 isolates was the AKSSuT phenotype; after 2002, isolates were distributed more widely among several resistance pat-

Table 2. Human- and bovine-source *Salmonella* Typhimurium isolates from Washington that were *Xba*I-PFGE type TYP035 or TYP187*

	Human			Bovine		
Year	TYP035	TYP187	Total no.† (%)‡	TYP035	TYP187	Total no.† (%)‡
2000	1	0	222 (0.5)	16	0	61 (26.2)
2001	1	0	174 (0.6)	18	0	47 (38.3)
2002	8	0	143 (5.6)	10	0	31 (32.3)
2003	13	0	153 (8.5)	5	0	13 (38.5)
2004	7	0	143 (4.9)	10	1	26 (42.3)
2005	18	0	124 (8.9)	12	2	29 (48.3)
2006	3	7	118 (8.5)	8	12	39 (51.3)

*For human isolates only a single isolate per individual was included, and for bovine isolates only independent isolates were included. PFGE, pulsed-field gel electrophoresis.
†Total number of unique *S.* Typhimurium isolates.
‡Percentage of the total that were part of the TYP035-TYP187 clade.

terns (Table 1). The predominant resistance pattern among isolates of TYP187 was AKSSuTCaz (Table 1).

Conclusions

The strain type reported here may be an emerging epidemic clone of MDR *Salmonella* with the potential to expand further. Since 2001, TYP035 has been infrequently reported in the National PulseNet Database, and most sporadic cases were from Washington. The TYP187 profile has never been reported outside of Washington. Comparison of PFGE patterns between Northwest and New York State *S.* Typhimurium isolates at the Cornell University Zoonosis Research Unit showed no occurrences of TYP035 or TYP187 among 23 bovine and 29 human *S.* Typhimurium isolates from the same period. These comparisons with isolates from other parts of the United States suggest that the emergence of the TYP035/ TYP187 clade is currently focused in the Pacific Northwest region but has the potential to become more widespread. In 2006, the Massachusetts Department of Public Health investigated an outbreak of salmonellosis associated with owl pellets among elementary school students (46 primary cases, 12 secondary cases) (*13*). This outbreak was caused by TYP035 *S.* Typhimurium, and the supplier of the owl pellets was located in a rural county in south-central Washington. This outbreak illustrates that epidemic clones may disseminate widely by means of unpredictable vehicles and that nonfoodborne transmission can have a major public health impact.

The TYP035/TYP187 clade first increased in the animal reservoir, followed by spillover into the human population, consistent with its being an emergent clone. Whether or not this strain will continue to expand, further characterization of this PFGE type and continued surveillance will be important steps for improving our understanding of epidemic *Salmonella* clones (*3*,*5*).

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Emergence of Human Rotavirus Group A Genotype G9 Strains, Wuhan, China

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Of 322 stool specimens collected from children with diarrhea from October 2005 through September 2006 in Wuhan, China, group A rotavirus was identified in 101 (31.4%). The most prevalent group A rotavirus genotype was G3P[8] (62.6%), followed by G1P[8](17.6%), G1+G3P[8](8.8%), G3P[4](6.6%), G1P[4](2.2%), and G9P[8](2.2%). The G9 strains were first detected in Wuhan.

Group A rotaviruses are the most common etiologic agents of severe diarrhea in infants and young children worldwide and are responsible for 2 million hospitalizations worldwide (*1*–*3*); in developing countries, these rotaviruses cause 400,000–500,000 deaths annually in children <5 years old. Previous molecular epidemiologic surveys have shown that 4 frequently observed genotype combinations—G1P[8], G2 P[4], G3 P[8], and G4 P[8]—are common worldwide (*3*,*4*) and, therefore, are prime candidates for current vaccine development strategies. Detailed epidemiologic studies have shown that rotavirus serotype G9 is emerging as an important human pathogen worldwide (*3*). Only 1 G9 strain was detected in Beijing between 1982 and 1997. However, G9 strains were identified in 3 regions in 1999; this increase coincided with the global trend. Our study involves the detection and characterization of G9 strains in Wuhan, a large city in central China; sequence analysis of the *vp*7 gene; and the phylogenetic analysis of the *vp*7 gene with that of G9 isolates in other regions of the world.

The Study

A total of 322 stool specimens were collected from children with diarrhea who were hospitalized in 3 hospitals from October 2005 through September 2006 in Wuhan. Collection procedures were in accordance with rotavirus surveillance protocols recommended by the World Health Organization. Viral RNA was extracted from stool specimens with the guanidine isothiocyanate method as described (*5*). Rotavirus electropherotypes were determined by polyacrylamide gel electrophoresis according to the method described by Herring et al. (*6*). The purified RNAs were examined by reverse transcription–PCR (RT–PCR) using the group A rotavirus–specific primers (Beg9/End9 and Con2/Con3) and then subjected to G genotype typing and P genotype typing by using a heminested RT–PCR strategy and electropherotyping (*5*,*7*). The result of G–P typing was confirmed by sequencing 10% of the total samples that were drawn randomly. Sequences of the RT–PCR products were analyzed with the Sequencher program (Gene Codes Corporation, Inc., Ann Arbor, MI, USA) and subsequently compared with the *vp*7 gene sequences of 11 G9 strains from the GenBank database using E-ClustalW (http://align.genome.jp); the phylogenetic tree was built by Molecular Evolutionary Genetics Analysis (MEGA) version 3.1 program (www.megasoftware.net).

Of 322 stool specimens, human group A rotavirus was detected in 101 (31.4%) case-patients. G typing of the 101 strains identified 67 as G3 (66.3%), 19 as G1 (18.8%), 8 as G1 and G3 mixed infection (7.9%), 4 as untypeable (4%), 1 as G4 (1%), and 2 as G9 (2%). Both rotavirus G and P types could be established in 91 strains, and 6 different combinations were identified (Table 1). G3P[8] (62.6%) was the most common combination detected, followed by G1P[8] (17.6%), G1/G3P[8] (8.8%), G3P[4] (6.6%), G1P[4] (2.2%), and G9P[8] (2.2%).

Two group A rotavirus strains were designated to G9 genotype by electropherotypes (Figure 1) and RT–PCR. The complete nucleotide sequence and deduced amino acid sequence of the *vp7* gene from the Wuhan G9 strain were compared with the *vp*7 gene sequences of 11 G9 strains from the GenBank database (Table 2). The comparison showed clearly that the *vp*7 of the Wuhan G9 isolate was closely related to the 2 G9 strains (XJ99–468, XJ04–652) isolated from XinJiang province in China (98.3%–98.4% identity on nucleotide sequence and 97.2%–97.5% similarity on amino acid sequence). Phylogenetic analysis also confirmed that the *vp*7 gene of the Wuhan G9 strain (CC597, Wuhan, China) clustered in the same branch with those of the XJ99–468 and XJ04–652 G9 strains (Figure 2).

Table 1. Distribution of G and P genotypes of rotavirus strains from October 2005 to February 2006 in Wuhan, China

G type	P type	Total no. (%) rotavirus strains
G1	P[4]	2 (2.2)
G1	P[8]	16 (17.6)
G3	P[4]	6 (6.6)
G3	P[8]	57 (62.6)
G9	P[8]	2 (2.2)
Mixed G1,G3	P[8]	8 (8.8)
Total		91 (100)

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Conclusions

In this study, we presented the distribution of group A rotavirus genotypes and the emergence of the novel G9 genotype of rotavirus in Wuhan City from October 2005 through September 2006. Comparison with the results of the surveillance from 2000 to 2005 in Wuhan showed that the G3P[8] genotype remains the most predominant. Our results are similar to those reported by Fang et al (*8*), indicating that G3P[8] serotype was the most common strain throughout China from 2001 through 2003.

An important result of this study was the first characterization of the G9 strain isolated from Wuhan during the rotavirus season 2005–2006. Sequence analysis of the *vp*7 gene of the Wuhan G9 isolate showed that it had high similarity (97.5% amino acid identity) to that of the XJ04–652 strain, which was isolated from the specimen of a 9-day-old infant girl in XinJiang province, China, in December 2004 (Table 2). In the past decade, the number of countries that have reported the detection of rotavirus G9 strains has increased dramatically. Apparently G9 rotaviruses are expanding on a global scale; currently, the G9 serotype is considered to be the fifth most common type worldwide. In Australia, although the serotype G9 was identified for the first time in the 1999–2000 season, a retrospective analysis showed the presence of 3 G9 isolates in the country. During the 1999–2000 and 2000–2001 seasons, it rocketed to the second most common G type and was responsible for 10% and 18.1% of the total group A rotavirus infections in the country, respectively (*9*). In the 2001–2002 season, the G9 serotype was the most important infecting rotavirus serotype in the country, representing 40.4% of the isolates (*10*). In Spain, previous studies had identified G1P[8] and G4P[8] as the predominant cocirculating strains from 1995 through 2004. However, these serotypes were displaced by G9P[8] during the 2005 season, when G9P[8] accounted for 50.6% of typed isolates in several regions of Spain (*11*). Similarly, G9 serotype was identified as the prevailing serotype in several Japanese cities from 1998 to 2000 with high prevalence rates of 52.9% to 71.4% (*12*). In 1995, the rotavirus G9P[8] emerged in Bangladesh and became the second predominant strain (27.7%) during the 2001–2005 rotavirus seasons (*13*); G9P[8] accounted for most (91.6%) circulating rotavirus strains in Thailand during the 2000–2001 season and has now become the most common genotype (*14*). In China, G9 strains were identified in 3 regions including Kunming, Lanzhou, and Qinhuangdao in 1999 and 2003 (*8*,*15*); only 1 strain was detected between 1982 and 1997 in Beijing (*8*). The incidence of G9 genotype increased from 0.9% to 4% from 1999 through July 2003, which coincided with an increased global incidence of G9

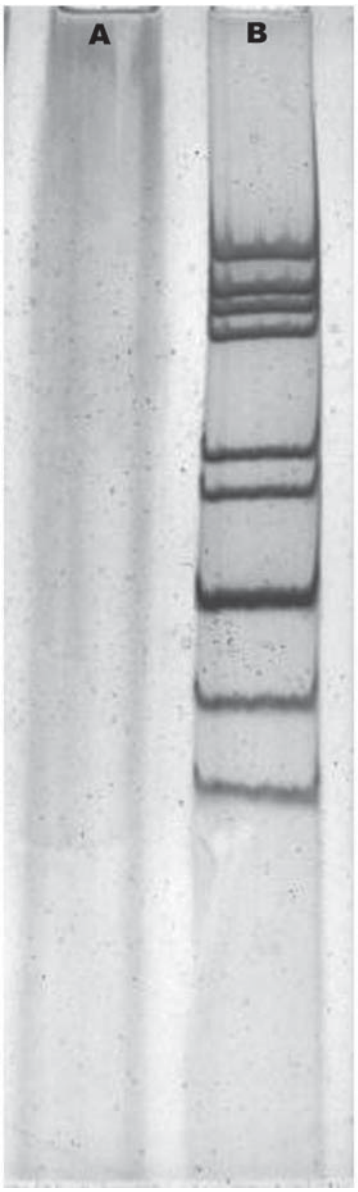

Figure 1. Polyacrylamide gel electrophoresis of rotavirus RNA. The viral RNAs were analyzed by electrophoresis in a polyacrylamide gel and visualized by silver staining. A, negative control; B, Wuhan G9 strain (CC589).

Table 2. Comparison of the nucleotide and amino acid sequence identity of *vp*7 gene of Wuhan G9 rotavirus strain (CC597) with those of 11 known G9 rotavirus strains

	Similarity, %	
Strain	Nucleotide	Amino acid
XJ04–652	98.4	97.5
XJ99–468	98.3	97.2
BD524	96.0	93.9
DE18	98.1	96.9
R136	98.0	96.6
KUMS04–5	98.2	96.0
02TW465	97.8	96.3
MG9–06	97.9	96.6
608VN	95.4	95.7
S25	97.6	96.6
JP32–4	90.7	92.3

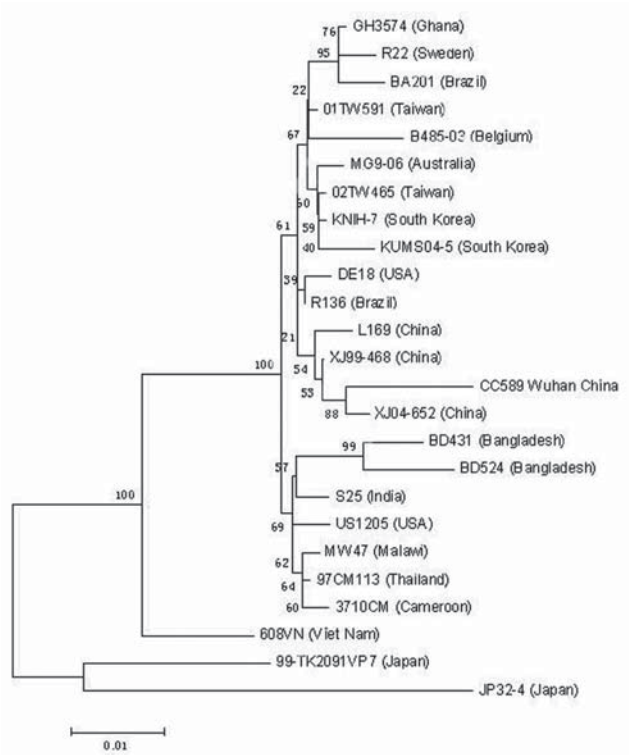

Figure 2. Phylogenetic analysis of the nucleotide sequences of the *vp7* gene of G9 strain isolated in Wuhan and G9 strains from different parts of the world. The bar indicates the variation scale. A phylogenetic tree was generated based on the neighbor-joining method using MEGA version 3.1 (www.megasoftware.net). GenBank accession numbers of the *vp7* gene sequences of group A rotavirus are the following: AY211068, AY196119, AY695811, DQ490173, AY307085, DQ096291, DQ990317, DQ056296, AY487877, AJ491163, AF438228, DQ321497, DQ321495, AJ250542, AJ250543, AJ491188, AF060487, AJ250544, AY866505, AY816184, AB091777, AB091756, AB176682, L79916, and EF197983.

strain in various countries. However, it remains a mystery why G9 strain has only now been detected at low frequency in China while it has been one of the most prevalent rotavirus types worldwide for more than a decade.

Since the rotavirus G9 strain is the most widespread emerging rotavirus serotype globally, continued surveillance of rotavirus infections in China is imperative. Surveillance programs can effectively monitor whether G9 rotavirus strains will rise in prevalence. This information will be crucial for the development and evaluation of more efficient vaccines.

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International Epidemic Intelligence at the Institut de Veille Sanitaire, France

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The French Institute for Public Health Surveillance monitors health events of potential international importance occurring worldwide to provide timely warning to French health authorities. We reviewed the nature and place of occurrence of the last 200 events. From an individual country's perspective, the need for multiple sources is emphasized.

Local epidemics may rapidly acquire international importance due to international travel and trade (*1*). With early warning, timely and adequate control measures can be adopted to prevent transmission. Epidemic intelligence is the systematic collection and collation of verified and unverified information from various sources, such as governments, United Nations organizations, nongovernmental organizations (NGOs), mass media, and personal communications (*2*). The Internet is transforming global disease surveillance (*3*). Information is selected by specific criteria, verified (if informal), and thoroughly analyzed before communication to the public.

The Institut de Veille Sanitaire (InVS)—the French Institute for Public Health Surveillance—set up an epidemic intelligence unit to monitor outbreaks worldwide along the lines of the World Health Organization (WHO) Department of Epidemic and Pandemic Alert and Response. At the national level, this unit's legal mandate is to detect, verify, and rapidly assess information on potential international health threats, which may affect populations in France or French nationals worldwide. Its main task is to inform French authorities, public health professionals, and other partners of these epidemic risks and to place events with excessive media coverage in the proper perspective. Information is structured and widely communicated weekly through the electronic Bulletin Hebdomadaire International (BHI) (www.invs.sante.fr/international/index.htm). To better assess the type, characteristics, and location of alerts documented by the unit, we reviewed the health events posted in the BHI, i.e., all confirmed information on potential international health threats that may affect populations in France or French nationals worldwide. We also examined initial signals and the use of various sources of international epidemic intelligence.

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The Study

We reviewed 200 events posted in the 32 BHI from May 17, 2006 to December 27, 2006. We examined event topics, geographic location (country and world region), onset date of the first case/outbreak, source, and publication date of the first signal, delay between first occurrence and the first signal, signal type (passive email alert vs. active manual search) and alarm status (first report vs. follow-up).

Potential sources were formal outbreak reports communicated by countries and supranational organizations (both official and NGO) posted on the Internet and scientific online forums such as ProMED-mail (available from www.promedmail.org) (*4*–*6*). A dedicated tool was used to collect information available on the Internet: the Global Public Health Intelligence Network (GPHIN) (available from www.phac-aspc.gc.ca/media/nr-rp/2004/2004_gphin-rmispbk_e.html) is a software codeveloped by WHO and Health Canada. GPHIN is a secure, restricted-access early warning system that gathers media reports of public health significance on a 24/7 basis (*7*). Like the medical intelligence system developed by the European community, the GPHIN is a multilingual system that provides relevant unverified information on public health events by monitoring global media sources in 7 languages. This automated process includes a filter for relevancy, but specific email alerts and the categorizing of information must be complemented by human analysis.

The highest proportion of events (53%, 105/199) occurred in Asia (Table 1). Of these events, 61% (122/199) were highly pathogenic avian influenza (HPAI) (H5N1) infections in animals or humans. These, combined with multicountry outbreaks of cholera (8%), chikungunya virus disease (7%), dengue (7%), and poliomyelitis (6%), were the most recurring topics posted in the BHI (Table 2).

The first signal's source could be identified in 88% (176/200) of events. News reports collected using the GPHIN were the most important initial sources of information, providing 36% (63/176) of all initial signals (online Appendix Table, available from www.cdc.gov/EID/content/13/10/1590-appT.htm). Of these 63 events, 37 (59%) were automatically forwarded by the GPHIN e-alert system, and 26 (41%) were detected through active searches. Official signals from the WHO network accounted for 29% (51/176) of all events posted in the BHI; ProMED-mail provided the first signal for 17% of the events. Of 176 events included in the BHI, 20% (35/176) were first detected only by manual and nonspecific Internet searches. Furthermore, 60% (105/176) of the posted events were first detected through informal sources that required extensive verification.

Table 1. Events posted in the BHI from May 17 through December 27, 2006, by world region*

Region	No. events		
	HPAI (H5N1)	Other	Total
Africa	21	29	50
Americas	0	11	11
Asia	80	25	105
Europe	20	9	29
Middle East	1	3	4
Total	122	77	199

*BHI, Bulletin Hebdomadaire International; HPAI, highly pathogenic avian influenza.

On average, delay between the first case of an outbreak (including retrospectively) and the first widely available signal was 3 months and 14 days (range 1 day–2 years, 10.5 months; n = 86). Among the 200 events posted, 107 were reported for the first time. For these, mean delay between the first case and the first signal was 1 month and 23 days (range 1 day–5 months, 16 days; n = 63). Based on a small sample of alerts, the mean delay for alert messages provided by the GPHIN was shorter (1 month, 19 days; n = 19) than that of the ProMED-mail (2 months, 4 days; n = 6).

Conclusions

As in the 2006 WHO report (*2*), influenza A (H5N1) HPAI cases and cholera outbreaks were the major topics included in the BHI. Alerts posted for this period mainly concerned Asia due to the occurrence of influenza A (H5N1). Due to the specific economic and political situations of each country, availability and sensitivity of information sources differed. Multiple information sources somewhat compensated for these differences. Delays between the occurrence of events and first reports reflect the following: 1) interval between the occurrence of a first case and development into a full-blown outbreak of international importance; 2) limitations of communicable disease surveillance, i.e., interval before an event is detected; 3) intrinsic limitations of epidemic intelligence, i.e., availability of information; and 4) the unavoidable prioritization of alerts, given time and resource constraints.

Table 2. Events posted in the BHI from May 17 through December 27, 2006, by topic*

Event	No. (%)
HPAI (H5N1) in animals	67 (34)
HPAI (H5N1) in human	55 (28)
Cholera	16 (8)
Chikungunya	13 (7)
Dengue fever	13 (7)
Poliomyelitis	11 (6)
Malaria	6 (3)
Japanese encephalitis	4 (2)
Adulterated alcohol intoxication	3 (2)
Crimean-Congo hemorrhagic fever	3 (2)
Plague	2 (1)
Yellow fever	2 (1)
Deaths following influenza vaccination	1 (0.5)
Measles	1 (0.5)
Micro-algae intoxication	1 (0.5)
Rift Valley fever	1 (0.5)
Viral meningitis	1 (0.5)
Total	200 (100)

*BHI, Bulletin Hebdomadaire International; HPAI, highly pathogenic avian influenza.

GPHIN was more efficient than any other information source used in this analysis, including ProMED-mail, both in terms of number of signals and rapidity of signal availability after event occurrence. Signals from GPHIN, however, are unverified media reports,and not all relevant events for our specific needs are electronically forwarded as e-alert. Permanent proactive searches using GPHIN or similar tools remain compulsory to address this limitation. Time-consuming human-operated screening of each report of the daily GPHIN list (500–2,000 reports/day) is needed as signal detection cannot be automated. Verification processes are essential because reports of outbreaks are widely disseminated and easily accessible to the public (*1*).

WHO and other supranational organizations, such as the European Centre for Disease Prevention and Control, monitor health events of international importance. However, these organizations cannot completely meet all needs of individual countries. Our experience at a national institute shows that implementation of epidemic intelligence should be specifically tailored to effectively monitor the health of a country's population and translate directly into public health action. For example, in 2005, an extensive cholera outbreak was detected and documented in Senegal, with far-reaching implications for Franco-Senegalese pilgrims. Information posted in the BHI is used by physicians in French tropical disease departments and travel clinics, who can provide timely information to travelers, and target clinical examinations of those returning with suggestive symptoms. The operational suspect case definition for influenza A (H5N1) in returning travelers is continuously updated as foci appear in various areas. Information is also forwarded to the French Ministries of Health and Foreign Affairs to alert a larger segment of the population through institutional websites or warnings on airport billboards.

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Dr Rotureau is an epidemiologist at the Tropical and International Department of the French Institut de Veille Sanitaire, Saint-Maurice, France. His research interests involve the study of ecologic, epidemiologic, and physiologic properties of Trypanosomatidae parasites and the diseases they cause.

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Foot-and-Mouth Disease Virus Serotype A in Egypt

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We describe the characterization of a foot-and-mouth disease (FMD) serotype A virus responsible for recent outbreaks of disease in Egypt. Phylogenetic analysis of VP1 nucleotide sequences demonstrated a close relationship to recent FMD virus isolates from East Africa, rather than to viruses currently circulating in the Middle East.

Foot-and-mouth disease (FMD) is caused by 7 immunologically distinct serotypes, O, A, C, Asia 1, South African Territories (SAT) 1, SAT 2, and SAT 3, which belong to the species *Foot-and-mouth disease virus* (genus *Aphthovirus*, family *Picornaviridae*). Several of these serotypes circulate currently or periodically in the Middle East and North Africa (*1*). In Egypt, routine prophylactic vaccination has been conducted with a locally produced serotype O vaccine. The last outbreak of serotype O was in June 2000, and other serotypes have not been reported since 1972 when serotype A occurred (*2*). This report describes an FMD serotype A virus responsible for recent outbreaks of disease in Egypt.

Clinical cases of FMD were first recognized on January 22, 2006, on a cattle farm at El Etehad in Ismailia, northeastern Egypt (Figure 1). Samples were submitted for laboratory investigation and serotype determination by using virus isolation, antigen ELISA, and reverse transcription–PCR (RT-PCR). Initial testing with antigen ELISA and RT-PCR assays suggested that multiple FMD virus (FMDV) serotypes may have been involved in the outbreak (data not shown), although only type A was later confirmed. On February 15, 2006, the Agriculture Ministry in Egypt notified international public health authorities (by reporting to the World Organization for Animal Health [OIE]) of 6 outbreaks of FMDV caused by serotype A in Ismailia and 12 additional outbreaks in 7 other Egyptian governorates: Alexandria (2 outbreaks), Behera (1 outbreak), Cairo (1 outbreak), Dakahlia (1 outbreak), Dumyat (5 outbreaks), Fayum (1 outbreak), and Menofia (1 outbreak). By April 6, 2006, 34 outbreaks of disease had been reported that affected >7,500 animals and involved an additional governorate (Kalubia). Most (96.7%) clinical FMD cases involved cattle; 411 cattle (mainly calves) reportedly died. Attempts to control the outbreaks were hampered by lack of an appropriate vaccine and concurrent outbreaks of highly pathogenic avian influenza. FMD became widespread in Egypt, with the following numbers of animals affected per month: 6,189 (January), 1,858 (February), 3,035 (March), 401 (April), and 297 (May). A locally produced bivalent FMDV vaccine, containing both O_1 and A/Egypt/2006 isolates, was released in mid-May 2006 for the first time in Egypt. No new cases have been reported since July 2006.

The Study

Clinical material from 5 cases (collected from 3 separate locations in Egypt; Table 1) was sent to the Food and Agricultural Organization of the United Nations (FAO) World Reference Laboratory for FMD (WRLFMD) at the Institute for Animal Health, Pirbright, United Kingdom, for confirmatory diagnosis and characterization of the causative FMDV strain(s). The possibility that these samples contained multiple FMDV serotypes was also investigated. FMDV isolates causing cytopathic effects in primary bovine thyroid (BTy) cell cultures were generated from all samples. The cell culture–grown virus isolates and original clinical submissions were identified as FMDV serotype A by antigen-detection ELISA (*3*).

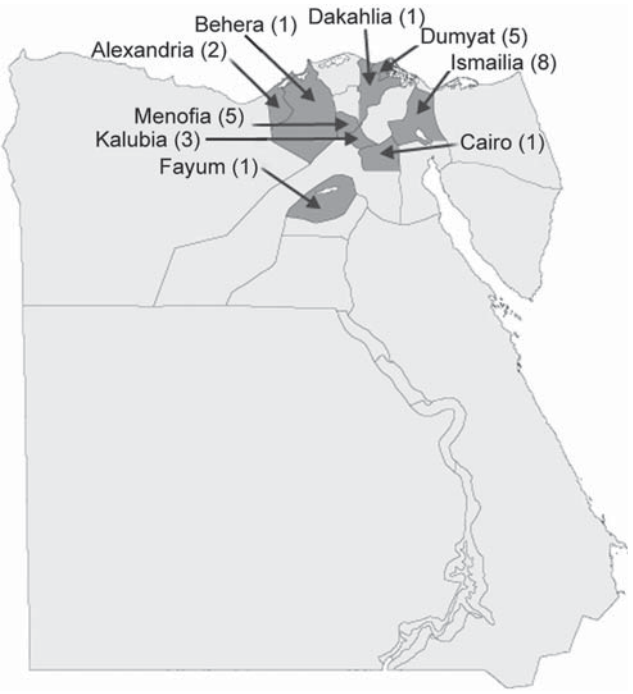

Figure 1. Locations and numbers of cases in the initial outbreaks of foot-and-mouth disease, Egypt, 2006.

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Table 1. Foot-and-mouth disease type A viruses examined in the study

WRLFMD ref. no. or virus name*	Location	Date collected	Species	GenBank accession no.
A/ARG/2000	Argentina	2000	Not known	AY593782
A/Trenquelauquen/ ARG/2001	Trenquelauquen, Argentina	Mar 31, 2001	Bovine	AY593786
A_{24}/Cruzeiro/BRA/55	Cruzeiro, Brazil	1955	Bovine	AJ251476
A/CAR/15/2000	Lahore Vina, Vina, Adamawa, Cameroon	2000	Bovine	EF208755
A/EGY/1/72	Alexandria, Egypt	May 13, 1972	Bovine	EF208756
A/EGY/1/2006	Ismailia, Egypt	Feb 9, 2006	Bovine	EF208757
A/EGY/2/2006	Ismailia, Egypt	Feb 9, 2006	Bovine	EF208758
A/EGY/3/2006	Ismailia, Egypt	Feb 9, 2006	Bovine	EF208759
A/EGY/4/2006	Fayoum, Egypt	Feb 16, 2006	Bovine	EF208760
A/EGY/5/2006	Domiat, Egypt	Feb 19, 2006	Bovine	EF208761
A/ETH/7/92	Shena, Ethiopia	Oct 3, 1992	Bovine	EF208765
A/ETH/1/94	Highland areas of Eastern Ethiopia	Feb 2, 1994	Bovine	EF208766
A/ETH/23/94	Nazret, East Shoa, Ethiopia	Mar 9, 1994	Not known	EF208767
A_5/Allier/FRA/60	Allier, France	1960	Bovine	AY593780
A/GAM/44/98	Gambia	Feb 4, 1998	Not known	EF208768
A_{10}/HOL/42	Groot-Ammers, the Netherlands	1942	Bovine	M20715
A/IND/17/77†	Tamil Nadu, India	1977	Bovine	AF204108
A/IRN/2/87	Mardabad, Kardaj, Tehran, Iran	Mar 11,1987	Bovine	EF208770
A/IRN/1/96	Zarnan, Shahriar, Tehran, Iran	Nov 13, 1996	Bovine	EF208771
A/IRN/22/99	Tabriz, East Azerbaijan Province, Iran	1999	Bovine	EF208772
A/IRN/1/2005	Ghalch-Sadri, Qom, Qom Province, Iran	Apr 4, 2005	Bovine	EF208769
A_{22}/IRQ/24/64	Mosul, Iraq	1964	Bovine	AJ251474
A_{21}/Lumbwa/KEN/64	Lumbwa, Kenya	1964	Bovine	AY593761
A_{23}/Kitale/KEN/64	Kitale, Kenya	1964	Bovine	AY593766
A/KEN/15/98	Meru, Kenya	Sep 8, 1998	Bovine	EF208774
A/KEN/16/98A	Nakuru, Kenya	Sep 15, 1998	Bovine	EF208775
A/KEN/29/2005	Embu, Eastern Province, Kenya	Aug 24, 2005	Bovine	EF208773
A/MAI/2/97	Mali	Not known	Not known	EF208776
A_{15}/Bangkok/TAI/60	Bangkok, Thailand	1960	Bovine	AY593755
A/TAI/118/87†	Sara Buri, Thailand	1987	Not known	EF208777
A/TAI/2/97	Thailand	1997	Not known	EF208778
A_{12}/UK/119/32	Kent, United Kingdom	1932	Bovine	AY593752

*WRLFMD, World Reference Laboratory for Foot-and-Mouth Disease.
†Not a WRLFMD reference no.

Total RNA was extracted from the first virus passage on BTy cells by using RNeasy kits (QIAGEN, Crawley, UK) for all 5 samples (EGY/1/2006–EGY/5/2006) (*4*). The complete VP1 region of the genome was amplified by RT-PCR by using 2 primer sets (A-1C562F/EUR-2B52R and A-1C612F/EUR-2B52R; Table 2) and the following thermal profile: 42°C for 30 min; 94°C for 5 min; 35 cycles of 94°C for 60 s, 55°C for 60 s, and 72°C for 90 s, followed by a final extension of 72°C for 5 min. The sequence of each amplicon was determined by cycle sequencing as previously described (*4*) but with the primers NK72, A-1C612F, and A-1D523R (Table 2). An unrooted neighbor-joining tree was constructed by using MEGA version 3.1 (*5*). The robustness of the tree topology was assessed with 1,000 bootstrap replicates as implemented in the program. Additionally, maximum parsimony (MEGA 3.1), minimum evolution (MEGA 3.1), and maximum likelihood (TREE-PUZZLE 5.2; [*6*]) trees were constructed; all 4 methods gave similar tree topologies (data not shown). Egyptian sequences shared a closer phylogenetic relationship with recent and historical isolates from East Africa rather than with contemporary serotype A viruses emerging from Iran, currently circulating in the Middle East and European Turkey (Figure 2).

Other conventional "typing" PCRs were performed to investigate whether additional FMDV serotypes were present in these samples. A multiplex agarose gel–based RT-PCR that targeted VP1 of O, A, C, and Asia 1 (primers P33, P38, P87–92, P40, P74–77) (*7*) generated a single band corresponding to the size expected (702 bp) for serotype A for all 5 samples (data not shown). In addition, a cocktail of primers (P1, P126, P150–153, P130, P159–161, P168–170) (*7*) recognizing VP1 of SAT1–3 serotypes did not show any bands after RT-PCR with these samples. However, amplicons of correct size (715 bp) were obtained after RT-PCR with samples EGY/1/2006, EGY/3/2006, and EGY/5/2006 when an additional primer set for SAT 1–3 VP1 (1D209F/2B208R) was used (*8*). Subsequent analysis of these SAT

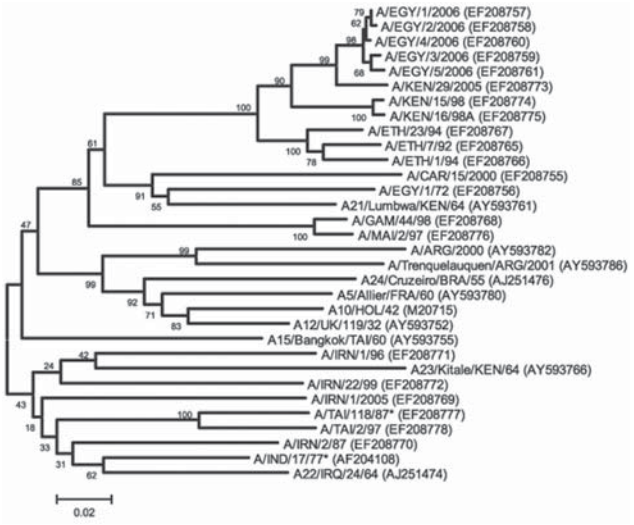

Figure 2. Midpoint-rooted neighbor-joining tree showing the relationships between the A Egypt 2006 virus isolates and other contemporary and reference viruses. Numbers indicate the percentage occurrence of the branches by the bootstrap resampling method. *Reference number not assigned by the World Reference Laboratory for Foot-and-Mouth Disease.

amplicons generated sequences that corresponded to serotype A, identical to the complete VP1 sequences of A/EGY/1/2006 and A/EGY/2/2006. Together, these findings support the conclusion that FMDV corresponding to a single serotype A was present in this material.

For vaccine selection, serologic tests were conducted to evaluate the extent of in vitro cross-neutralization of A/EGY/1/2006 and A/EGY/2/2006 by antisera produced against available FMDV vaccine strains (*9*). The match (r_1 value) against the vaccine strains A_{22}/Iraq/64 and A/Iran/96 that are regularly used elsewhere in the Middle East was less than the cut-off value of 0.3 ($r_1 = 0.23$ and 0.24, respectively), whereas an acceptable match ($r_1 = 0.42$) was found against the A/Eritrea/98 vaccine strain that is of East African origin. However, A/Eritrea/98 vaccine is not in routine production nor held in vaccine reserves and was therefore not available for immediate supply. A recent in vivo study demonstrated that a high potency A_{22}/Iraq/64 vaccine could provide clinical protection against challenge with the new A/EGY/2006 virus (B. Haas, pers. comm., 2006). High-potency vaccines are known to protect even when relationship values are lower than the normal cut-off values (*10*).

Conclusions

Local interpretation of agarose-based RT-PCR assays and sequence data led the Egyptian authorities to initially suspect the involvement of at least 2 serotypes, A and SAT 2. However, tests performed at the WRLFMD conclusively showed the presence of a single serotype, A, in the samples received from Egypt. Unofficial reports suggest that the disease was introduced by animals imported from Ethiopia for slaughter (*11*). This hypothesis is consistent with the results of the molecular typing, which suggested a relation between strains of Egyptian and East African origin. The molecular typing confirms only that through the trade in live cattle, an East African type A strain was introduced, which was not contained at the quarantine station. The origin of the infection is unclear, since the animals in quarantine may have acquired infection at various points during shipment, including possible contaminated pens or other animals on board the ship, at the port before loading, or in transit from Ethiopia to the port of loading. Veterinary inspection of the quarantined animals also detected cases of lumpy skin disease (LSD), and possibly the origin of the LSD epidemic in Egypt in 2006 may relate to the Ethiopian animal trade, which is supported by the reports of LSD epidemics in Ethiopia in 2005. Undoubtedly, the lack of reporting of disease preimportation or at the quarantine stations did not assist the authorities in controlling the disease. Because imported animals may acquire infection at any point up until their arrival, they must be vaccinated and tested for the absence of FMDV nonstructural proteins.

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Table 2. Oligonucleotide primers used for RT-PCR and sequencing*

Primer name	Primer sequence (5′→3′)	Sense	Gene	Position†
A-1C562F	TACCAAATTACACACGGGAA	Forward	VP3	3123–3142
A-1C612F	TAGCGCCGGCAAAGACTTTGA	Forward	VP3	3173–3193
EUR-2B52R	GACATGTCCTCCTGCATCTGGTTGAT	Reverse	2B	3963–3988
NK72	GAAGGGCCCAGGGTTGGACTC	Reverse	2A/2B	3897–3917
A-1D523R	CGTTTCATRCGCACRAGRA	Reverse	VP1	3748–3766

*RT-PCR, reverse transcription–PCR.
†Position on the genome of A_{21}/Lumbwa/KEN/64 (GenBank accession no. AY593761).

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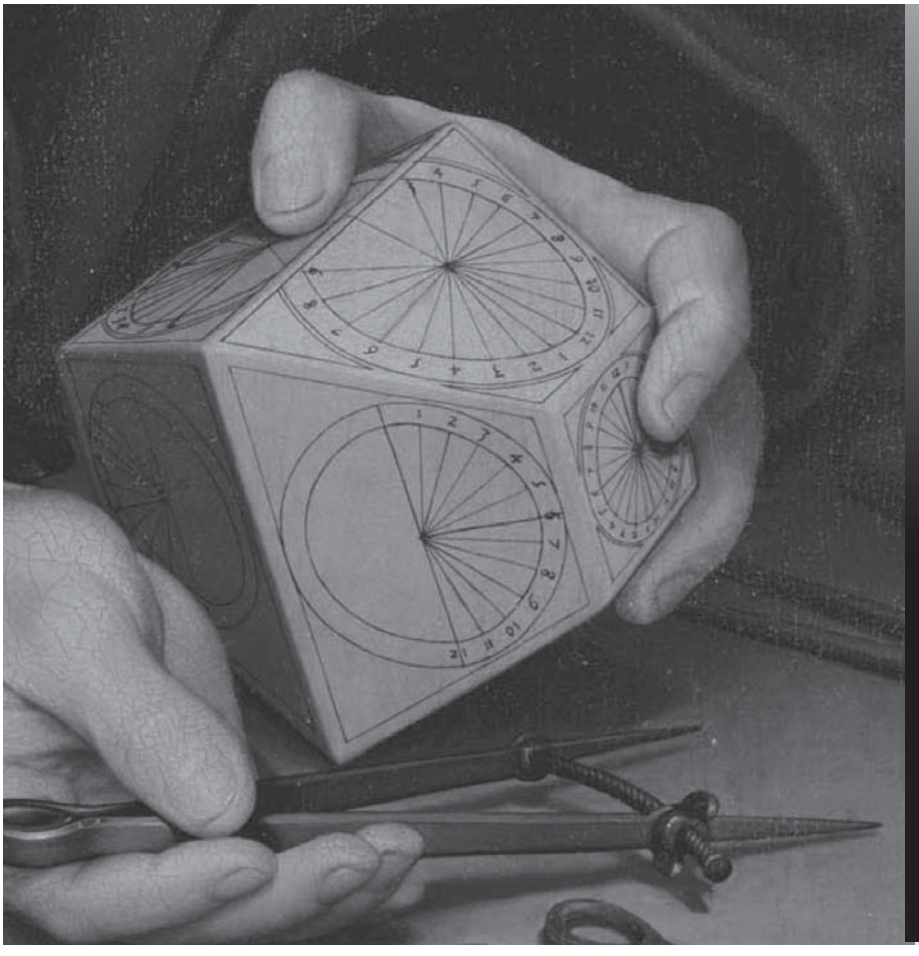

Malaria Diagnosis and Hospitalization Trends, Brazil

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Maria das Gracas C. Alecrim,†
Monica R.F. Costa,† and Gerald V. Quinnan Jr*

We focused on rates of malaria in the state of Amazonas and city of Manaus, Brazil. *Plasmodium vivax* accounted for an increased number and rate of hospital admissions, while *P. falciparum* cases decreased. Our observations on malaria epidemiology suggest that the increased hospitalization rate could be due to increased severity of *P. vivax* infections.

The study of malaria prevalence in the state of Amazonas and city of Manaus indicates an increase in the percentage of hospitalized *Plasmodium vivax* patients and an overall increase in malaria cases caused by this parasite. Our observations on malaria epidemiology and case treatment suggest that the increased hospital admissions are associated with a higher frequency of severe disease associated with *P. vivax* infections. Amazonas includes most of the Brazilian Amazon Region, where malaria has been controlled but never eradicated. Since the 1980s, there has been a reemergence of malaria, which appears to coincide with changing malaria control policies associated with the ending of the Malaria Eradication Campaign (*1*,*2*).

From January through August 2003, the number of cases nationwide was reduced by 2.6%, when compared with the same period in 2002. However, this change did not represent a uniform reduction in the number of malaria infections within the country. The states of Amazonas, Rondônia, and Tocantins reported increases of 82.9%, 14.7%, and 10.3%, respectively (*3*). Perhaps the best indicator of what has been occurring with malaria control during the past 5 years is reflected in recent statistics for malaria in Amazonas and the city of Manaus. During 2002 and 2003, the number of malaria cases reported in Amazonas increased 103.3% (*4*).

An observational study conducted in the reference center for diagnosis and treatment of malaria in Amazonas (Fundação de Medicina Tropical do Amazonas [FMT-AM]) described severe disease, including thrombocytopenia with hemorrhagic manifestations during infection with *P. vivax*. In that series, 46 (61.3%) of 75 patients admitted to the hospital for treatment of *P. vivax* malaria were classified with severe disease using predetermined criteria (*5*). We considered increased case severity as the need to hospitalize patients for treatment. Our primary goals were to present the epidemiology of malaria in Amazonas and the city of Manaus from 1980 to 2006 and to describe the overall rates, prevalence, and admission rates of malaria caused by *P. falciparum* and *P. vivax*.

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The Study

We extracted total yearly cases of malaria and population size in Amazonas from the database maintained by the Brazilian Ministry of Health (DATASUS, 2004), National Foundation of Health (*6*–*8*), and Secretary of Surveillance in Health (*3*,*9*). Data from FMT-AM were extracted from the malaria logbooks (for the years before the Foundation started publishing the reports) and from the Quarterly reports (for the years that the Foundation published the reports). All malaria cases diagnosed and referred for treatment are maintained (1989–1994) and quarterly reports are published by the FMT-AM (*10*). Quarterly reports published from 1995 to 2004 provided the total number of malaria diagnoses, case-patients admitted to the hospital, and number of deaths. Data from 2005 and 2006 were obtained by one of the authors (M.R.F. Costa) directly at FMT-AM (Subgerência de Arquivos Médicos e Contas Hospitalares). The hospital protocol is to exclude mixed infections by additional testing. We collected and tabulated data from these sources by year, parasite species, admissions, and percent admissions (Table). Percent admission was calculated as the total number of case-patients admitted to the hospital due to the specified parasite, divided by the total number of malaria cases caused by that same parasite in FMT-AM during that year, multiplied by 100.

Malaria cases from all causes in Amazonas, 1980–2006, are presented in Figure 1. An irregular increase is noted since 1988, reaching a peak in 1999, followed by a decline in 2001, only to rise again in the following years. A decrease was observed in 2006, but the data are not final. Figure 1 also shows the total number of malaria cases diagnosed at FMT-AM; fluctuations observed are very similar in direction and relative magnitude to those found statewide.

The number of infections due to *P. falciparum* and *P. vivax* diagnosed annually at FMT-AM are shown in Figure 2, panel A. The percentage of diagnosed case-patients admitted to the hospital, by parasite and year, is presented in Figure 2, panel B. In 1989, 264 (20%) of the patients with a diagnosis of *P. falciparum* infection were admitted to the hospital, while only 26 (0.85%) of those infected with *P. vivax* required admission. While *P. falciparum* remains the main cause of malaria admissions,

Table. Total malaria cases in the state of Amazonas, Brazil, 1980–2006, and malaria case-patients diagnosed and admitted at FMT-AM by parasite, 1989–2006 *

		FMT–AM					
			Plasmodium falciparum		*P. vivax*		Other causes‡
Year	Amazonas†	Malaria, all causes	No. case-patients	No. admitted (%)	No. case-patients	No. admitted (%)	No. case-patients
1980	4,447	–	–	–	–	–	–
1981	8,169	–	–	–	–	–	–
1982	13,142	–	–	–	–	–	–
1983	10,299	–	–	–	–	–	–
1984	8,528	–	–	–	–	–	–
1985	11,196	–	–	–	–	–	–
1986	15,319	–	–	–	–	–	–
1987	15,233	–	–	–	–	–	–
1988	19,392	–	–	–	–	–	–
1989	34,944	4,347	1,262	264 (20.92)	3,043	26 (0.85)	42
1990	28,479	3,037	839	175 (20.86)	2,175	15 (0.69)	23
1991	45,849	5,765	664	179 (26.96)	5,076	23 (0.45)	25
1992	37,885	5,083	670	118 (17.61)	4,398	29 (0.66)	15
1993	55,364	10,157	2,834	325 (11.47)	7,284	24 (0.33)	39
1994	68,287	7,469	1,433	199 (13.89)	5,948	44 (0.74)	88
1995	52,602	5,765	1,049	174 (16.59)	4,518	30 (0.66)	198
1996	70,044	6,206	1,333	201 (15.08)	4,686	18 (0.38)	187
1997	94,382	10,483	1,871	186 (9.78)	8,506	175 (2.06)	106
1998	114,748	10,854	1,751	217 (12.39)	9,004	116 (1.29)	99
1999	167,722	19,967	4,459	341 (7.65)	15,238	155 (1.02)	270
2000	96,026	12,266	2,541	177 (6.97)	9,227	147 (1.59)	498
2001	48,385	4,315	813	127 (15.62)	3,443	95 (2.76)	59
2002	70,223	88,711	992	106 (10.69)	7,808	263 (3.37)	71
2003	143,343	30,017	2,213	150 (6.78)	27,679	677 (2.45)	125
2004	152,440	27,169	5,727	257 (4.49)	21,228	345 (1.63)	214
2005	229,330	31,243	8,698	264 (3.52)	22,174	378 (1.70)	371
2006	190,378	16,182	3,363	175 (4.31)	12,672	161 (1.27)	147

*2005–2006 data obtained at the Malaria Laboratory and Epidemiology Department of the FMT–AM by M.R.F.C. FMT-AM, Fundação de Medicina Tropical do Amazonas; –, data not available.
†Total malaria cases in the state of Amazonas.
‡Includes *P. malariae* infections and mixed infections (*P. falciparum* + *P. vivax*).

we observed a significant increase in *P. vivax* admissions: the mean percent admissions from 1989 to 1996 was 0.59% (standard deviation [SD] 0.18), increasing to 1.91% (SD 0.74) from 1997 to 2006. This relative increase in *P. vivax* malaria requiring admission to the hospital for treatment was disproportionate to the change in numbers of cases and to the relative frequency of *P. vivax* cases over *P. falciparum* malaria cases.

Conclusions

We presented the epidemiology of recent malaria cases in the State of Amazonas and city of Manaus, emphasizing the emergence of severe *P. vivax* malaria. Assuming that patients requiring hospital admission were sicker than those treated as outpatients, we observed that malaria transmission in this region was continuous and fluctuated in intensity. *P. vivax* was consistently the main cause of malaria, but the number of patients with *P. vivax* requiring hospital admission increased significantly in recent years. Changes in control operations were linked to the reestablishment of malaria in major urban areas of the Amazon basin, e.g., Belém (*11*). In Manaus, this could have had an impact on *P. vivax* disease manifestations and severity but did not seem to have affected the severity of disease caused by *P. falciparum,* perhaps because the current policy of early diagnosis and treatment has been reported to have a greater impact on disease caused by *P. falciparum* than *P. vivax* (*12*,*13*).

In this study, we assumed that case definition and criteria for admission at FMT-AM, for both *P. vivax* and *P. falciparum* malaria, were relatively constant (http://www.fmt.am.gov.br/). Our data showed that the likelihood of hospital admissions for case-patients diagnosed with *P. vivax* malaria increased substantially after 1996, while the percentage of *P. falciparum* admissions declined. The decreasing rate of admission for *P. falciparum* malaria during the later years of our study supports the interpretation that the criteria for admission to FMT-AM were not loosened. It is possible that referrals to FMT-AM from elsewhere in the region increased during this period, but that would likely

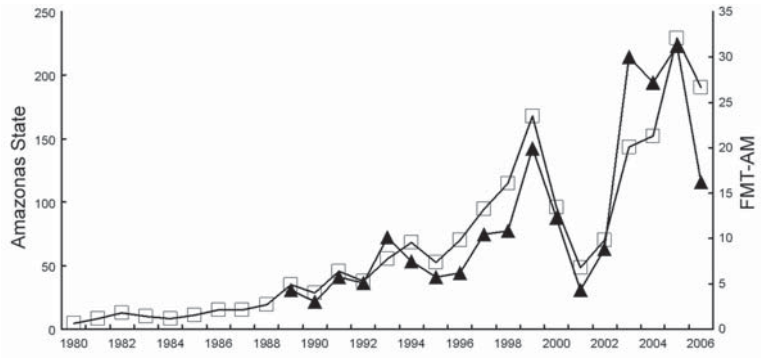

Figure 1. Malaria diagnoses (in thousands) according to blood smears positive for *Plasmodium falciparum* or *P. vivax* in Amazonas, Brazil, (open squares) and Fundação de Medicina Tropical do Amazonas (FMT-AM) (solid triangles), 1980–2006.

affect *P. falciparum* admissions too. Based on these considerations, we interpret the data as suggestive of an increased illness associated with *P. vivax* infections in the region.

In this study we did not attempt to describe the specific disease manifestations that were the basis for admissions of individual patients. However, recent reports described a range of unusual manifestations of *P. vivax* infection elsewhere (*14*), consistent with the disease manifestations reported in Manaus (*5*).

Biologic aspects of the human host, vector, and parasite and changes in the environment contribute to the epidemiology of malaria. Our data demonstrate that malaria is a growing health burden in the Amazon Region of Brazil and that disease caused by *P. vivax* is a substantial and increasing threat to the health of the population in Manaus. More studies are needed to understand the complex mechanisms of this disease and its impact on susceptible populations.

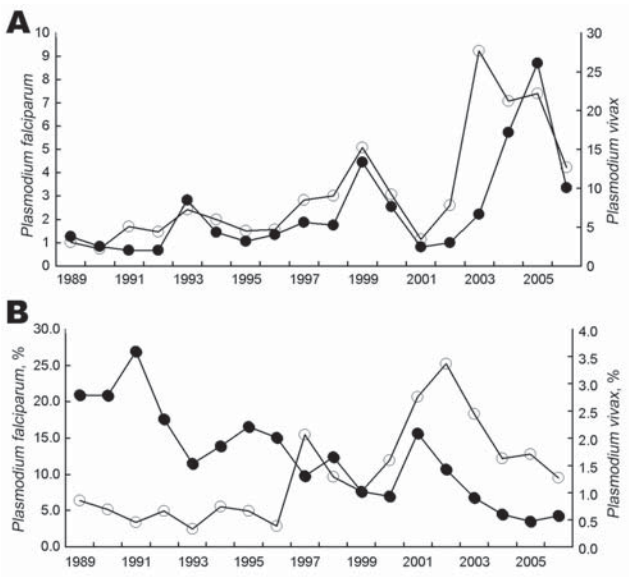

Figure 2. Malaria diagnoses by parasite species, *Plasmodium falciparum* (solid circles) and *P. vivax* (open circles), at the Fundação de Medicina Tropical do Amazonas (FMT-AM), Amazonas, Brazil, 1989–2006. A) Total numbers of diagnoses (in thousands). B) Percentage of infections resulting in hospital admission.

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Multifocal Avian Influenza (H5N1) Outbreak

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During March 2006, an outbreak of highly pathogenic avian influenza (H5N1) occurred in multiple poultry farms in Israel. The epidemiologic investigation and review of outbreak mitigation efforts uncovered gaps in planning for and containing the outbreak, thus affording valuable lessons applicable to other countries in similar settings.

On March 16, 2006, samples taken from a commercial turkey farm in southern Israel due to unexpected mortality rates (>0.7% per day) were positive for avian influenza subtype H5 by PCR. Highly pathogenic avian influenza (HPAI) subtype H5N1 was later confirmed by virus isolation. Eight more outbreak foci in commercial poultry farms in small settlements were identified within 2 weeks (Table 1). We briefly describe key findings of the outbreak investigation and lessons learned from our outbreak mitigation experience.

The Study

Epidemiologic investigation was performed by a joint team of veterinary and public health epidemiologists, administrators, and law enforcement officials. Descriptive epidemiology as well as cross-matching of available records were also performed to identify common factors associated with ≥2 foci and to establish potential flow of events. These records included log books of the affected farms, which contained daily mortality rates, identification of vehicles entering, personnel working and visiting the farm in the previous 30 days, and affiliated slaughterhouses.

In February 2006, influenza virus (H5N1) was detected for the first time in Egypt (*1*); in March 2006, outbreaks were detected simultaneously in the Palestinian Authority's Gaza Strip and Israel. Later in March 2006, a single case was detected in Jordan (*2*). Molecular characterization of the isolates from Israel and Gaza performed in the Veterinary Services Central Laboratory showed that they were different from influenza (H5N1) viruses recently isolated in Indonesia (*3*); they belonged to a single strain and were closely related to other HPAI (H5N1) strains isolated during this period in European, Asian, and African countries.

Turkey farms, accounting for 10% of Israeli poultry farms, were unproportionally involved in this outbreak (6/9 outbreak foci). The relative prevalence of turkey farms in the southern district near the Gaza Strip (50% of farms); the close interactions between personnel at farms of the same poultry type; and the higher susceptibility of turkeys to avian influenza virus (*4*) may be plausible explanations.

Several epidemiologic links between outbreak foci were identified (Table 1). These links and the near-simultaneous detection of several outbreak foci specifically on turkey farms, increase the likelihood that the virus disseminated through use of shared vehicles or by personnel. Alternatively, the involvement of 2 heavy breeder farms (farms F, H) characterized by strict biosafety procedures to prevent such transmission, and the fact that all 9 farms used open sheds, may support the role of migratory birds in disease transmission.

Because all epidemiology-trained veterinarians were assigned to regional outbreak containment at multiple foci, initiation of coordinated epidemiologic investigation in the farms was delayed by up to 10 days. Therefore, precrisis allocation of designated epidemiology-trained veterinarian investigators and joint investigation team training could be an important component of avian influenza preparedness plans.

The key control measures taken, the case definitions used, and the guiding principles for oseltamivir prophylactic treatment are summarized in Table 2. Israeli-Palestinian cooperation allowed coordination of cross-border mitigation efforts (*5*). Overall, these control measures enabled full outbreak containment within 17 days, without further recurrences (as of August 2007).

Rapid recruitment of teams willing and able to take part in culling and burial proved highly challenging. The Israeli Ministry of Defense was therefore assigned to coordinate and execute these efforts (through its civilian contractors) and did so effectively. Teams involved in poultry eradication activities were instructed to use N95 masks, disposable gowns, and safety goggles. Yet in hindsight, the investigation showed that, in some cases, the equipment was not used properly (e.g., gowns left open, mask lowered to uncover the nose) due to the challenging physical conditions in the hot and humid poultry houses. Shorter work shifts within the farms and better education of uninitiated workers are therefore key logistical aspects of preparedness.

Oseltamivir chemoprophylaxis (75 mg/day until 7 days after last exposure to poultry) was given to all culling teams, including poultry workers in the 3-km protection zones surrounding the infected farm. This policy was in accordance with European Center for Disease Prevention and

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Table 1. Confirmed highly pathogenic avian influenza (H5N1) outbreaks in Israel*

Focus ID	District	Poultry type	Biosecurity standards	Date (2006)			Epidemiologic links (identifiers)			
				Increased no. deaths	Report	Diagnosis	Culling	FS	SH	Vet.
A	Southern	Meat type turkeys	Normal	Mar 14	Mar 15	Mar 16	Mar 17	A	A,B	A
B	Southern	Meat type turkeys	Normal	Mar 14	Mar 16	Mar 16	Mar 18	B	A	B
C	Southern	Meat type turkeys	Normal	Mar 15	Mar 16	Mar 17	Mar 20	A	B	A
D	Jerusalem	Meat type turkeys	Normal	Mar 15	Mar 16	Mar 17	Mar 18	A	A	D
E	Southern	Broilers	Normal	Mar 13	Mar 19	Mar 19	Mar 21	A,C	C	E
F	Southern	Heavy breeders	High	Mar 19	Mar 19	Mar 19	Mar 21	D	*	F
G	Jehuda and Samaria	Meat type turkeys	Normal	Mar 21	Mar 21	Mar 22	Mar 23	D	*	G
H	Jerusalem	Heavy breeders	High	Mar 28	Mar 28	Mar 28	Mar 30	A	*	E
I	Southern	Meat type turkeys	Normal	Mar 30	Mar 31	Mar 31	Apr 1	A	*	B

*No slaughtering took place in the 30 days before or during the outbreak period. ID, identifier; FS, feed supplier; SH, slaughterhouse; Vet., veterinarian.

Control guidelines (*6*), but not with the guidelines of Centers for Disease Control and Prevention (*7*) or the World Health Organization (*8*) that recommend against providing prophylactic treatment to low-risk exposure groups. This extensive approach proved helpful in recruiting culling workers, relieving their fears, and in reassuring the local population potentially exposed to the infected poultry. Also, only a minute fraction (425 prophylactic courses) of the Israeli pandemic preparedness stockpile had to be used.

Timely and full (market price) compensation to farmers was key in encouraging prompt reporting and achieving trust and cooperation of poultry owners in culling and gathering epidemiologic data. Culling was performed by administering organophosphate poison in the flock's drinking water after 24 hours of water deprivation. This method proved lacking, as not all birds died as a result of this process. In certain cases, birds had to be manually slaughtered, a method that potentially exposes workers to increased risk for infection. Alternative culling methods such as use of asphyxiating foam are now considered for future outbreaks. The birds were buried above large polyethylene sheets within or in close proximity to the farm, and lime was applied to accelerate decomposition. Composting, a more environmentally friendly method that prevents ground water contamination (*9*), is considered for healthy birds culled in the protection zone. Only a few valuable birds (i.e., in zoos) were vaccinated with stockpiled H5N2 vaccines, because in some cases these vaccines may increase circulation of H5N1 viruses by allowing asymptomatic infections (*10*,*11*) potentially leading to continuous silent spread of the disease among birds (and subsequently to humans).

Table 2. Veterinary and public health measures taken during the highly pathogenic avian influenza (H5N1) outbreak in Israel, by proximity to infected poultry

Measure taken	Location by proximity to outbreak focus			
	Infected flock	Protection zone (<3 km)	Surveillance zone (3–10 km)	Outside outbreak area (>10 km)
Management of poultry	Stamping out	Stamping out	Active surveillance: transportation of poultry and hatching eggs allowed only following PCR testing of samples within the previous 72 h	Passive and active surveillance
Poultry products management	Destroyed	Destroyed	Released for consumption after clinical examination of the laying flocks proved negative	No restrictions
Poultry contacts monitoring	Self-monitoring	Self-monitoring	None	None
Case definition of human suspected avian influenza	Close contact with poultry and any ILI*	Close contact with poultry and severe ILI†	None	None
Oseltamivir prophylaxis to poultry contacts	All poultry contacts (including all culling and burial teams)	All poultry contacts (including all culling and burial teams)	None	None

*ILI, influenza-like illness: respiratory symptoms and fever (>37.5°C).
†ILI as defined above, in severity that requires hospitalization.

The Israeli public proved quite attentive to risk communication efforts as shown by the results of a national telephone survey conducted at the peak of the outbreak by the Israeli Center for Disease Control. Among a random sample of Israelis ≥21 years of age, 34 (62%) of 552 interviewees who were aware of the outbreak and generally consumed poultry products did not reduce poultry consumption at all due to the outbreak. In contrast, a recent preevent survey in the United States has shown that 40% of respondents would stop eating poultry products altogether if the H5N1 virus was detected. (*12*). This outbreak was also not associated with a massive increase in "worried well" hospital admissions. Only 24 patients (21 adults and 3 children) came to local hospitals due to self-defined or general practitioner-defined suspected avian flu during March 2006. Five of these 24 persons (4 adults and 1 child) indeed met the case definition of suspected case and were hospitalized, but none had laboratory-confirmed H5N1 infection. These results are probably derived, at least in part, from the effective frontline risk communication efforts of the district health officers who offered guidance to local general practitioners and the anxious public at the outbreak scene.

Conclusions

Preparedness planning for avian influenza should account for the unique challenges associated with a simultaneous multifocal outbreak, including personnel recruitment and allocation; coordination of all parties involved in outbreak mitigation and investigation; simultaneous culling and disposal in multiple sites; and coordinated central and local risk communication efforts. Outbreak containment, even in these settings, could be achieved without the use of vaccines, which should be kept as a measure of last resort. Case definition and antiviral prophylactic policies may be revised ad hoc according to the unfolding events and in response to the medical and psychological needs of each population. The lessons learned and described in our study may serve to refine preparedness plans elsewhere in view of the increasing global dissemination of this virus.

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Rapid Field Immunoassay for Detecting Antibody to Sin Nombre Virus in Deer Mice

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We developed a 1-hour field enzyme immunoassay (EIA) for detecting antibody to Sin Nombre virus in deer mice (*Peromyscus maniculatus*). The assay specificity and sensitivity were comparable to those of a standard EIA. This test will permit identification of rodents with antibody to this and perhaps other hantaviruses.

Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are rodentborne or insectivoreborne viruses; some are recognized causes of human hemorrhagic fever with renal syndrome or hantavirus pulmonary (or cardiopulmonary) syndrome (HPS) (*1*). The normal transmission cycle is rodent to rodent, without arthropod intermediate hosts. Each hantavirus has a single principal reservoir host, which suggests a coevolutionary relationship (*2*). In North America, the principal cause of HPS is Sin Nombre virus (SNV) because of the geographically widespread nature of its rodent host, the deer mouse (*Peromyscus maniculatus*), the most common mammal in North America.

As with other rodent reservoirs that harbor unique hantaviruses, most, if not all, deer mice become persistently infected without discernible pathologic consequences (*3*,*4*), which makes distinguishing infected from uninfected deer mice by simple observation impossible. Development of a field-relevant technique for detection of antibody to SNV would be of value; the technique could be exploited for further investigations of the virus–reservoir host interactions and characteristics and to determine whether experimental infections of deer mice with SNV accurately parallel natural infections (*3*,*4*).

Commonly used serologic tests for deer mice require a minimum of 3–5 hours to complete (*2*,*5*,*6*) and thus are impractical to use in the field in a single day without putting the rodents at risk for death from heat, cold, dehydration, trap injuries, and other hazards while tests are being conducted. We modified a previously described protein-A/G horseradish peroxidase enzyme-linked immunosorbent assay (PAGEIA) to detect antibodies to SNV in deer mice (*7*). The test can be completed in ≈1 hour under relatively primitive field conditions. The assay has advantages over more laborious assays used for similar purposes and, because it is mammal-specific rather than species-specific, we expect this assay will be applicable to serologic tests of mammals of many other species.

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The Study

A fragment of the S segment (nt 43–394) encoding part of the nucleocapsid was cloned into pET21b with a C-terminal His tag to produce a 15-kDa truncated antigen (*8*) for use in the assay. Deer mice were trapped near Fort Lewis, Colorado, and blood was collected as previously described (*9*); whole blood was diluted in (1:100) 1 mL of phosphate-buffered saline (PBS) in 96 deep-well plates (P-DW-11-C, Axygen, Union City, CA, USA) at time of collection to expedite sample loading. The remainder of the blood was frozen on dry ice and returned to the laboratory for additional testing.

Wells of 96-well polyvinyl chloride plates (Falcon 353912, BD Biosciences, San Jose, CA, USA) were coated with 100 μL of 2 μg/mL recombinant nucleocapsid in PBS and blocked (0.25% gelatin in PBS) a week in advance. Wells were washed in the field 3× with 200 μL of PBS (pH 7.0) by using an 8-channel pipettor, and blood in PBS was added from the deep well plate; positive and negative (1:100) controls (diluted in PBS) were included. Plates then were incubated at ambient temperature (range ≈23°C–29°C) for 30 min. After 3 more washes with PBS/0.5% Tween-20, 100 μL of pretitrated staphylococcal protein-A/streptococcal protein-G horseradish peroxidase conjugate (Pierce Biotechnology, Inc., Rockford, IL, USA) diluted 1:1,000 in PBS was added for 30 min. Plates again were washed 3× with PBS-Tween-20, and 100 μL of activated ABTS substrate was added to each well. After 15 min of incubation at ambient temperature, wells were scored by using a 0–4+ system, with 0 indicating no reaction (i.e., clear, no color) and 4+ representing the strongest signal (i.e., dark green color). Samples deemed 1+, 2+, 3+, or 4+ were considered positive (very weak, weak, strong, very strong, respectively). Samples were retested under laboratory conditions with PAGEIA and standard Centers for Disease Control and Prevention (CDC) enzyme immunoassay (EIA) (*5*).

Blood samples from 222 deer mice were collected during 3 trapping sessions in the summer of 2006, and 39 samples were scored as positive in the field by PAGEIA; 183 were negative by the field PAGEIA, repeat laboratory PAGEIA, and the standard EIA in the laboratory. One sample (HA-2564) was scored negative by field and laboratory PAGEIA, but (low) positive (optical density [OD] of 0.327) by conventional EIA (Table).

Table. Comparison of results of PAGEIA and standard EIA for detection of antibody to Sin Nombre virus (SNV) in blood samples from 40 deer mice captured in southwest Colorado, 2006*

Accession no.	Field PAGEIA score†	Laboratory PAGEIA OD‡	Laboratory PAGEIA score§	Standard EIA OD¶	Standard EIA score#
HA-2548	3+	1.254	Pos	0.903/0.066	Pos
HA-2552	4+	2.406	Pos	1.083/0.113	Pos
HA-2554	4+	1.788	Pos	1.395/0.058	Pos
HA-2558	4+	2.383	Pos	1.462/0.055	Pos
HA-2560	4+	1.913	Pos	1.378/0.086	Pos
HA-2564	0	0.001	Neg	0.327/0.055	Pos
HA-2565	1+	0.236	Pos	0.715/0.046	Pos
HA-2567	4+	2.123	Pos	1.485/0.080	Pos
HB-2604	4+	2.065	Pos	1.161/0.067	Pos
HB-2608	2+	0.855	Pos	0.653/0.095	Pos
HB-2612	1+	0.282	Pos	1.136/0.071	Pos
HB-2616	1+	0.311	Pos	0.458/0.001	Pos
HB-2617	2+	0.517	Pos	0.819/0.008	Pos
HB-2618	2+	0.494	Pos	1.085/0.009	Pos
HB-2622	3+	1.254	Pos	1.519/0.029	Pos
HB-2628	1+	0.493	Pos	0.220/0.082	Neg
HB-2630	3+	1.609	Pos	0.681/0.008	Pos
HA-2570	4+	1.970	Pos	0.389/0.024	Pos
HA-2578	4+	2.101	Pos	1.185/0.017	Pos
HB-2642	4+	2.784	Pos	1.294/0.063	Pos
HA-2601	4+	2.699	Pos	0.921/0.121	Pos
HA-2609	2+	0.608	Pos	0.228/0.042	Neg
HA-2612	4+	2.482	Pos	1.072/0.085	Pos
HA-2616	1+	0.331	Pos	0.076/0.059	Neg
HB-2681	3+	0.977	Pos	1.392/0.048	Pos
HB-2682	3+	1.095	Pos	1.326/0.042	Pos
HA-2634	4+	3.010	Pos	0.863/0.014	Pos
HA-2647	4+	2.824	Pos	0.720/0.023	Pos
HA-2666	4+	2.682	Pos	0.477/0.028	Pos
HB-2706	1+	0.836	Pos	0.324/0.032	Pos
HB-2710	2+	0.664	Pos	0.155/0.035	Neg
HB-2712	3+	1.599	Pos	0.345/0.033	Pos
HB-2717	1+	1.098	Pos	0.293/0.027	Pos
HB-2720	3+	2.581	Pos	0.630/0.039	Pos
TS-0830–6	2+	0.889	Pos	0.799/0.097	Pos
TS0830–7	1+	0.000	Neg	0.030/0.033	Neg
TS-0830–8	3+	2.014	Pos	0.800/0.024	Pos
TS-0830–9	4+	1.949	Pos	0.884/0.054	Pos
TS-0830–18	4+	2.112	Pos	1.180/0.021	Pos
TS-0830–20	3+	1.427	Pos	0.820/0.072	Pos

*PAGEIA, protein-A/G horseradish peroxidase enzyme-linked immunosorbent assay; EIA, enzyme immunoassay; OD, optical density; Pos, positive; Neg, negative.
†Field scores were based upon visual inspection without instrumentation, with 0 as negative, 1+ as very weak, 2+ as weak, 3+ as strong, and 4+ as very strong, relative to positive and negative control samples.
‡Field-collected samples were retested by PAGEIA under laboratory conditions and the OD reported here. The instrument was blanked on the negative control sample.
§OD >0.200 above the negative control was considered positive.
¶For laboratory EIA, OD was recorded for diluted (1:100) blood with both SNV antigen (numerator) and control antigen (denominator).
#A sample tested with SNV antigen and having an OD $\geq$0.300 was considered positive if the OD of that sample with the control antigen was $\leq$0.150. In regard to sample HBV-2717, the OD of the laboratory EIA with antigen was 0.293, very near the acceptable minimum, and the background was 0.027, which is very low; this sample was considered provisionally positive.

Of the 39 samples that were scored positive in the field, 5 discrepancies between these and laboratory tests were found (Table). One sample (TS-0830–7) scored as 1+ in the field was determined to be negative on subsequent laboratory testing by both PAGEIA and conventional EIA. The other 4 samples (HB-2628, HA-2609, HA-2616, HB-2710) were scored as positive by field and laboratory PAGEIA but negative by conventional EIA. In the field, each of these samples was scored as 1+ or 2+ and had ODs of 0.331–0.664 by laboratory PAGEIA. However, ODs ranged from 0.076 to 0.228 by conventional EIA.

The PAGEIA results were similar to results of conventional EIA, with a specificity of 82.9% (184 negatives/222 total rodents) versus 84.7% (188/222) for conventional EIA. The sensitivity of the PAGEIA was 97.1% (34 positive by PAGEIA/35 positive by conventional EIA).

Conclusions

We have modified an existing serologic assay so that it is suitable for use in the field. The assay relies on a staphylococcal protein-A and streptococcal protein-G horseradish peroxidase conjugate (*10*). Each protein has the capacity to bind to the Fc portions of antibodies, including immunoglobulin M (IgM) and IgA for protein A (*11*,*12*), but has highest affinity for IgG subclasses of many mammalian species.

All samples scored 3+ or 4+ were also positive in laboratory tests when results were read by using a spectrophotometer. Thus, we are confident that such samples in the field will indicate seropositive animals. Because we are suggesting that this assay be used for identifying seropositive rodents and not for determining seroprevalence (although it appears to be adequate for those studies as well) and to be conservative, we considered only samples that appeared dark green (3+ and 4+) in the field assay to be positive with relative certainty. To minimize the complexity of the PAGEIA under field conditions, we did not use a negative control antigen to assess nonspecific reactivities of serum samples. Use of this test will allow deer mice with antibody to SNV to be identified. Deer mice are the population most likely to be naturally infected with that virus, and those rodents can be retained for further testing and for studies of tissues, live cells, and body fluids to be used for subsequent laboratory investigations, such as for determining cellular immunologic responses, viremia levels, viruria levels, and virus shedding in excreta and secreta.

Additional limitations of the PAGEIA are similar to those of other serologic tests. PAGEIA can detect only seropositivity, which is not necessarily indicative of current infection or of current shedding of virus. It also binds only with high affinity to IgG; thus, it is not useful for discriminating other immunoglobulin classes, such as IgM, the presence of which usually indicates recent infection.

Because of the broad mammalian species specificities of a protein-A and protein-G conjugate, the rapid PAGEIA likely can be used to test for antibodies to other antigens in other mammals. Lee et al. (*7*) characterized the reactivities of protein A and protein G with IgG from rodents of several species. They found that serum specimens from both sigmodontine rodent species (deer mice and hispid cotton rats, *Sigmodon hispidus*) they tested were recognized by protein-A and/or protein-G conjugates. Similar laboratory-based PAGEIAs have also been used to detect antibody to antigens of agents causing other infectious diseases, including severe acute respiratory syndrome coronavirus-like viruses and Nipah virus in bats (*13*–*15*).

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Super-Sentinel Chickens and Detection of Low-Pathogenicity Influenza Virus

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Chicken interferon-α administered perorally in drinking water acts on the oropharyngeal mucosal system as an adjuvant that causes chickens to rapidly seroconvert after natural infection by low-pathogenicity Influenza virus. These chickens, termed super sentinels, can serve as sensitive early detectors of clinically inapparent infections.

Early detection of low-pathogenicity type A influenza virus (LPAI) circulating among chickens is important for 3 reasons: 1) these are the most prevalent strains in nature and can cause substantial losses for commercial poultry producers (*1*), 2) these strains can contribute genetic material to high-pathogenicity type A influenza virus (HPAI) (*2*), and 3) the H5 and H7 LPAI strains can mutate to HPAI with catastrophic effects in birds and with the potential for transmission to humans with lethal consequences (*3*). Kuiken et al. reported that an HPAI (H7N7) isolate was observed in February 2003 in the Netherlands, which most likely originated in free-living ducks and had evolved into a highly pathogenic variant after introduction into poultry farms (*4*). Although subsequent serologic screening of poultry showed that the H7 influenza virus had been affecting the Dutch poultry industry several months before the major epidemic, its presence had not been recognized (*4*). Our study addresses this problem by using a novel method that causes chickens to seroconvert under conditions in which LPAI would otherwise go undetected. This report shows that recombinant chicken interferon-α (rChIFN-α) (*5*) administered perorally in drinking water (*6*) acts as an adjuvant to produce a super-sentinel chicken that is a sensitive and early detector of clinically inapparent LPAI.

The Study

In 2003, the first clue to an aberrant condition in a commercial flock of laying hens in Connecticut was signaled by a drop in feed consumption and then in egg production. It took 6–7 weeks from the time tracheal samples were sent to a diagnostic laboratory to confirm the diagnosis of LPAI (H7N2) infection at National Veterinary Services Laboratory (NVSL) (N. Adriatico, pers. comm.). One such isolate, A/CK/CT/72/2003(H7N2), was obtained from the US Department of Agriculture, NVSL, Ames, Iowa, and used throughout this study to determine whether the peroral administration of rChIFN-α under conditions found to ameliorate Newcastle disease (*6*), infectious bronchitis (*7*), and infectious bursal disease (*8*), would similarly affect avian influenza. We reasoned that if the spread of LPAI could be slowed or prevented, the probability of its mutating to HPAI would be proportionately reduced, thereby lowering the chances of transmission to humans. In the course of this study, we observed a strong adjuvant effect of rChIFN-α administered in drinking water under conditions of virus transmission that mimic natural infection in chickens. This led to the concept of the super-sentinel chicken described here.

Three-week-old specific-pathogen-free (SPF) white leghorns (Charles River Specific Pathogen Free Avian Supplies [SPFAS], Inc., Storrs, CT, USA) were tagged and divided into 2 groups of 10 chickens each. Two birds in each group were overtly infected intravenously or intranasally with 10^6 infectious particles, measured as plaque-forming particles in primary chicken kidney cells (Charles River SPAFAS, Inc.). This strain of LPAI (H7N2) required a high inoculum to ensure infection (data not shown), comparable to that reported for another LPAI (H7N2) strain evaluated in SPF chickens (*9*). The 8 remaining cage mates in each group served as sentinel birds naturally subject to infection by the respiratory tract, ingestion of fecal material, or both. One group of birds received plain drinking water; the other group received drinking water that contained 2,000 U/mL rChIFN-α. The water was provided ad libitum and changed daily. Water consumption was the same in both groups, as determined from the amount remaining after a known volume was provided each day (data not shown). With a half-life of 3–5 days in water at room temperature (*6*), this concentration of interferon (IFN) delivered an average dose of $\approx 3 \times 10^5$ U rChIFN-α/bird/day. Fourteen days post overt infection (dpi), the rChIFN-α-water was replaced with plain water for the remaining 14 days of the study. This dose of rChIFN-α was sufficient to ameliorate Newcastle disease (*6*).

Following overt infection of 2 birds per cage, and the natural cross-infection of the 8 cage mates, serum samples were taken from each of the 10 birds at the intervals indicated in Figure 1. This figure shows data from 2 independent studies that used agar gel precipitin (AGP) tests to detect antibody against avian influenza virus nucleoprotein and M1 antigens. This qualitative test demonstrated that of the 16 naturally infected chickens given plain water, none seroconverted over the 28-day period they were exposed to the 2 infected cage mates. In marked contrast, of the 16 naturally infected chickens given water containing IFN, 14

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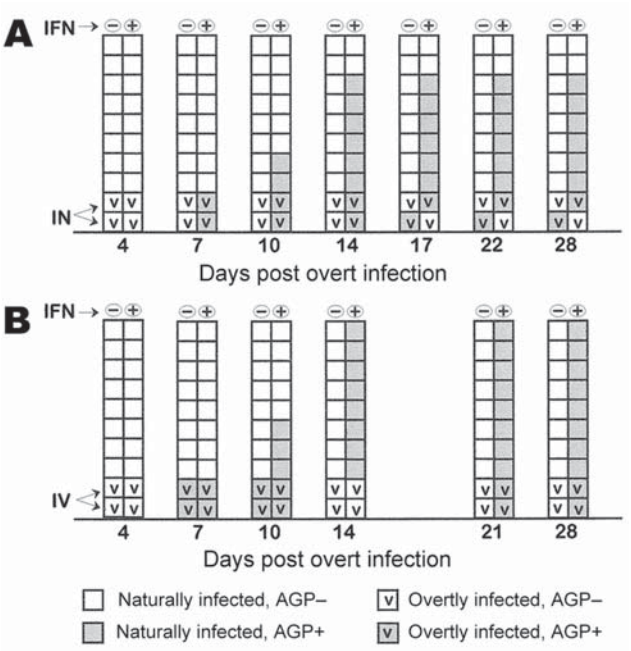

Figure 1. Seroconversion in specific-pathogen-free white leghorns after infection with influenza A/CK/CT/72/2003 (H7N2) as measured by agar gel precipitin (AGP) tests for avian influenza virus nucleoprotein and M1 antigens. Each box represents 1 chicken; (–), water; (+), water plus recombinant chicken interferon-α at 2,000 U/mL. IFN, interferon; IN, intranasal; IV, intravenous. A and B are independent trials. Serum samples were obtained at the times indicated on days post infection for overtly infected birds.

were seropositive by 14 dpi and remained so during the 28-day test period.

Figure 2 shows the number of seroconverted birds in a third study as quantified by hemagglutination inhibition (HI) titer (HI U/mL) of serum samples taken at the time intervals indicated as dpi. None of the 8 naturally infected birds given plain water seroconverted during the 28 days of the trial. In contrast, the 8 naturally infected chickens raised on IFN-water all seroconverted by 10 dpi (8/8), as did the overtly infected birds. Similar results were observed in 2 other trials. In all, 4 independent comparable trials were conducted, representing 2 AGP and 2 HI tests (Table 1). The marked contrast in the fraction of naturally infected birds that seroconverted on plain water and IFN-water is evident.

Although the sensitivity of LPAI to the action of IFN is well documented (*10*,*11*), rChIFN-α in the drinking water may have been exacerbating the infection, thereby leading to high levels of virus and antigen and high levels of seroconversion. This possibility was tested by using quantitative real-time reverse transcriptase–PCR to determine the amount of avian influenza virus in tracheal samples at 2, 4, and 10 dpi. Table 2 shows that within the error expected

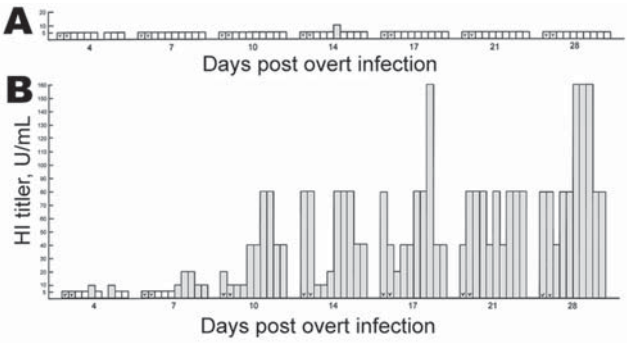

Figure 2. Seroconversion in sentinel specific-pathogen-free white leghorns after natural infection with influenza A/CK/CT/72/03 (H7N2) from overtly infected birds as quantified by hemagglutination inhibition (HI) tests for hemagglutinin (HA) antigen. The titer in HI U/mL is plotted as a function of days post overt infection of 2 birds in each group. The key is similar to that of Figure 1, except the assay is for HI. A, water only; B, water plus recombinant chicken interferon-α at 2,000 U/mL. Results of 1 trial are shown; 2 other trials gave similar results.

from testing individual chickens, the amount of infectious particle equivalents were not significantly different in birds given plain water or IFN-water. Thus, that more avian influenza virus antigen was produced in chickens that were given IFN-water is an unlikely explanation.

Conclusions

Although the role of IFN as an adjuvant when delivered perorally has been established in mammals (*12*), our data demonstrate for the first time, to our knowledge, that avian IFN administered in drinking water to naturally infected chickens lowers the threshold of antigen required to stimulate the adaptive immune response to an LPAI isolate. As a consequence, the action of perorally administered rChIFN-α in effect creates super-sentinel chickens that seroconvert in response to levels of antigen that would otherwise go undetected. Super-sentinel chickens would thus provide a novel means of detecting otherwise inapparent infections of LPAI, thereby buying time for its control or eradication.

Table 1. Seroconversion in influenza A virus–infected 3-week-old chickens given water or water + interferon*

	No. chickens		
Infection type†	Water	Water + interferon‡	Total
Overtly infected	4/8§	8/8	16
Sentinel	2/32	31/31	63
Combined	6/40	39/39	79

*Represents 4 independent trials.
†Overtly infected birds were mixed with uninfected sentinel cage mates, the latter to become infected naturally.
‡Recombinant chicken interferon-α at 2,000 U/mL (*5*).
§No. positive birds/total no. receiving treatment, scored by agar gel precipitin or hemagglutination inhibition tests.

Table 2. Influenza A virus infectious particle equivalents (IPE) in tracheal swabs from sentinel chickens given water or water + interferon and infected naturally*

Day postinfection	Water (IPE/mL)	Water + interferon† (IPE/mL)
2	1,112 ± 1,353‡	760 ± 632
4	1,234 ± 764	463 ± 484
10	1,325 ± 398	2,113 ± 1,834

*Each cage contained 2 overtly infected birds and 8 cage mates as sentinels. Only sentinel birds are reported. Chickens were 3 weeks old at the start.
†Recombinant chicken interferon-α in water at 2,000 U/mL (*5*).
‡Mean ± SD, n = 8. Quantitative real-time reverse transcriptase–PCR analysis with influenza A virus standard: IPE/mL.

We envision the introduction into a large flock of a number of small cages containing chickens in which IFN-water replaces plain water. These super-sentinel chickens will serve as sensitive early detectors of LPAI, like the proverbial canary used in mines to detect low levels of toxic gases. Because of the cross-reaction between chicken and turkey IFN-α (*5*,*13*), super-sentinel turkeys could likely be created in a similar manner. Super-sentinel birds could be replaced every month and possibly returned to production.

All strains of chickens tested, including those in the People's Republic of China, have proved to be sensitive to the action of rChIFN-α (*14*). Genetically engineered production of rChIFN-α (*15*), treatment with it optimized for dose and duration, and its long half-life in water may make it economically feasible to convert many birds in a flock to super-sentinel status. It also may be prudent to set up super-sentinel birds in areas of high risk for avian influenza virus outbreaks, such as live-bird markets. Surveillance of other families of birds might be possible with species-specific IFN. Further studies are required to test these possibilities and the extent to which rChIFN-α functions as an adjuvant with other strains of avian influenza virus and chickens.

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Human Metapneumovirus Infection among Children, Bangladesh

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We confirmed circulation of human metapneumovirus (HMPV) among children with febrile and respiratory illness in an urban slum in Dhaka, Bangladesh, during active surveillance in 2001. HMPV was the most common single virus identified among febrile children and appears to contribute to the high rates of illness in this population.

Human metapneumovirus (HMPV) is the newest member of the family *Paramyxoviridae*, in the subfamily *Pneumovirinae*, shared with respiratory syncytial virus (RSV) (*1*). It appears to have 2 distinct genetic subgroups (*2*,*3*). HMPV was first described in a population of children in the Netherlands in 2001 (*1*) and has subsequently been linked with lower respiratory tract illness (LRTI) in children and adults (*2*,*4*). Although HMPV independently contributes to LRTI, some studies report more severe cases when HMPV is a coinfectant with RSV (*5*,*6*) or influenza (*7*); other studies have found no synergy (*3*).

The Study

As previously reported (*8*), we undertook fever surveillance in Kamalapur, an urban community in Dhaka used by the International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B) as a field site since 1998. The site has 7 geographic strata and 379 clusters. We randomly selected clusters within strata and enrolled all households within those clusters for surveillance, after obtaining informed written consent.

Field research assistants (FRAs) screened for fever across all ages among 5,000 households once weekly using standardized calendar questionnaires. FRAs referred children <13 years of age who reported fever for any duration, or anyone ≥13 years who reported fever for ≥3 days, to our onsite clinic where study physicians conducted standardized history and physical examinations. If an axillary temperature of ≥38°C was confirmed, physicians collected 3–5 mL of blood from children <5 years and persons ≥5 years, respectively, as well as convalescent blood samples 14 days later. Blood samples were allowed to clot and then centrifuged to obtain serum.

We retrospectively selected serum samples to test for respiratory viruses from patients <13 years of age who had cough for 1–3 days and fever of ≥38.5°C; we also selected paired serum samples negative for dengue by immunoglobulin M antibody capture (MAC)–ELISA. These samples were sent to the Centers for Disease Control and Prevention (CDC; Atlanta, Georgia, USA) for testing by hemagglutination inhibition for influenza and enzyme immunoassay for RSV; parainfluenza types 1, 2, and 3; adenovirus; and HMPV by using standard methods (*9*,*10*). A positive acute HMPV infection was defined as a ≥4-fold rise in titer between acute-phase and convalescent-phase samples.

Statistical analysis was performed by using StataSE Release 9.2 (StataCorp, College Station, TX, USA). We compared continuous variables between groups by using analysis of variance. For univariate analysis of categorical variables, we used 2 × 2 tables; for multivariate analysis, we used conditional logistic regression to determine strength of association between HMPV infection and potential explanatory covariates to obtain relative odds (RO) and 95% confidence intervals (CIs); p values were obtained by using the Fisher exact test. This study was approved by the research review and ethical review committees of ICDDR,B and the Institutional Review Board of CDC.

From December 6, 2000, through December 5, 2001, 889 persons came to our clinic with fever, and blood samples were collected from 888 (99.9%). Of the 889, 775 (84.9%) were self-referred; 114 (93.4%) of 122 were referred by FRAs during the same period. Of the 888 sampled patients, we selected serum samples from 128 children <13 years of age who had paired samples, documented fever >38.5C, cough for 1–4 days before first blood collection, and negative test results for dengue antibodies by MAC-ELISA. These samples were tested by hemagglutination inhibition against influenza virus A (H1N1 and H3N2) and influenza type B. Among these, 107 paired samples had sufficient remaining serum to be tested for other respiratory viruses, including HMPV.

Table 1 shows the distribution of all virus infections detected by serologic testing of 107 paired specimens. Of 60 infections detected among these children, 20 (33.3%) were caused by HMPV, the largest single group after influenza (although more than either influenza A or B alone). HMPV was detected in the dry premonsoon season from January through the end of June (Figure).

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Table 1. Viruses detected in children <13 years of age by serology, Kamalampur, Bangladesh, December 2000–December 2001

Virus	No. infections	% (N = 60)	Cumulative %
Human metapneumovirus	20	33.3	33.3
Respiratory syncytial virus	3	5.0	38.3
Adenovirus	4	6.7	45.0
Parainfluenza virus 3	9	15.0	60.0
Influenza (H1N1)	8	13.3	73.3
Influenza (H3N2)	2	3.3	76.6
Influenza B	14	23.3	99.9

We found no demographic differences in subgroup analysis by age group (<5 years and ≥5 years) or between children with acute HMPV infection and noninfected children (Table 2). Also, no differences were found in the reported history of fever duration or other complaints associated with febrile or respiratory illness in this population before treatment.

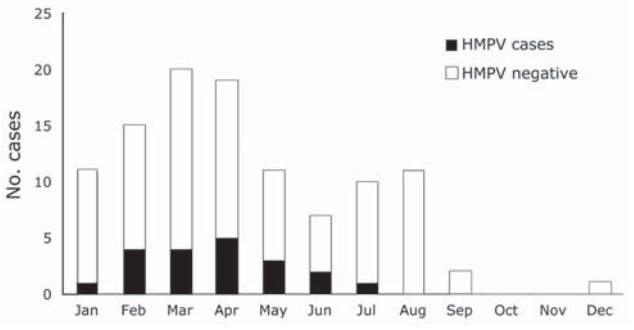

Figure. Human metapneumovirus (HMPV) infection in children <13 years of age, Kamalapur, Bangladesh, 2001.

Clinical findings (Table 2) showed no differences in mean fever or proportion of children with high fever (≥39°C). However, compared with non-HMPV infection, acute HMPV infection was 3.5 times more likely (95% CI 1.02–11.24) to be associated with clinical pneumonia in all children and 4.8 times more likely (95% CI 0.90–23.86) to be associated with altered mental status (irritability/lethargy) in children <5 years old (Table 2). Only 1 child with acute HMPV infection had coinfection with another virus (influenza A).

Conclusions

To our knowledge, ours is the first reported finding of HMPV in Bangladesh demonstrating substantial contribution of HMPV to febrile and lower respiratory tract illness in children <13 years of age. Our report substantiates that of a study from India (*11*). In a hospital study conducted in Bangladesh, a virus was isolated in only 33.3% of children with LRTI (*12*). Although HPMV was unknown at that time,

Table 2. Demographic and clinical characteristics of children <13 years of age presenting with fever, Kamalampur, Bangladesh, December 2000–December 2001*

Variables†	HMPV positive (N = 20)	HMPV negative (N = 87)	Relative odds (95% CI)	p value‡
Mean age, y§ (SD, 95% CI)	4.5 (2.6; 3.2–5.7)	4.5 (3.1; 3.9–5.2)	–	0.951
Mean age for 0–4 age range, y§ (SD, 95% CI)	2.9 (1.4; 2.1–3.8)	2.3 (1.2; 2.0–2.7)	–	0.113
Mean age for 5–12 age range, y§ (SD, 95% CI)	7.3 (1.9; 5.5–9.0)	7.7 (1.8; 7.1–8.4)	–	0.556
Children <5 y, no. (%)	13 (65.0)	52 (59.8)	1.25 (0.41–4.08)	0.801
Male gender, no. (%)	9 (45.0)	49 (56.3)	0.63 (0.21–1.89)	0.457
Duration of fever prior to clinic,§ d (SD, 95% CI)	3.1 (0.8; 2.7–3.5)	3.1 (1.7; 2.7–3.4)	–	0.983
Symptoms				
Headache, no. (%)	8 (40.0)	34 (39.1)	1.04 (0.33–3.10)	1.000
Body pain, no. (%)	4 (20.0)	25 (28.7)	0.62 (0.14–2.20)	0.580
Rhinorrhea, no. (%)	16 (80.0)	60 (69.0)	1.80 (0.51–8.06)	0.419
Difficulty breathing, no. (%)	3 (15.0)	4 (4.6)	3.66 (0.48–23.48)	0.119
Normal activity/behavior, no. (%)	15 (75.0)	64 (73.6)	0.73 (0.22–2.20)	0.620
Fever,§ °C (SD, 95% CI)	39.2 (0.6; 39.0–39.5)	39.1 (0.6; 39.0–39.2)	–	0.899
High fever (≥39°C), no. (%)	10 (50.0)	49 (56.3)	0.78 (0.26–2.32)	0.627
Respiratory rate§ (SD, 95% CI)	42 (11; 36–47)	41 (9; 39–43)	–	0.808
Crepitations (rales) or wheezing, no. (%)	11 (55.0)	28 (32.1)	2.57 (0.85–7.86)	0.072
Altered mental status,¶ no. (%)	5 (25.0)	8 (9.2)	3.29 (0.73–13.19)	0.065
Altered mental status¶ if <5 y#, no. (%)	5 (38.5)	6 (11.5)	4.79 (0.90–23.86)	0.035
Pneumonia/LRTI diagnosis, no. (%)	8 (40.0%)	14 (16.1)	3.48 (1.02–11.24)	0.029

*HPMV, human metapneumovirus; CI, confidence interval; SD, standard deviation; LRTI, lower respiratory tract illness.
†Categorical variables report N persons (percent of persons with characteristic in each group). Compared by 2 x 2 table, odds ratio with 95% CI.
‡2-tailed Fisher exact text.
§Continuous variables. Means compared by using Student t test.
¶Irritability, lethargy.
#N = 65 children <5 y.

and thus would not have been reported, the contribution of viruses to LRTI in children is often underreported by studies that have focused on bacterial infection identification or that did not include collection of paired serum samples to detect viral infections (*3*). HMPV has likely been a major factor in pneumonia and bronchiolitis in this population, as it has in others (*1*,*2*,*11*). In this study, HMPV was not only significantly associated with pneumonia, but with lethargy, an indicator of severe pneumonia in young children. Given the high rates of illness and death from pneumonia in this population (*8*), this association has important implications for disease control strategies. HMPV was also found in the dry pre-Monsoon season, when incidence of pneumonia peaks in this population. Similarly, parainfluenza peaks from March–April. In contrast, influenza occurs before and during the Monsoon season (March–August).

Our pilot study to assess the possible effects of HMPV on LRTI in children in Bangladesh had the following limitations: 1) healthy control patients were not included in the study; 2) the study was not originally designed to look for respiratory viruses; 3) fever was a main selection criterion and may have biased selection of more severe illnesses (a previous pneumonia study indicated that <33% of children in this environment with severe pneumonia have fever (*13*), perhaps substantially underestimating HMPV prevalence); 4) the observation period of 1 year may not represent the typical seasonal pattern; 5) case identification was based on serologic test results, and some children may have had a subclinical immune response or acute-phase samples may have been collected too late to observe a significant rise in titer, thus underestimating prevalence of disease; and 6) the study included insufficient cases to analyze viral interaction. To more clearly define the role of HMPV and other respiratory viruses in this population, and to improve disease control strategies, fever surveillance targeting a broader range of clinical syndromes over a sustained period is needed.

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Ciprofloxacin-Resistant *Neisseria meningitidis*, Delhi, India

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Decreased susceptibility of *Neisseria meningitidis* isolates to ciprofloxacin emerged from an outbreak in Delhi, India. Results of antimicrobial susceptibility testing of the meningococcal isolates to ciprofloxacin and further sequencing of DNA gyrase A quinolone-resistance–determining region confirmed the emergence of ciprofloxacin resistance in the outbreak.

Neisseria meningitidis serogroup A is the major cause of meningitis outbreaks worldwide, especially in African and Asian countries, including India. Meningococcal disease is endemic in India, and sporadic cases of meningococcal meningitis have occurred in Delhi in previous years (*1*). During 1966, 616 cases of meningitis were reported; case-fatality rate was 20.9%. In 1985, an outbreak of greater magnitude had 6,133 cases with 799 deaths (13%). An outbreak of meningococcal meningitis also occurred in Delhi during April–July 2005 (*1*), and the disease reappeared in January–March 2006.

When a sporadic case or epidemic occurs, the close contacts need to receive a vaccine and chemoprophylaxis with antimicrobial drugs to cover the delay between vaccination and protection. Recently, ciprofloxacin and ceftriaxone have been established as acceptable alternatives to rifampin for prophylaxis of meningococcal disease (*2*). Because ciprofloxacin can be used in single doses during meningococcal epidemics, ciprofloxacin is the chemoprophylactic agent of choice (*3*). Because meningococcal disease is a serious and rapidly progressing illness, monitoring the trends in the resistance to antimicrobial agents is important. To date, ciprofloxacin-resistant serogroup A *N. meningitidis* has not been reported anywhere in the world. Four reports of sporadic instances of decreased susceptibility to ciprofloxacin: serogroup B from France in 1999 and Spain in 2002, serogroup C from Australia in 1998, and serogroup Y from Argentina in 2002 (*4*–*6*). For the first time, to our knowledge, we report the emergence of decreased susceptibility of serogroup A *N. meningitidis* to ciprofloxacin from the 2005 outbreak in Delhi.

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The Study

A total of 444 meningococcal cases and 62 deaths due to meningococcal meningitis serogroup A were reported in Delhi during April–July 2005. The reappearance of meningococcal cases was reported in Delhi during January–March 2006 (177 meningococcal cases and 17 deaths). The meningococcal cases were reported from the major hospitals and were characterized by sudden onset of fever with petechial rash, neck stiffness, and altered sensory functions. All age groups were affected, but the highest proportion was in those 15–30 years of age; male patients accounted for 71% of cases and female patients 29%.

Fourteen *N. meningitidis* clinical isolates were collected from the major hospitals in Delhi. All these isolates were from patients with meningococcal meningitis from the outbreak. The strains were isolated mainly from cerebrospinal fluid and a few from skin swabs; the primary culture, isolation, and serogrouping (Latex agglutination kit, Wellcogen, Wellcome, Dartford, UK) were performed in the respective hospitals. *N. meningitidis* strains from Ranbaxy Research Laboratories culture collection (RRL-1 and RRL-2) were used as reference serogroup A strains. In addition, 11 clinical isolates collected during the January–March 2006 recurrent outbreak were also included for selective antimicrobial drug susceptibility testing in this study.

The MICs of antimicrobial agents against these isolates were determined by the agar dilution method on Mueller-Hinton agar plates with 5% sheep blood as recommended by Clinical and Laboratory Standard Institute (CLSI) guidelines (*7*). The pulsed-field gel electrophoresis (PFGE) method used in this study was based on the procedures described by Popovic et al. (*8*). Two isolates (Ap-II 420 and Ir-I 442) from the outbreak were used for genotyping as described by Maiden et al. (*9*) and analyzed by using the multilocus sequence typing (MLST) database (*10*).

Because the *N. meningitidis* isolates showed resistance to ciprofloxacin, the DNA gyrase A quinolone-resistance–determining regions (QRDRs) of 2 ciprofloxacin-sensitive and 7 ciprofloxacin-resistant meningococcal strains from this outbreak and 2 reference strains were sequenced. The following primers were used for QRDR study. The forward primer was 5′-CGTACTGTACGCGATGCACGA-3′; the reverse primer was 5′-TTTCGCCATGCGGATTTCGGT-3′. The genomic DNA was isolated from the strains and PCR was performed as described by Shultz et al. (*11*).

The MIC pattern of the clinical isolates of *N. meningitidis* from the outbreak showed that they were susceptible

to β-lactam antibiotics penicillin, ampicillin, and a third-generation cephalosporin, ceftriaxone. The MIC against ceftriaxone for all the clinical *N. meningitidis* isolates was <0.001 μg/mL. Of 14 strains, 12 showed decreased susceptibility to all the fluoroquinolones, especially to ciprofloxacin (MIC 0.25 μg/mL) (online Appendix Table, available from www.cdc.gov/EID/content/13/10/1614-appT.htm). In addition, all these 12 strains were resistant to nalidixic acid (MIC >16 μg/ml). A notable shift in antimicrobial susceptibility was not observed in these isolates against protein synthesis inhibitors (Online Appendix Table). Eleven meningococcal isolates from the recurrent outbreak showed resistance to ciprofloxacin as well as nalidixic acid (Table). The break points are based on the CLSI guidelines (*7*).

The PFGE patterns of the strains from the outbreak (9 strains) were indistinguishable, and they appeared to be from the same origin (Figure 1). The antimicrobial drug resistance patterns were not distinguishable from the PFGE types. The PFGE pattern of the strains from the outbreak was different from that of the reference strains RRL-1 and RRL-2. The housekeeping genes of the ciprofloxacin-resistant strain (Ir-I 442) showed no alteration or mutation, and they were identical to the ciprofloxacin-sensitive strain (Ap-II 420). *Neisseria* MLST analysis with the MLST database showed that these isolates were similar to the outbreak strains from Dhaka, Bangladesh (2002), and Nigeria (2003) (*10*). In addition to the endemicity in Delhi, the migration of persons or visitors from other countries may have contributed to the spread of the serogroup A outbreak.

The sequencing of the *gyrA* QRDRs of ciprofloxacin-resistant strains of *N. meningitidis* showed 4 amino acid differences. One of these encoded a conservative threonine substitution at position 91 (Thr-91 → Ile); the other 3 changes were synonymous: Asn-103 → Asp, Ile-111 → Val, and Val-120 → Ile (Figure 2). Gyrase A changes have been reported in clinical isolates of resistant meningococci in positions 91 (Thr-91 → Ile) and 95 (Asp-95 → Asn and Asp-95 → Gly) (*4*–*6*). The *N. meningitidis gyrA* gene shares 95% identity with the *N. gonorrhoeae gyrA* gene. The GyrA substitution in meningococci at position 91 (Thr-91 → Ile) was equivalent to Ser-91 → Ile, reported in fluoroquinolone-resistant *N. gonorrhoeae* from Japan (*12*). In meningococci as well as in gonococci, the mutation at position 91 causes fluoroquinolone resistance. For

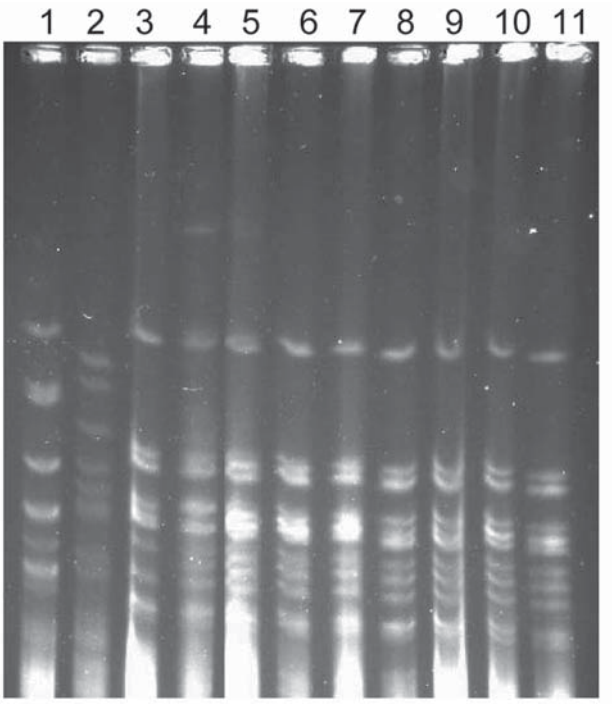

Figure 1. Pulsed-field gel electrophoresis (PFGE) pattern of the *Neisseria meningitidis* isolates from the outbreak. The chromosomal DNA digested with SpeI enzyme was separated by clamped homogeneous electric fields PFGE (BioRad, Hercules, CA, USA). 1, RRL-1; 2, RRL-2; 3, SFDJ 723; 4, Ap-II 420; 5, SFDJ E-95; 6, IR-I 442, 7, IR-II 440; 8, SFDJ E-100; 9, SFDJ 184; 10, SFDJ E-79; 11, NICD 18.

Table. Antimicrobial susceptibility pattern of *Neisseria meningitidis* isolates from the recurrent outbreak during January– March 2006, Delhi, India*

Isolate	MIC (μg/mL)									
	PEN	CRO	RIF	CIP	GAT	MXF	LVX	SPX	NOR	NAL
SFDJ M-1	0.125	0.008	0.004	0.125	0.03	0.125	0.125	0.06	0.125	>16
SFDJ M-2	0.125	0.008	0.004	0.25	0. 25	0.125	0.03	0.06	0.25	>16
SFDJ 691	0.03	<0.001	0.125	0.25	0.25	0.5	0.25	0.25	0.5	>16
SFDJ 306	0.03	0.008	0.008	0.125	0.06	0.125	0.25	0.06	0.25	>16
SFDJ 651	0.06	0.002	0.03	0. 5	0.125	0.25	0.25	0.125	0.5	>16
SFDJ 568	0.125	0.002	0.06	0.25	0.125	0.25	0.25	0.125	0.5	>16
SFDJ 668	0.06	0.002	0.06	0.25	0.125	0.25	0.125	0.125	0.25	>16
SFDJ 270	0.125	0.008	0.015	0.125	0.06	0.125	0.125	0.125	0.25	>16
SFDJ MA-3	0.06	0.004	0.125	0.5	0.25	0.25	1	1	1	>16
SFDJ 339	0.125	<0.001	0.25	0.25	0.25	0.25	0.25	0.25	0.25	>16
SFDJ 58	0.125	0.008	0.004	0.125	0.06	0.125	0.03	0.06	0.25	>16

*PEN, penicillin; CRO, ceftriaxone; RIF, rifampin; CIP, ciprofloxacin; GAT, gatifloxacin; MFX, moxifloxacin; LVX, levofloxacin; SPX, sparfloxacin; NOR, norfloxacin; NAL, nalidixic acid.

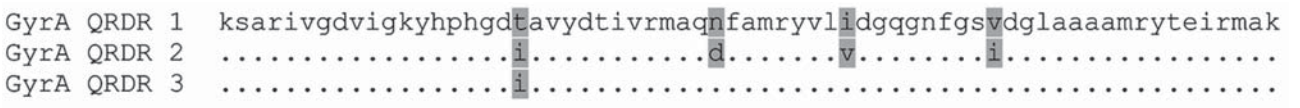

Figure 2. Comparison of GyrA quinolone-resistance–determining region (QRDR) for the alteration of amino acids in *Neisseria meningitidis* isolates. GyrA QRDR 1, ciprofloxacin-sensitive strains RRL-1, RRL-2, SFDJ 723, Ap-II 420; GyrA QRDR 2, ciprofloxacin-resistant strains SFDJ E-100, SFDJ E-79, SFDJ E-63, NICD 18, SFDJ E-95, IR-I 442; GyrA QRDR 3, ciprofloxacin-resistant strain IR-II 440. Ciprofloxacin-sensitive RRL-1 and RRL-2 were the reference strains, and the other 9 strains were from the outbreak. The shaded positions of the amino acids denote changes in GyrA QRDR of ciprofloxacin-resistant *N. meningitidis* in Thr-91 → Ile, Asn-103 → Asp, Ile-111 → Val, and Val-120 → Ile. The mutation of amino acid at conservative position 91 (Thr-91 → Ile) is responsible for the decreased susceptibility to fluoroquinolones, and the other 3 changes are synonymous.

example, 1 *N. meningitidis* strain from 2005 outbreak, IR-II 440, showed the mutation only at position 91 (Thr-91 → Ile), and ciprofloxacin resistance was observed.

Conclusions

The antimicrobial drug susceptibility of the clinical isolates and further sequencing of the QRDRs of gyrase A of the strains confirms the emergence of ciprofloxacin resistance in the outbreak in Delhi. In addition to chemoprophylaxis, fluoroquinolone consumption in the community, for a range of infections may be, in part, responsible for the emergence of ciprofloxacin resistance in *N. meningitidis* isolates. Lack of *N. gonorrhoeae* isolate response to ciprofloxacin has been reported in Delhi (*13*). Drift in susceptibility of *N. gonorrhoeae* to ciprofloxacin caused therapeutic failure. Ciprofloxacin treatment failure in cases of typhoid fever has also been reported (*14*). To date, to our knowledge, no failure of ciprofloxacin as chemoprophylaxis for *N. meningitidis* has been reported. To predict clinical outcome, further detailed pharmacokinetic/pharmacodynamic (PK/PD) analysis is needed to determine the impact of the PK/PD parameters on resistance selectivity (*15*).

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Compliance with Exclusion Requirements to Prevent Mumps Transmission

To the Editor: Control of communicable diseases often relies in part on school and workplace exclusion. Exclusion policies are also likely to play a role in pandemic influenza control and currently are used as policy for control of several vaccine-preventable diseases, including mumps (*1*). Mumps virus is typically present in saliva from 2–3 days before to 4–5 days after onset of parotitis. However, virus has been isolated from saliva as early as 6 days before and as late as 9 days after the first signs of salivary gland involvement (*2*).

In Illinois, persons with mumps are excluded from school and the workplace for 9 days after onset of parotitis (*3*) to reduce transmission of mumps virus. However, exclusion policy is not consistent among all states. For example, persons diagnosed with mumps in Iowa are excluded from school and the workplace for 5 days, whereas persons with mumps in New York and California are excluded for 9 days.

Illinois experienced a mumps outbreak during 2006 that resulted in 796 cases. We describe a telephone survey administered during April–June 2006, to a convenience sample of 174 persons ≥9 days after onset of parotitis during this outbreak to assess compliance with school and workplace exclusion requirements. The survey response rate was 68% (174/257).

Among 94 (54%) persons with mumps who had attended school, 85 (93%) of 91 spent time at home after they began experiencing parotitis, and 6 (7%) of 91 did not stay home from school. Most persons were told by local health department staff, student health services staff, or their medical provider to remain at home for 9 days. Among persons with mumps who spent some time away from school, 48 (56%) of 85 remained at home for ≥9 days. However, 37 (44%) of 85 persons did not remain at home for the entire exclusion period (median 5 days; range 1–8 days). Among 111 (64%) persons who worked outside the home, 93 (87%) of 107 spent time at home after they began experiencing parotitis.

Among persons who spent time away from work, 53 (57%) of 93 remained at home for ≥9 days. However, many persons (41%, 38/93) remained at home for fewer than the 9 days required by the state (median 5 days, range 1–8 days) after onset of parotitis. Reasons for complete noncompliance (not remaining at home from work during any part of the exclusion period) included not feeling ill enough to remain at home (50%, 7/14) and not receiving a diagnosis until after the exclusion period had elapsed (36%, 5/14) (Table). Because almost 80% of these noncompliant persons acknowledged being told not to work, lack of such instruction did not play a major role in this subset of cases.

Despite public health control measures, including expanded vaccination recommendations (*4*) and school and workplace exclusion, mumps cases in Illinois increased 90% from 419 during January 1, 2006, through May 17, 2006, to 796 through December 31, 2006. Given limited resources of local health departments, monitoring and ensuring compliance with exclusion control measures are likely to be a barrier in control of mumps, and these difficulties should be recognized as a potential issue in pandemic influenza planning. Additional studies targeting reasons for failure to comply and how to improve compliance will be useful preparedness activities.

An examination of whether exclusion for 9 days rather than only 5 days is a more effective mumps transmission control measure is also needed, given the difficulty with ensuring complete compliance for the full 9 days. Evidence for 9 days of shedding of mumps virus was based on a small number of experimentally infected children (N = 15), 8 of whom were asymptomatic (*2*). However, mumps exclusion policy states that 9 days is needed for persons with symptoms of parotitis. In addition, the population studied included no specimens from adults, although the exclusion policy derived from these data applies to persons of all ages. Finally, exclusion policy based only on parotitis may be feasible but would not affect persons with subclinical and nonspecific clinical infections who may shed mumps virus. A uniform evidence-based policy for exclusion is needed.

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Table. Reasons reported by 14 persons with mumps for not remaining home from work for any part of the exclusion period

Reason	Frequency,* no. (%)
Did not feel ill enough to miss work	7 (50)
Did not receive mumps diagnosis until after exclusion period	5 (36)
Was not told to remain at home	3 (21)
Could not financially afford to miss work	2 (14)
Too busy to miss work	1 (7)
No sick leave available	1 (7)

*Some persons reported >1 reason for not remaining home from work.

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Alveolar Echinococcosis, Lithuania

To the Editor: Alveolar echinococcosis (AE), a serious zoonosis caused by the tapeworm *Echinococcus multilocularis*, has been reported in neighboring countries of Lithuania in recent years (*1*–*4*), but no published epidemiologic information is available. The red fox (*Vulpes vulpes*), the main definitive host of *E. multilocularis* in Europe (*1*), and important intermediate rodent hosts (e.g. *Arvicola terrestris, Microtus arvalis*) are present in Lithuania (*5*), but to date they have not been investigated systematically. The helminth fauna of carnivores in Lithuania had been investigated in a study in 1976, but no record was made for *E. multilocularis* (*6*). Notably, *E. multilocularis* has recently been identified in 1 of 5 muskrats (*Ondatra zibethicus*) captured in the Šilutė district of Lithuania (*7*). The objectives of our study were to estimate the prevalence of *E. multilocularis* in definitive hosts and to gather first information concerning AE in humans in Lithuania.

From 1997 to July 2006, 80 AE cases have been diagnosed at the reference hospital for AE, the Hospital of Tuberculosis and Infectious Diseases in cooperation with the Santariškių Clinic, Vilnius University. Diagnoses were based on serologic testing using ELISA (Bordier Affinity, Crissier, Switzerland) and Western blot (LDBIO, Lyon, France) or imaging methods (ultrasound scan, computed tomography). In 6.7% of the cases identified by imaging techniques, serum antibodies were not detected by ELISA. Diagnoses in all cases were confirmed by histopathologic examination or typical liver lesion morphologic features. Most of the cases were registered in the past 5 years (10–16 cases/year in 2002–July 2006 compared with 0–4 cases/year in 1997–2001). In 26 (33%) of 80 patients, metacestodes were found in the bilateral liver lobes; in 20 (25%) metacestodes were found in the right lobe. Metacestodes had also spread into extra hepatic tissues and metastasized to the right lung, right kidney, spleen, and genitals in 18 (23%) of the patients. AE was diagnosed in 62 (78%) of patients in the third to fourth clinical stage of the disease, according to the PNM (P, parasitic mass in the liver; N, involvement of neighboring organs; M, metastasis) classification: P2-3N0-1M0, P4N1M1 (*8*); twelve (15%) patients died, 7 of them within 4–24 months after diagnosis. The patients' ages varied from 21 to 83 years (mean age 58 years). Women were more frequently infected (63%) than men (38%), which could be explained by women's more frequent involvement in gardening. Eighty-one percent of AE patients were farmers or persons involved in agricultural activities. Most AE patients originated in the northwestern and northeastern parts of Lithuania, but cases were recorded from many parts of the country (Figure), which suggests that the whole territory of Lithuania should be considered as an AE-endemic area.

To assess the prevalence of *E. multilocularis* in definitive hosts, the small intestines of 206 hunted red foxes were collected from randomly selected districts from October 2001 to April 2004 and examined following strict safety precautions by the sedimentation and counting technique. *E. multilocularis* was detected in 118 red foxes (57.3%, 95% confidence interval [CI] 50.2%–64.1%). The tapeworm was present in foxes from most tested localities; the highest prevalence of 62.3% (CI 49.0–74.4%) was observed in the Kaunas district (Figure). The median worm burden per infected fox was 56 (1–20, 924) in this district. The high prevalences of *E. multilocularis* in foxes in the examined areas support the hypothesis that foxes play the key role as definitive

Figure. Number of patients (shown in **boldface**) diagnosed with human alveolar echinococcosis at the Hospital of Tuberculosis and Infectious Diseases, Vilnius University, from 1997 through July 2006 in districts of Lithuania. No. *Echinococcus multilocularis*–positive/no. red foxes (*Vulpes vulpes*) (shown in *italics*) investigated during 2001–2004 is indicated for some of the districts.

hosts in the biology of this tapeworm in Lithuania.

In the framework of an epidemiologic investigation on *E. granulosus*, the contents of small necrotic lesions (size 3–8 mm) found in 21 randomly collected pig livers from small family farms in the southwestern part of Lithuania were also investigated by PCR (*9*); 3 lesions were positive for AE. Further, 2 of 34 dogs from rural areas in the southwestern part of Lithuania shed taeniid eggs in feces that were positive for *E. multilocularis* on examination with a multiplex PCR (*10*).

The high number of human AE cases and the high prevalence of *E. multilocularis* in definitive wild hosts document that AE is of emerging concern in Lithuania. However, this study cannot conclusively document a recent extension of the parasite's range and an increase of the infection pressure. Clearly, the identification of AE in pigs and of *E. multilocularis* in dogs from small family farms demonstrates that transmission of *E. multilocularis* occurs in rural environments in close proximity to the population.

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Human Bocavirus and Gastroenteritis

To the Editor: We read with great interest the recent study by Vicente and colleagues, who suspect the human bocavirus (HBoV), a newly detected parvovirus initially described as a respiratory pathogen, to be a possible causative agent of gastroenteritis in children (*1*). These researchers investigated the presence of HBoV DNA in 527 stool samples from ambulatory patients (<36 months of age) with unrelated respiratory symptoms. Of these stool samples, 48 (9.1%) were positive for HBoV DNA. Other enteric pathogens were found in 58% of all HBoV-positive fecal samples.

A close taxonomic relationship exists between HBoV and bovine parvovirus, an animal virus capable of causing gastrointestinal symptoms in cattle (*2*). Taking into account the assumed high tenacity of this parvovirus against environmental factors and hospital-grade disinfectants (*3*,*4*), we believe the possibility of fecal-oral transmission, in addition to transmission via respiratory droplets, has to be considered in interpreting the observations of Vicente et al. (*1*).

Gastroenteric symptoms have been described in up to 25% of all patients with respiratory HBoV infections (*5*–*7*). Although these observations suggest that HBoV may contribute to gastroenteritis or even be a causative agent, further studies are needed. Such studies should include control groups of asymptomatic patients and should test stool samples for HBoV. The correlation between detection of HBoV and clinical symptoms of gastroenteritis needs further confirmation in animal models, which are still not available. Nevertheless, the study by Vicente et al. did not clarify the extent of respiratory symptoms in patients with HBoV-positive stool samples. Taking into account the nature of parvovirus particles, we believe the virus likely passed through the gastrointestinal tract, as patients frequently swallow virus-containing sputum or nasal secretions. Thus, the observation that HBoV is an enteric pathogen should be considered a preliminary finding. Finally, we suggest that the role of HBoV should be investigated through histologic examination of mucosa biopsy specimens (e.g., from patients with chronic gastrointestinal diseases) to confirm pathogenicity.

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In Response: We thank Schildgen et al. (*1*) for their comments on our study, with which we basically agree. Our main findings were the possibility of fecal–oral transmission of the recently discovered human bocavirus (HBoV) and the high frequency with which it has been detected in the feces of infants and young children with gastroenteritis (*2*). For us, this latter finding suggested that HBoV is an enteric pathogen, although we cannot rule out the possibility that the fecal elimination was unrelated to the diarrhea in these patients. The possible pathogenic role of this virus in acute respiratory infections has also been questioned; consequently, several types of studies have been proposed to obtain evidence of pathogenicity and proof of disease causation (*3*,*4*).

Schildgen et al. suggest the possibility that HBoV could be detectable in feces after patients swallow respiratory tract secretions. Most of the children with gastroenteritis in our study showed no signs of respiratory infection. Swallowing respiratory tract secretions when one does not have respiratory inflammation would probably produce a quantity of virus too small to be detected in feces in the high frequency with which it was detected in our study. Therefore, we believe that the fecal elimination was more likely produced by HBoV replication in the intestinal mucosa. The studies proposed by Schildgen et al. would undoubtedly help elucidate this issue.

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Burning the Rat

Al Zolynas

I find him lying by the door,
legs outstretched as if he died in mid-leap.
I pick him up by the tail.
He feels loose, beyond the first stiffness of death.
His molecules have realized the futility of hanging on;
they know the party's over, it's time to head home.

Suddenly, I want to burn this rat.
I surprise myself at how much I want this.
I want to save him from the slow
decay, the fetid rearrangement
of his parts --or so I tell myself.
But mostly, I want to see him burn.

I drop him on the wire screen
that covers the forty-gallon drum
I use for burning garbage.
I light the fire.
I am strangely satisfied.
As I expected, his whiskers furl
into quick question marks and are gone;
his fur bubbles, then turns black and dry.

The tail, the long nightmare of a tail,
holds on longer than I thought.

Hours later, it is the only thing left,
a white length of ash
like the backbone of something prehistoric
seen from a great distance.

Rembrandt van Rijn (1606–1669). The Rat Catcher (1632). Etching (13 cm × 14 cm).
Courtesy of the "Sordid and Sacred Collection" of John Villarino

Rats, Global Poverty, and Paying the Piper

Polyxeni Potter*

"I frankly consider him a great virtuoso," said Italian painter Guercino of Rembrandt van Rijn. Guercino (1591–1666) was referring to the legendary master not as painter or portraitist but as etcher because it was etchings that built Rembrandt's reputation during his lifetime (*1*). The first to fully exploit this technique, which dates back to 14th-century armor ornamentation, he left behind some 300 fine prints, a benchmark for posterity. Most were small, displayed not on walls but in albums like early photographs, rested on tables for a closer look.

Etchings are created by drawing on a resin- or wax-coated metal plate with a needle. The plate is immersed in acid, which "bites" or eats away the lines where the metal is exposed. Rembrandt viewed this technique as drawing. Attracted to its spontaneity, he practiced it throughout his career and defined it with inventiveness and flair. His genius lay in the light touch of the draftsman, not the heavy hand of the professional printmaker. Using the needle as brush or pen, he created lines that flowed across the plates, varied in texture and tone from being carved more deeply or immersed longer in the acid bath (*2*).

For even greater tonal variation, Rembrandt experimented with drypoint—drawing lines directly into the soft surface of the copper plate. These lines held more ink and formed velvety black, rich shadows. Combined etching and drypoint limited copies from a single plate, sometimes to as few as 15, from possible hundreds. His finest prints are rare and unique, even if created by a reproductive process. Unlike other 17th-century artists, who did the drawing, then turned the task over to printmakers, he did the total job himself, able to alter the drawing throughout the process and create, from the same plate, prints that were not identical. The paper used, common European white or thin absorbent, ivory-yellow or light gray Japanese, also produced remarkable variations.

On these small plates, Rembrandt etched life in his native Holland. To capture natural movement, he ran a theatrical studio, where apprentices played out gestures for each other and enacted scenes from the streets of Amsterdam and the fringes of society. Enlivened with dramatic light and shadow, these indelible scenes established his reputation as master storyteller. They described an urban culture conscious of boundaries and criteria for inclusion and exclusion; focused on work, thrift, and restraint; and overwrought with vagabonds, landlopers, beggars, tricksters, outsiders—a population both created and demonized by society and portrayed in sordid detail by period art (*3*).

Earlier painters, Hieronymus Bosch (c.1450–1516) for one, depicted beggars as indistinguishable from demons. Peter Bruegel the Elder (c.1525–1569) painted peasants as objects of mirth, and Adriaen van de Venne (1589–1662) capped his unsparing images of the poor with ironic humor and ridicule. Some of Rembrandt's prints show the poor in unflattering and compromising situations, but he was indebted to Jacques Callot (c.1592–1635), whose etchings he collected. They humanized the poor and allowed them individuality and seriousness (*4*).

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Rembrandt may have learned compassion from his own misfortunes. His life, which might have been one of comfort, recognition, and wealth, turned into a journey of adversity, marred by the deaths of his wife and young children, bankruptcy, and social rejection in his later years. Bitter and disillusioned, he continued to produce earthy street scenes crowded with beggars, peddlers, the underclass, whose faces were oddly reminiscent of his own portraits or those he painted in religious scenes.

In the disorderly intimacy of the streets that so fascinated Rembrandt, economic delinquency manifested itself in more than just the unwanted poor. Stray animals, a hog here and there, tame pigeons, cats, rabid dogs, roamed unchecked. And rats, most prolific, most hated and feared, for as the poet put it, "They fought the dogs and killed the cats, / and bit the babies in the cradles, / and ate the cheeses out of the vats, / And licked the cooks' own ladles ..." (*5*).

The house rat, or black rat, arrived in Europe around 400 to 200 BCE and quickly established a commensal relationship with the locals in homes, ships, river banks, and sewers, generating brisk business for the rat poison peddlers, common street venders. The Pied Piper of Hamelin, a legend immortalized by the Brothers Grimm, recounted rat infestation so severe in that German town, in 1284, it required special intervention.

When Europe was overrun by the Black Death in 1348, Giovanni Boccaccio, who lived through it in the city of Florence, wrote in The Decameron, "It began with yong children, male and female, either under the armepits, or in the groine by certaine swellings, in some to the bignesse of an Apple, in others like an Egge ..." (*6*). His description led to later speculation that the disease was bubonic plague caused by *Yersinia pestis* and spread by fleas carried by the black rat. In A Journal of the Plague Year, Daniel Defoe's chronicle of the great plague of London in 1665, rats were named as suspects, "All possible Endeavours were used also to destroy the Mice and Rats, especially the latter" (*7*).

In The Rat Catcher, on this month's cover, Rembrandt's light hand scratched a telling rat's tale in a local transaction between an itinerant peddler and a homeowner. Looking on is the peddler's diminutive assistant, holding a container with rat poison or ferrets trained to hunt rats. The peddler's extended hand holds poison. The homeowner reaches from behind the half-closed double door but is repulsed. A basket on the long pole is filled with live rats. One on top is poised to jump. Others hang dead from the base. Hairy and unkempt, the peddler himself looks like a rat in his tattered furry cape and long sword. Domestic clutter frames the entrance to the cottage. Etched softly in the background is a prowling cat.

The Rat Catcher was a popular print, copied 11 times in the 17th century (*8*). The scene struck a nerve because it contained much more than a transaction on a village lane. At the threshold of this cottage, separated by the closed half door, met but remained apart, two sections of society: the rooted and the vagrant outsider. What Rembrandt managed to convey was the humanity of all, not just the squeamish owner but the vagrants too, social outcasts though they were. The assistant wears a wistful glance. The peddler, solid on his feet, has a pet perched trustingly on his shoulder.

A carrier of bubonic plague, epidemic typhus, trench fever, ratbite fever, leptospirosis, hantavirus pulmonary syndrome, salmonella poisoning, and many other infections, the rat is still a suspect around the world, destroying as much as one third the global food supply each year, killing domesticated animals, damaging buildings and furnishings (*9*). Social inequity also continues, perpetuating a cycle of poverty and disease (*10*). This cycle cannot be ignored for, as Boccaccio and Defoe reported, disease cannot be fenced out of prosperous areas. "... [T]he plague bacillus never dies ... it bides its time in bedrooms, cellars, trunks, and bookshelves," wrote Albert Camus, in The Plague, "and perhaps the day would come when, for the bane and the enlightening of men, it would rouse up its rats again and send them forth to die in a happy city" (*11*).

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

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Epidemiology of *Streptococcus dysgalactiae* subsp. *equisimilis* in Tropical Communities, Australia

Genetic Diversity of Clonal Lineages in *Escherichia coli* O157:H7 Model

Complete list of articles in the November issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

October 4–7, 2007
45th Annual Meeting of IDSA
San Diego, CA, USA
Contact: 703-299-0200
http://www.idsociety.org

October 11–13, 2007
American Medical Writers Association (AMWA) 2007 Annual Conference
Marriott Atlanta Marquis
Atlanta, GA, USA
http://www.amwa.org/default.asp?Mode=DirectoryDisplay&id=344

October 18–20, 2007
European Scientific Conference on Applied Infectious Disease Epidemiology
Stockholm, Sweden
http://www.escaide.eu

October 24, 2007
Progress Against Malaria: Developments on the Horizon
New York Academy of Sciences Conference Center
New York, NY, USA
http://www.nyas.org/mraconf

November 3–7, 2007
American Public Health Association Annual Meeting: Politics, Policy and Public Health
Washington, DC, USA
http://www.apha.org/meetings

November 21–24, 2007
Genetics and Mechanisms of Susceptibility to Infectious Diseases
Institut Pasteur
Paris, France
http://www.pasteur.fr/infosci/conf/sb/host_genetics

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To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). In 50–150 words, describe timely events of interest to our readers. Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

EMERGING
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Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles see below and visit http://www.cdc.gov/ eid/ncidod/ EID/instruct.htm.

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Instructions to Authors

Manuscript Preparation. For word processing, use MS Word. Begin each of the following sections on a new page and in this order: title page, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, figure legends, appendixes, and figures. Each figure should be in a separate file.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

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Tables and Figures. Create tables within MS Word’s table tool. Do not format tables as columns or tabs. Send graphics in native, high-resolution (200 dpi minimum) .TIF (Tagged Image File), or .EPS (Encapsulated Postscript) format. Graphics should be in a separate electronic file from the text file. For graphic files, use Arial font. Convert Macintosh files into the suggested PC format. Figures, symbols, letters, and numbers should be large enough to remain legible when reduced. Place figure keys within the figure. For more information see EID Style Guide (http://www.cdc.gov/ncidod/EID/style_guide.htm).

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Types of Articles

Perspectives. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and a brief biographical sketch of first author. Articles in this section should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may also address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

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Research Studies. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary, and a brief biographical sketch of first author—both authors if only 2. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should be under 3,500 words and should include references, not to exceed 40. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), a one-sentence summary of the conclusions, and brief biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., “The Study” and “Conclusions.” Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and a brief biographical sketch of first author—both authors if only 2. Dispatches are updates on infectious disease trends and research. The articles include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no figures or tables.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. All letters should contain material not previously published and include a word count.

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