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July 2012

International Health

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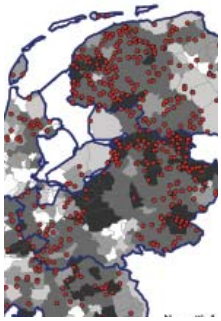

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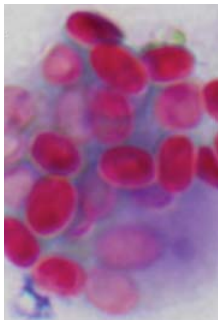

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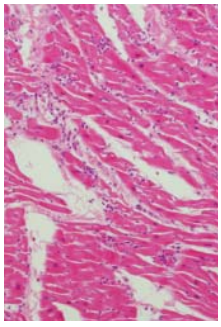

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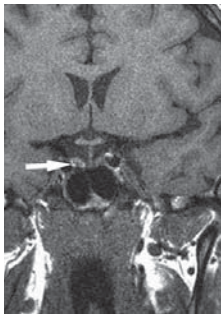

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World Health Organization Perspective on Implementation of International Health Regulations

Maxwell Charles Hardiman and World Health Organization Department of Global Capacities, Alert and Response[1]

In 2005, the International Health Regulations were adopted at the 58th World Health Assembly; in June 2007, they were entered into force for most countries. In 2012, the world is approaching a major 5-year milestone in the global commitment to ensure national capacities to identify, investigate, assess, and respond to public health events. In the past 5 years, existing programs have been boosted and some new activities relating to International Health Regulations provisions have been successfully established. The lessons and experience of the past 5 years need to be drawn upon to provide improved direction for the future.

Throughout the >60 years that the World Health Organization (WHO) has been in existence, member states have made use of the constitutional provision that permits the Health Assembly to adopt regulations concerning sanitary and quarantine requirements and other procedures designed to prevent the international spread of disease (*1*). In 1951, the first such regulations, the International Sanitary Regulations, were adopted and focused on 6 communicable diseases requiring coordinated international measures to control their transmission between countries (*2*). By the 1990s, they had been amended and renamed the International Health Regulations (IHR); their application was reduced to only 3 diseases, and they were considered inadequate for addressing the increasingly globalized nature of health risks. In 1995, the Health Assembly called on the WHO secretariat to develop revised regulations that were more relevant to worldwide public health challenges (*3*–*5*). A process of intensive and wide technical consultation was followed by a series of intergovernmental negotiations in which WHO member states took control of the draft and negotiated additions and amendments to every aspect before agreeing to a final version in time for it to be adopted at the 58th Session of the Health Assembly (*6*).

Since entering into force in 2007, the IHR have provided a legally binding global framework to support national and international programs and activities aimed at preventing, protecting against, controlling, and providing a public health response to the international spread of disease (*7*). Although the IHR contain articles directed toward several facets of public health security, they can be broadly summarized into 2 main areas: urgent actions to be taken with respect to acutely arising risks to public health and strengthening of national systems and infrastructure (referred to as core capacities). This article provides an overview of selected contributions to these areas made during the past 5 years. It is written from the perspective of the WHO department charged with coordinating implementation of the IHR at WHO global headquarters in Geneva and seeks to identify major achievements and continuing challenges.

Establishment of National IHR Focal Points

One of the early demonstrations of global commitment to implementation of the IHR has been the successful establishment of National Focal Points (NFPs) in all but 1 of the states parties to the IHR. (States parties to the IHR include all WHO member states, the Holy See [an observer to the World Health Assembly], and Liechtenstein.) NFPs are national centers, not individual persons, that occupy a critical role in conducting the communications aspects

Author affiliation: World Health Organization, Geneva, Switzerland

DOI: http://dx.doi.org/10.3201/eid1807.120395

[1]Members who contributed to this article: Anouk Berger, Stella Chungong, Sophia Desillas, Paula Gomez, Fernando Gonzalez-Martin, Daniel Menucci, Varvara Mouchtouri, Isabelle Nuttall, Bruce Plotkin, Rajesh Sreedharan, and Jun Xing.

of the IHR, within their countries and internationally (*8*). They are responsible for proactively notifying WHO of relevant health events, responding to WHO secretariat requests for event-related information, and ensuring that messages and advice from WHO are disseminated to the relevant actors within the country. Since 2007, NFPs have been increasingly diligent in updating and confirming their contact details to WHO on an annual basis as required by the regulations. NFPs are officially sanctioned to work with WHO on IHR implementation and provide feedback to WHO on country needs and concerns for this task. Staff members who work in NFPs are a major audience for WHO training materials. The engagement of NFPs in the scientific evaluation of the IHR notification procedures has indicated that a high proportion of NFPs had a good understanding of the notification procedures and had accessed WHO training materials on this issue and has indicated that agreement was high in terms of events that must be notified when applying the procedures (*9*). NFPs have access to the contact details of all other NFPs through a password-protected website that enables direct communication among countries at the NFP level. For events that do not require WHO coordination (such as routine tracing of contacts for an infectious disease associated with international travel), such direct communications have been useful.

Not all NFPs are able to function as expected. For example, some contact details fail to work for urgent communications, some NFPs indicate that procedures for round-the-clock communications are not yet established, and delays in responding to requests for event information often occur. Studies have indicated that NFPs know how to assess events under the IHR. Their participation in event-related communications is increasing; however, their role has been primarily providing official and accurate information on events that first gain WHO attention through informal sources such as media reports. Among the reasons identified for such less-than-optimal performance is that some NFPs lack authority or access to the necessary authority, resulting in delays in obtaining clearance for communications. Such lack of authority is also identified as a barrier to the effective intersectoral collaboration that is envisioned as critical to the NFP role within their national situation. Although NFPs generally recognize the value of engaging with government sectors outside the health ministry, they lack the convening power needed to establish solid and reliable linkages.

Pilot Testing of IHR-Implementation Course

A key WHO objective is to strengthen the human resources available to countries to set up and manage systems for securing global public health under the IHR framework. In partnership with established educational institutions, the WHO secretariat has been pilot testing an IHR-implementation course, which promotes a global harmonized understanding and application of the IHR framework.

The IHR-implementation course is for public health professionals, mainly those belonging to NFPs but also those from other related sectors from national or international organizations in public and private sectors. The course is delivered over 5 months as on-the-job training. The 210 total learning hours consist of 12 weeks of distance learning with tutoring and a 6-week break used to finalize assignments and prepare for the 2-week face-to-face session.

The first 3 pilot IHR-implementation courses have been operated by the WHO Department of Global Capacities, Alert and Response in collaboration with the University of Pretoria, South Africa; Georgetown University Law Center, USA; the University of Geneva, Switzerland; and Institut Bioforce Développement, France. Implementation of the courses involved the contributions of several WHO departments: Food Safety, Zoonoses and Foodborne Diseases; Protection of the Human Environment; Health Action in Crises; and Health Systems and Services. WHO Regional Offices have been mobilized to identify and sponsor participants.

The IHR-implementation courses have been delivered in English to 89 participants from 57 countries in all 6 WHO regions. Post-training evaluation of the first 2 courses conducted in 2011 indicated that the course content was relevant to participants' work, improved their understanding of IHR, and increased their confidence when dealing with the topic. Competencies developed have been put into practice, and material from the course has been re-used at the national level. The opportunity to engage with peers from other countries during and after the course was considered especially valuable.

In light of the positive evaluation and continuing need, organization of additional courses at the national level is planned. A need to provide the course in languages other than English requires new institutional partners and additional resources. Some of the IHR-implementation course contents are being developed into stand-alone modules for potential integration into other established training opportunities such as field epidemiology training and Masters of Public Health programs.

Monitoring of Progress of IHR National Core Capacities

One of the most substantial obligations introduced by the IHR is the commitment of states parties to develop, strengthen, and maintain national capacities to identify, investigate, assess, and respond to public health events in their territories and to develop, strengthen, and maintain

routine and emergency public health capacities at certain designated points of entry. These obligations were introduced in acknowledgment that effective national systems are the essential underpinning to any global health security and that such systems are the mechanisms needed to prevent many public health events from reaching the level of international significance. The IHR capacities are described in functional terms in Annex 1, and a major milestone toward implementation has been to reach a consensus on the scope and technical components that can be expected to contribute to the required functionality.

For surveillance and response, the capacities are grouped under the following 8 main headings:

- National legislation, policy and financing
- Coordination and NFP communications
- Surveillance
- Response
- Preparedness
- Risk communication
- Human resources
- Laboratory

A range of potential health hazards can fall under the IHR capacity requirements. These hazards have been identified as infectious, zoonotic, food safety, chemical, and radiologic/nuclear.

To help states parties assess their capacity, a monitoring framework was developed. The framework represents a consensus of technical expert views drawn globally from WHO member states, technical institutions, partners, and from within WHO. The framework incorporates current knowledge and concepts that have been successfully used to monitor capacity-development activities. It builds on the experts' knowledge of current capacities of states parties, existing regional and country strategies for capacity development, and other available resources and tools, particularly other tools used for IHR core capacity assessment by states parties. Using a checklist of 20 indicators, the IHR monitoring process assesses status of implementation in 8 areas of core capacity, development of capacities at points of entry, and development of capacities for the IHR-relevant hazards.

An annual questionnaire is used to collect data on the core capacities; country responses are stored in a secure database at WHO, accessible only to IHR NFPs and the secretariat through use of tools that ensure country confidentiality. The questionnaire is made available in several formats, including through the Internet. To ensure that the full spectrum of relevant hazards is covered, NFPs are advised to lead the process of completing the questionnaire, in close collaboration with officials responsible for the various capacity areas and including other sectors.

Outputs of the monitoring framework include country profiles for all reporting countries and detailed NFP reports on strengths, weakness, and gaps; profiles for the 6 WHO regions; and aggregated global reports for the World Health Assembly. This information has enabled states parties to measure progress and identify where improvements are needed, thereby providing evidence for program planning, recommendations, and decision making. At the global level, this monitoring information is used by the secretariat to comply with the Health Assembly request for an annual report on IHR implementation from WHO, including information provided by states parties and on the secretariat's activities. Thus, WHO governing bodies can take account of the progress when directing secretariat activities. The analysis also enables better identification of the priority areas toward which the secretariat and other development partners can focus their support to countries.

From a total of 194 states parties, the questionnaire elicited 128 and 156 responses for 2010 and 2011, respectively. Because not all states parties responded to the questionnaire, the reports produced might not completely reflect IHR core capacity development strengths and weaknesses at the regional and global levels. Evaluating implementation status in nonresponding countries is challenging, especially because some of these countries face the greatest implementation difficulties. With the goal of improving the validity and consistency of self-reported data, several multicountry workshops and trainings have been held and standardized data collection and analysis tools have been promoted. Such challenges are also being addressed by identifying several supplementary information sources that might partially reflect national IHR capacities and including such information in an additional report to the 2012 Health Assembly.

The biggest challenge involved in implementing the IHR is ensuring that the IHR core capacities are present in all countries of the world. Ensuring IHR core capacities is also the area in which the IHR have the greatest potential to make a major contribution to world health; as the process approaches a key 5-year milestone on June 15, 2012, all efforts are being refocused on this issue.

Interagency Collaboration for Public Health at Points of Entry

Although many IHR provisions address international travel and transport and public health activities at points of entry (ports, airports, and ground crossings), these have not been areas in which WHO or many member states had strong preexisting programs. Attention has therefore

been focused on leveraging interagency and multisectoral collaboration at all levels to achieve the public health objectives. For example, the Cooperative Arrangement for the Prevention of Spread of Communicable Disease through Air Travel project (*10*) is an initiative of the WHO sister agency the International Civil Aviation Organization, through which countries can receive support for realizing IHR objectives relating to air travel. Other collaborations include the International Tourism Response Network (*11*), regional networks such as the Risk Assessment Guidance for Infectious Diseases Transmitted on Aircraft project (initiated by the European Centre for Disease Prevention and Control) (*12*), and the European Commission ship sanitation training network project (*13*). To facilitate information sharing and coordination among authorities responsible for health measures and development of IHR core capacities at points of entry, WHO supports a specialized network for ports, airports, and ground crossings: the PAGnet (*14*). During the 2011 nuclear accident in Japan, the 2010–11 cholera epidemic in Haiti, and the 2009 influenza A (H1N1) pandemic, PAGnet offered a communication platform to public health officials at points of entry around the world, facilitating timely information sharing on response measures that helped avoid overreaction and unnecessary barriers to international travel and trade.

Although assessments have shown many IHR capacities at certain points of entry in several countries, countries differ widely in the levels of capacity, the allocation of responsibilities, and the priority given to this area of public health. This heterogeneity makes it more difficult to provide guidance and advice that is relevant to the national and local contexts of all ports, airports, and ground crossings around the world. Private industry and commercial organizations, which involve a variety of governmental sectors in addition to health, are key actors for the implementation of IHR provisions affecting travel and transportation. WHO must use its convening power, its neutrality, and its focus on public health objectives to help the disparate actors reach consensus.

Pandemic Influenza and Convening of the Emergency Committee

Around the world, many IHR provisions are used daily. Thus far, however, the full range of provisions relating to global emergencies have been applied to only 1 event: the 2009–2010 influenza pandemic. The IHR define a category of events with the term "public health emergency of international concern." The WHO director-general follows defined procedures to determine which events are so characterized. The key practical outcomes of such a determination are the provision of relevant information to all states parties, the convening of an IHR Emergency Committee to advise the director-general regarding the event, and the issuance of IHR temporary recommendations.

The first IHR Emergency Committee was convened on April 25, 2009, to advise the WHO director-general about the determination of the first public health emergency of international concern under the IHR. That this first meeting of the Emergency Committee took place by teleconference within 48 hours of the decision to convene it demonstrated that the procedures established by the IHR could work in practice. The continued work of this committee, providing advice to the director-general for more than a year, demonstrates the commitment of its members to support the governments of the world and WHO in their responses to the emergency. During the influenza pandemic, the NFP network developed much-needed momentum and provided early information and situation updates as the virus was identified around the world. The WHO secretariat was able to provide updates, announcements, and advice to countries through the event information site for NFPs with timing that was coordinated with its provision of public information.

The duration of the public health emergency of international concern posed several challenges for the procedures established for IHR implementation. For example, the decision to protect the impartiality of the advice given by members of the IHR Emergency Committee (by not publishing their names until after their work was completed) was not helpful when their work went on for more than a year and was under intense media speculation. Also, the rules adopted for temporary recommendations were designed to allow them for only a limited amount of time, which was just barely compatible with the pandemic experience. The IHR did not prevent several countries from applying restrictive travel- and trade-associated measures not recommended by WHO, although several such measures were discontinued or modified after communication with the WHO secretariat. The IHR Review Committee was concerned by the restrictive measures and provided recommendations on how they can be more effectively addressed (*15*).

Establishment of External IHR Review

The potential to learn lessons from the 2009–2010 pandemic influenza experience and the need to address public concerns regarding the WHO response led to the establishment of the first IHR Review Committee. The remit of this committee was expanded (by the WHO Executive Board from a periodic review of the functioning of the IHR, as required under IHR Article 54) to include an independent, external review of the international response to pandemic influenza. Although the secretariat provided administrative and logistic support, the committee, under the chairmanship of Harvey Fineberg, enjoyed complete autonomy in interpreting their mandate, defining their

methods of work, and identifying their evidence. In doing so, they followed the requirements of the IHR in ensuring states parties the opportunity to observe and engage in formal committee meetings. After more than a year, the committee delivered its final report to the 64th Health Assembly, at which the approach taken was commended and the recommendations were endorsed by the member states. Despite findings that WHO faced systemic difficulties and some shortcomings in addressing the influenza pandemic, the committee concluded that the actions taken were motivated by public health concerns and found no evidence of misconduct. The 15 recommendations in this report have gone on to form a major component of the biennial work plans of the relevant WHO departments.

The exhaustive work of the IHR Review Committee made heavy demands on the time of its expert members and on WHO resources. WHO should take advantage of the exceptional opportunity to learn from this analysis of the pandemic experience.

The IHR allow review committees to give advice broadly on the functioning of the regulations, and it can be foreseen that in future years, committees will need to be convened with markedly different tasks, for example, advising on the granting of a second round of extensions to the core capacity time frame. At such time, the working methods of such a future review committee will need to be reassessed to fit with its mandated task.

Conclusions

The IHR are a legal tool designed to contribute to the achievement of public health goals, in which success is seen and measured in improvements to public health rather than adherence to any particular article of the document. At the same time, given the large number of initiatives for and influences on public health outcomes, it will always be hard to tease out and identify the specific contributions of such an instrument to global health. This article indicates some of the direct effects that IHR implementation is having on public health practice. Where states and WHO are building on preexisting programs, the IHR have boosted continuing commitment and momentum. An example at the international level is the WHO program for management of acute public health events; an example at the country level is the program to strengthen capacity in public health laboratories. In addition to boosting existing programs, some new activities relating to IHR provisions have been successfully established, such as the NFP network and the Emergency Committee.

The lessons and experience of the past 5 years need to be drawn upon to provide improved direction for the future. The member state–driven negotiations provide a legacy of ownership and commitment from countries, which continues to be evident in the nature and number of interventions concerning IHR during meetings of WHO governing bodies. As we approach the 5-year target date of June 2012, the immediate challenge is for WHO and the states parties to live up to the intention of the IHR national core capacity requirements and to make the best use of the opportunity for countries to continue their efforts beyond that date as anticipated under the extension procedure provided by the IHR.

Dr Hardiman is team leader within the WHO Department of Global Capacities, Alert and Response, which focuses on the legal and procedural aspects of IHR implementation. His research interests are detection and response to disease outbreaks and protection against the international spread of disease.

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Address for correspondence: Maxwell Charles Hardiman, Department of Global Capacities Alert and Response, World Health Organization, 20 Avenue Appia, 1211 Geneva, Switzerland; email: hardimanm@who.int

Assessment of Public Health Events through International Health Regulations, United States, 2007–2011

Katrin S. Kohl, Ray R. Arthur, Ralph O'Connor, and Jose Fernandez

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Learning Objectives

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

- Describe overall potential public health emergencies of international concern (PHEIC) from states parties posted by WHO on a secure Web portal between July 2007 and December 2011
- Describe potential PHEIC from the United States posted by WHO on a secure Web portal between July 2007 and December 2011
- Describe potential benefits of having the IHR framework for notification in place, as well as strategies for reporting and sharing information with the WHO

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Disclosures: ***Katrin S. Kohl, MD, PhD, MPH; Ralph O'Connor, PhD;*** *and* ***Jose Fernandez, PhD,*** *have disclosed no relevant financial relationships.* ***Ray R. Arthur, PhD,*** *has disclosed the following relevant financial relationships: owns stock in Vivus Inc.*

Under the current International Health Regulations, 194 states parties are obligated to report potential public health emergencies of international concern to the World Health Organization (WHO) within 72 hours of becoming aware of an event. During July 2007–December 2011, WHO assessed and posted on a secure web portal 222 events from 105 states parties, including 24 events from the United States. Twelve US events involved human influenza caused by a new virus subtype, including the first report of influenza A(H1N1)pdm09 virus, which constitutes the only public health emergency of international concern determined by the WHO director-general to date. Additional US events involved 5 *Salmonella* spp. outbreaks, botulism, *Escherichia coli* O157:H7 infections, Guillain-Barré syndrome, contaminated heparin, Lassa fever, an oil spill, and typhoid fever. Rapid information exchange among WHO and member states facilitated by the International Health Regulations leads to better situation awareness of emerging threats and enables a more coordinated and transparent global response.

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (K.S. Kohl, R.R. Arthur, R. O'Connor); and Department of Health and Human Services, Washington, DC, USA (J. Fernandez)

DOI: http://dx.doi.org/10.3201/eid1807.120231

Global air travel makes it possible for most countries to be reached from a country furthest away within a day, and some countries are connected by direct flights to >70 other countries. Just as persons and goods travel rapidly around the world, so too can pathogens. The outbreak of severe acute respiratory syndrome (SARS) in 2003 continues to symbolize the real possibility of rapid international disease spread of an emerging pathogen (*1*). It also raised awareness that global disease threats can go undetected and unreported to the point that control efforts are extremely difficult because major spread has often already occurred.

The experience with SARS led to the call for more transparent and rapid sharing of information on health risks and public health measures between countries and the World Health Organization (WHO) (*2*). In 2005, the World Health Assembly adopted revised International Health Regulations (IHR) with the declared purpose to "prevent, protect against, control and provide a public health response to the international spread of disease in ways that are commensurate with and restricted to public health risks, and which avoid unnecessary interference with traffic and trade" (*3*). The IHR legally bind 194 WHO states parties, including all WHO member states. One of the key principles inspiring the IHR is open, fast, and secure information exchange about disease emergence and response activities. The IHR provide a platform for dialog in form of national focal points (NFPs), which are always-available points of contacts in each IHR state party for all IHR-related information exchange with WHO and other NFPs, and through provision of a secure web portal, the IHR Event Information Site (EIS), which is accessible by all NFPs.

The IHR went into effect in the United States on July 18, 2007, with the explicit reservation that the United States assumes its obligations "in a manner consistent with its fundamental principles of federalism," an acknowledgment that responsibilities in the United States under these Regulations are shared between the Federal Government and the States. In addition, the United States specifically understands that all countries have an obligation to notify to WHO potential public health emergencies of international concern (PHEICs) "irrespective of origin or source, whether they involve the natural, accidental or deliberate release of biological, chemical or radionuclear materials" (*4*). In this report, we focus primarily on application of IHR assessment and reporting requirements within the United States for rapid sharing of information on potential PHEICs.

Assessment and Reporting per IHR Obligation

A critical feature of reporting under the current IHR compared with international reporting requirements detailed in the previous version of the IHR in 1969 is that states parties not only report events on the basis of a prescribed list of diseases, but also on the basis of a list of assessment criteria for any event with the potential for international spread, even if the source or cause of the event is unknown. Annex 2 of the IHR provides the decision instrument for assessing and notifying WHO of a potential PHEIC (*3*). A PHEIC is defined by Article 1 of the regulations as an extraordinary event that may "constitute a public health risk to other States through international spread of disease" and "potentially require a coordinated international response" (Article 1, Definitions, IHR). In addition to any disease with a risk for international spread, certain listed diseases must always be assessed, and 4 diseases (human influenza caused by a new virus subtype, wild-type poliomyelitis, smallpox, and SARS) must always be immediately reported to WHO.

The 4 criteria that guide the assessment are the following: 1) is the public health effect of the event serious?; 2) is the event unusual or unexpected?; 3) is there a major risk for international spread?; and 4) is there a major risk for travel or trade restrictions? If 2 of the 4 criteria are met, the event must be reported to WHO (*3*). The IHR document further provides examples to guide states parties in application of these criteria, and WHO has developed a guidance document for the application of the decision instrument (*5*). Once an event is reported to WHO, information is assessed by WHO and the states parties concerned, and further actions to be taken by WHO are determined. These actions include sharing information about the event with the global community by the secure EIS portal, providing technical assistance, and escalating the assessment to the level of the WHO director-general for considering if the event is determined to be a PHEIC.

Rapid information exchange in the context of the IHR is defined as a 48-hour period for states parties to assess an emerging event, and an additional 24 hours to report the event to WHO, if the assessment indicates that the event may constitute a PHEIC. The 48-hour assessment period begins once the national level of government becomes aware of the event. Just as the report of a potential PHEIC to WHO is meant to be the beginning of a constructive dialogue between states parties and WHO, states parties are obligated to respond to any inquiries from WHO about disease events within their borders within 24 hours, even if those events have otherwise not been reported to WHO. For example, in 2008, the WHO IHR Contact Point for the Americas, hosted by the Pan American Health Organization, requested an assessment of a measles outbreak in the United States as a potential threat to the global measles elimination initiative. Although information regarding this outbreak had already been released (*6*) at the time of the request, the Pan American Health Organization used IHR communication

channels to receive a formal assessment to better gauge the US response capability and alert other countries with more in-depth information about a potential threat to their measles elimination activities. The United States provided an assessment that indicated that the outbreak did not meet the criteria required for formal notification as a potential PHEIC.

In another situation, the IHR framework enabled the US public health community to better understand the risk to travelers exposed to a rabid animal in a game resort in Kenya in 2011 (*7*). Because the US government first became aware of this event through returning travelers, and was concerned about travelers from other countries who also may have been exposed, we used the IHR reporting structure to successfully engage WHO to assist with global contact-tracing activities. Because decision criteria for potential PHEICs have been accepted by all IHR states parties, these criteria provided an a priori accepted basis for our rapid and transparent joint assessment with the Kenyan Ministry of Health. Although no formal notification of a potential PHEIC was made to WHO, use of the IHR framework enabled all parties involved to better understand and respond to the exposure risk.

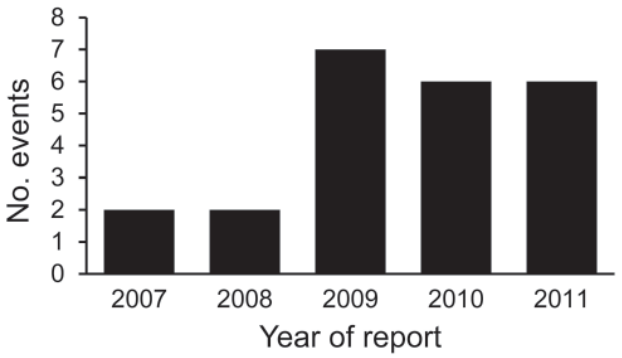

Figure. Twenty-four public health events in the United States assessed by the World Health Organization and posted on the International Health Regulations information site, July 2007–December 2011. There was 1 event of botulism and 1 event of *Salmonella* sp. infection in 2007; 1 event related to heparin and 1 event of *Salmonella* sp. infection in 2008; 5 events of influenza, 1 event of *Escherichia coli* infection, and 1 event of *Salmonella* sp. infection in 2009; 3 events of influenza, 1 event of Lassa fever, 1 event related to an oil spill, 1 event of *Salmonella* sp. infection, and 1 event of typhoid fever in 2010; and 4 events of influenza, 1 event of Guillain-Barré syndrome, and 1 event of *Salmonella* sp. infection in 2011.

Assessment of US Public Health Events as Potential PHEICs

During July 2007–December 2011, WHO posted 222 events from 105 member states assessed by WHO on the IHR EIS, including 24 events from the United States (Figure). Half of the events from the United States involved human influenza caused by a new virus subtype (12 events), followed by *Salmonella* spp. outbreaks (5 events) (*8*–*12*) and 1 event each for botulism (*13*), contaminated heparin (*14*), *Escherichia coli* O157:H7 (*15*), an oil spill (*16*), Lassa fever (*17*), Guillain-Barré syndrome (*18*), and typhoid fever (*19*) (Table).

Such events may involve no human illness, but must demonstrate the potential risk for human disease. For example, one of the considerations for reporting the oil spill along the US Gulf Coast in 2010 was the potential for a change in ocean currents that may have led to the international dispersion of oil with potential harm to human activities, e.g., coastal fishing. Events may involve only 1 case of disease, e.g., several reports by the Centers for Disease Control and Prevention (CDC) of influenza caused by a new virus subtype involved 1 case; some reports included ≥2 unrelated cases; and other reports included small clusters of influenza cases. Some events may be assessed when only a few cases are identified, e.g., the outbreak of typhoid fever was assessed when 9 cases from 2 states were confirmed. For other events, hundreds of cases were identified by the time of the assessment. For example, the outbreak of *Salmonella* Typhimurium infection was assessed at a time when >500 patients from 43 states had been identified.

Three events serve as examples for assessment and reporting practices in the United States for potential PHEICs and may assist others in their interpretation of the IHR assessment criteria in the decision instrument. In the first example, the first 2 cases of what later became known as influenza A(H1N1)pdm 09 infection were identified at CDC on April 17, 2009, and reported to WHO by the US Department of Health and Human Services as a potential PHEIC the same day (*20*). WHO determined that the event met all 4 assessment criteria because it involved a new subtype of influenza virus, which was likely to be highly transmissible; the first 2 cases clustered in time without an apparent epidemiologic link; 1 of the case-patients had traveled to Mexico within the incubation period; and major media attention was potentially impairing international travel or trade. On April 25, 2009, the WHO director-general determined that the event constituted a PHEIC on the basis of additional information from the United States and Mexico (*21*), and declared a pandemic 7 weeks later (*22*). No other public health event, including other novel influenza strains reported by the United States or any other country, has so far been determined to be a PHEIC.

The second example involves the second most frequently reported pathogen by the United States under the IHR, i.e., different strains of *Salmonella* spp., in this case, *Salmonella enterica* serotype Enteritidis infection (*9*). On October 27, 2011, CDC reported an outbreak of *S. enterica*

serotype Enteritidis associated with pine nuts from Turkey as a potential PHEIC to WHO. At the time of reporting to WHO, 42 cases of *S. enterica* serotype Enteritidis infection with an identical genetic fingerprint and onset dates during August 20–October 8 had been reported to CDC from 6 states. Nineteen (63%) of 30 patients interviewed had consumed these pine nuts, and ill persons had purchased the pine nuts from bulk bins of the same grocery store chain.

Table. Noninfluenza public health events in the United States, July 2007–December 2011, assessed by WHO as potential public health emergencies of international concern and posted on the IHR secure web portal*

Event by year of assessment (reference)	Assessment determination by WHO per IHR criteria† 1	2	3	4	Description of event at time of assessment
2007					
Botulism (*13*)	X	X	X	X	Four cases associated with a canned food product were identified in 2 states for the first time in 40 y in the United States. The company exported food items to ≥8 countries.
Salmonella enterica serovar Wandsworth infection (*10*)	X	X		X	Fifty-seven cases were identified in 18 states in the United States. *Salmonella* spp. can cause serious illness in specific risk groups. Implicated products were sold in the United States and Canada. Other national health authorities are requesting additional information about the outbreak.
2008					
Heparin (*14*)	X		X	X	Contaminated heparin products identified in ≥2 countries were associated with life-threatening clinical events. The United States Food and Drug Administration enacted an Import Alert for the products.
S. enterica serovar Saintpaul infection (*11*)	X	X	X	X	Several hundred cases with the same genetic fingerprint have been identified in 40 states, the District of Columbia, and Canada; illness onset was during April–July 2008. This organism was a previously rare cause of salmonellosis in the United States. The implicated food items (raw hot peppers) grown in Mexico were recalled.
2009					
Escherichia coli O157:H7 infection (*15*)	X	X	X		A multistate outbreak in 70 persons (25 were hospitalized, 7 showed development of hemolytic uremic syndrome) was associated with eating raw, refrigerated, prepackaged cookie dough that was exported to numerous other countries.
S. enterica serovar Typhimurium infection (*12*)	X	X	X	X	Several hundred cases have been reported in 43 states with an onset during September–December 2008. The outbreak was associated with peanut-containing products, an unusual vehicle for this organism. At least 30 countries may have received the products, and a food recall was implemented.
2010					
Lassa fever (*17*)	X		X		Four days after travel on 3 connecting flights involving 3 continents, a patient was hospitalized for sore throat, diarrhea, thrombocytopenia, and given a diagnosis 5 d later. No high-risk contact was identified, but awareness of travel-associated cases is essential.
Oil spill (*16*)	X	X			An accident at an oil drilling rig off the US coast resulted in an ongoing leak from the well. Modeling suggested that high winds might distribute oil over a wider area, which may potentially affect coastal fisheries and other human activity in other countries.
S. enterica serovar Montevideo infection (*8*)	X		X		Several hundred cases were identified in 42 states with an onset during July 2009–January 2010. The implicated food item (salami) was exported to 8 countries and was recalled.
Typhoid fever (*19*)	X	X	X		Nine confirmed cases were reported to CDC from 2 states; 7 persons were hospitalized. Consumption of frozen mamey fruit pulp was epidemiologically linked to the outbreak. The pulp was manufactured in Guatemala and shipped throughout the United States and possibly other countries.
2011					
Guillain-Barré syndrome (*18*)	X	X	X		Twenty-three suspected cases were clustered in time and place along the United States–Mexico border, possibly associated with *Campylobacter jejuni.*
S. enterica serovar Enteritidis infection (*9*)		X	X	X	Forty-two cases were reported in 6 states linked to consumption of pine nuts imported from Turkey. The product was recalled.

*WHO, World Health Organization; IHR, International Health Regulations; X, yes; CDC, Centers for Disease Control and Prevention.
†1, Is the public health effect of the event serious?; 2, Is the event unusual or unexpected?; 3, Is there a major risk for international spread?; 4, Is there a major risk for international travel or trade restrictions?

During the assessment by senior public health scientists, it was determined that the event was unusual in that pine nuts had not been associated with *Salmonella* spp. outbreaks and thus constituted an unusual vehicle of transmission.

In addition, it was determined that a major risk for international spread and potential for trade restrictions were present because the pine nuts were imported from Turkey and similarly exported to Canada. However, the event did not meet the criterion for a serious effect on public health. Given that *Salmonella* spp. are estimated to contribute to 11% of all domestically acquired foodborne illness and >1 million estimated illnesses each year (*23*), this outbreak was not particularly large compared with other *Salmonella* spp. outbreaks. Because 3 of the 4 assessment criteria from the IHR decision instrument were met, WHO was formally notified of the event. A PHEIC was not determined by WHO, but the event was posted as a WHO-assessed public health risk on the IHR EIS. In the United States, the product was recalled from the grocery store chain, and no new cases were identified 44 days after the beginning of the outbreak.

The third example is a joint report by the United States and Mexico for a binational cluster of cases of acute flaccid paralysis (*18*). At the time of reporting, 23 suspected cases of Guillain-Barré syndrome were identified in a localized area along the United States–Mexico border. Several of the case-patients had evidence of infection with the enteric bacterium *Campylobacter jejuni*, which has been associated with Guillain-Barré syndrome. The event was determined to have a potentially serious effect on public health because several hospitalizations had been reported. The event was also determined to be unusual or unexpected because the local incidence of acute flaccid paralysis had doubled, compared to the expected rate for the same time frame and location. The joint assessment stated that the event posed a major risk for international spread because cases had been reported in the border area in Mexico and in the United States. However, because of localized spread, albeit between 2 countries, the event was not deemed to potentially lead to travel or trade restrictions. At the time the event was reported, because it met 3 of the 4 IHR assessment criteria, the definitive diagnosis, Guillain-Barré syndrome, or the underlying cause for the outbreak (later believed to have been caused by diarrheal illness likely linked to contaminated water systems), were not yet known. This report was not determined to be a PHEIC by WHO, but was posted as a WHO-assessed public health risk on the IHR EIS, as were regular updates on the progress of the outbreak investigation.

The ability of the United States to assess a public health event under the assessment criteria of the IHR decision instrument depends on the following: 1) the federal government becomes aware of an event; 2) federal, state, and local subject matter experts investigating the event are familiar with IHR reporting obligations; 3) and functional surveillance systems are in operation. The ability to determine to report an event requires minimum epidemiologic assessment capacities, including a certain level of expert judgment, and close collaboration with involved parties (e.g., local and state health departments, other federal agencies, or foreign governments). In the United States, we reported >10% of all events posted on the IHR EIS as events assessed by WHO by using the criteria for public health risk for international concern since the IHR went into effect.

Overall, events posted on the IHR EIS represent events that occurred in ≈60% of states parties. Taking into account that the implementation of the IHR is a collective learning process, this might reflect the need to define the purpose of the IHR EIS and be explicit about the threshold for assessment and posting. For example, not all notified events from the United States were posted as WHO-assessed events on the IHR portal, but some were used for public health action by WHO; for example, notifications to WHO of international air travelers with extensively drug-resistant tuberculosis resulted in contact-tracing activities in several countries. In other situations, states parties might be less prone to initiate and sustain a dialog with WHO through the IHR communication channels because of their limited capacity to detect unusual health events or restrictive information sharing policies. An example of this reluctance includes incomplete reporting of new cases of poliomyelitis.

This information signals the need for additional resources to implement the IHR globally. WHO is collaborating closely with its member states to meet IHR requirements for core capacities for surveillance, including the capacity to detect events of potential international public health concern and rapidly assess and report these events to WHO. Although the decision instrument allows for user judgment and experience with resultant lack in specificity (*24*), it can serve as an aid toward the goal of rapid and transparent reporting by states parties. By June 2012, states parties were expected to meet the minimum core capacities for surveillance and response, and development of designated air ports, sea ports, and ground crossings, unless they request a 2-year extension from WHO.

Contribution of IHR to Global Information Exchange

Although states parties are documenting their progress toward implementation of the IHR requirements, the IHR has already fostered transparency and speed of sharing information on emerging health threats globally. Provision of secure web portals for public health events and designation of NFPs enable access to PHEIC assessments of other countries and enhance direct exchange of public

health information between countries. For example, in 2011 in the United States, we were notified directly at least once a month by NFPs in other countries about an outbreak or possible exposure to an infectious disease that might merit public health follow-up by US public health officials, e.g., contacting a traveler about possible exposure to an infectious disease.

The IHR serve as a reminder of our obligation to the global community, which may get lost in an outbreak investigation and staging of domestic control efforts, and provide a framework for WHO to coordinate a globally harmonized response. This obligation was put to test during the influenza A(H1N1)pdm09 virus outbreak, just 2 years after the IHR went into effect. Although the weaknesses of some countries in detecting and reporting novel influenza strains came to light, the level of coordination through regular regional consultations by the WHO director-general and secure and rapid information exchange on the IHR web portal on new cases and response strategies (*25*) were unprecedented and a welcome improvement to the less coordinated response during the SARS outbreak in 2003. In the first 6 months of the influenza A(H1N1)pdm09 outbreak alone, 517 event updates were posted on the IHR web portal. Continuing to strengthen the capacity of WHO member states to detect, analyze, and report emerging health threats remains a priority for WHO.

Many countries do not rely solely on rapid information exchange within the IHR framework or on traditional surveillance systems to learn about emerging health threats in their own or other countries. For example, nontraditional surveillance based on the widespread availability of the Internet and advances in informational technology over the past 15 years that have provided access to media reports can be used as a rich and useful source for early warning of disease threats, even in situations in which the disease or the etiologic agent are unknown. Event-based surveillance has become a critical part of the global biosurveillance programs of WHO (*26*), the US government, and other countries. The IHR provide a common framework for disease detection and information sharing, including confirmation of media-based reports, but also for in-depth consultation and coordinated response for global threats.

Conclusions

Reporting of potential PHEICs under the IHR framework is not complete when simply counting the number of states parties (n = 105) who reported events that were posted on the IHR web portal in the past 5 years. However, having the IHR framework for notification in place enables improved global connectivity through better situational awareness and built-in global consultation provisions for response. Over time, the global public health community will come to a shared understanding of what merits IHR reporting to WHO, and will build the IHR assessment into their routine detection and response activities. Such a standardized approach in a secure information exchange environment will provide some assurance that not only will persons, goods, and pathogens travel rapidly around the world, but so will information regarding risks to global public health.

Dr Kohl is deputy director of the Division of Global Migration and Quarantine, Centers for Disease Control and Prevention, Atlanta, GA. Her research interests are implementation of the international health regulations and improving health of globally mobile populations.

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Address for correspondence: Katrin S. Kohl, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30333, USA; email: kkohl@cdc.gov

International Health Regulations—What Gets Measured Gets Done

Kashef Ijaz, Eric Kasowski, Ray R. Arthur, Frederick J. Angulo, and Scott F. Dowell

The global spread of severe acute respiratory syndrome highlighted the need to detect and control disease outbreaks at their source, as envisioned by the 2005 revised International Health Regulations (IHR). June 2012 marked the initial deadline by which all 194 World Health Organization (WHO) member states agreed to have IHR core capacities fully implemented for limiting the spread of public health emergencies of international concern. Many countries fell short of these implementation goals and requested a 2-year extension. The degree to which achieving IHR compliance will result in global health security is not clear, but what is clear is that progress against the threat of epidemic disease requires a focused approach that can be monitored and measured efficiently. We developed concrete goals and metrics for 4 of the 8 core capacities with other US government partners in consultation with WHO and national collaborators worldwide. The intent is to offer an example of an approach to implementing and monitoring IHR for consideration or adaptation by countries that complements other frameworks and goals of IHR. Without concrete metrics, IHR may waste its considerable promise as an instrument for global health security against public health emergencies.

The global spread of severe acute respiratory syndrome highlighted the need to detect and control disease outbreaks at their source (*1*,*2*). The 2005 revised International Health Regulations (IHR) were established as a legally binding agreement providing a framework for improving detection, reporting, and response to public health emergencies of international concern (public health emergencies) (*3*). The global implementation of IHR began on June 15, 2007, and in an unusual episode of international consensus, all 194 WHO member states ratified the agreement. When implemented, IHR should improve global capacity to detect, assess, notify, and respond to public health threats. Properly and fully implemented, IHR should usher in a new global era of international communication, cooperation, and unprecedented security against the epidemic threats that have plagued humanity since ancient times. But there is a problem.

After enactment of the revised IHR in June 2007, all member countries were required to develop and implement a minimum of core public health capacities by June 2012, the 5-year anniversary of IHR's enforcement. Many countries did not meet the deadline and have requested a 2-year extension. In an era of limited resources, competing priorities, and political challenges, achievement of the IHR implementation goals, even with an extension, will be a challenge. Focusing efforts toward IHR implementation and capacity building and enabling all countries to measure progress toward IHR implementation is, therefore, essential. Toward this end, concrete goals and metrics for 4 of the 8 core capacities were developed by the WHO Collaborating Center for IHR Implementation of National Surveillance and Response Capacity at the Centers for Disease Control and Prevention with other US government partners in consultation with WHO and national collaborators worldwide (Table 1). This approach is in alignment with WHO's IHR framework and facilitates measurement of implementation activities. The framework focuses on 4 of the core capacities (human resources, surveillance, laboratory, and response) and builds on WHO's IHR Monitoring Framework by defining simple standards for these capacities (*4*). The focus on these 4 capacities should not imply that they are more important than other capacities (legislation, policy, and financing; coordination; advocacy and national focal point communications; preparedness; and risk communication) because implementation of IHR

Author affiliations: World Health Organization Collaborating Center for International Health Regulations Implementation of National Surveillance and Response Capacity, Atlanta, Georgia, USA; and Centers for Disease Control and Prevention, Atlanta

DOI: http://dx.doi.org/10.3201/eid1807.120487

Table 1. Goals, targets, and intended use for 4 core capacities for focusing International Health Regulations implementation

Capacity	Goal	Target/measure	Intended use
Human resources	Ensure adequate numbers of trained personnel are available to support the response to a public health emergency	A national workforce plan and 1 trained field epidemiologist for every 200,000 persons	Document that a workforce plan exists and is maintained and updated, and monitor annual progress toward the goal of 1 trained field epidemiologist for every 200,000 persons.
Surveillance	Ensure that surveillance systems capable of detecting selected potential public health emergencies in any part of the country are established and functioning	Surveillance infrastructure that demonstrates the ability to detect ≥3 of 5 syndromes indicative of a potential public health emergency of international concern	Monitor and evaluate the effectiveness of the surveillance system, and identify areas for improvement within the country's public health surveillance infrastructure.
Laboratory	Ensure access to laboratory diagnostic capabilities that can identify a range of emerging epidemic pathogens by using the full spectrum of basic laboratory testing methods	Ability to perform 10 core diagnostic tests for confirmation of indicator pathogens from any part of the country	Assess/measure capacity for detection will by using external/internal quality assurance for each of the 10 core tests and indicator pathogens using standard methods.
Response	Ensure countries have adequate rapid response capacity for public health emergencies	At least 1 functioning rapid response team per major administrative unit	Maintain an adequate number of rapid response teams with the necessary training, appropriate personnel, and regular outbreak responses.

requires implementation of all 8 capacities. The intent is to assist partner countries in better focusing efforts, to improving efficiency at IHR implementation, and to better monitoring and evaluating progress. Focusing on the subset of IHR core capacities also will provide a foundation for an all-hazards approach for addressing public health emergencies regardless of cause. We describe the rationale, targets, and definitions for these 4 goals and means by which countries can use the data collected through monitoring and evaluation indicators for measuring progress related to these 4 core capacities.

Human Resources

A well-trained cadre of public health professionals at the national health authorities at a country's central and local levels is needed for timely detection and response to public health emergencies. There is a worldwide shortage of public health professionals who are trained in public health practice and have had competency-based public health field experience. Building the cadre of field-trained epidemiologists available to monitor disease trends, inform decision makers about potential disease threats, and guide response during a public health emergency should be one of the first priorities in implementing the IHR.

The aim of the human resource goal is to ensure adequate numbers of trained personnel for response to a public health emergency. Specific targets to measure progress toward completion of this goal are a fully adopted national workforce plan and ≥1 trained field epidemiologist per 200,000 population who are active in the public health sector (*5*). Although the workforce plan cannot ensure that trained professionals remain in the public health sector, it will at least indicate a government's commitment to public health through stability of the public health workforce. These concrete indicators enable measurement of incremental progress and are specific enough to enable tracking of success and clear documentation of failure.

Surveillance

Disease surveillance is a cornerstone of public health practice. It provides for systematic and ongoing collection of data that help identify and detect disease-related aberrations that might constitute public health emergencies. Additionally, surveillance for key disease syndromes provides the foundation for interpreting signals of possible emergencies and early notification of outbreaks of potentially devastating diseases (*6*). The following 5 syndromes have internationally recognized standards for syndromic surveillance: severe acute respiratory syndrome, acute neurologic syndrome, acute hemorrhagic fever, acute watery diarrhea with dehydration, and jaundice with fever (*7*,*8*).

The metrics focus on the ability to detect public health emergencies with a target of documenting that ≥3 of these syndromes have surveillance systems in place that meet the respective international standards. These metrics will assist countries in ensuring that efforts at disease surveillance are effective and that systemic incentives are appropriately aligned to provide early warning for a potential public health emergency. The 3 syndromes chosen will depend on national disease control priorities. These surveillance systems should include early warning surveillance data and laboratory findings, which should be analyzed by trained epidemiologists.

Information for syndromic surveillance collected at the clinic or hospital level can help generate village- and district-level alerts. An alert investigation unit can then investigate these alerts, including an in-depth epidemiologic analysis. On the basis of the outcome of the analysis, rapid

response teams can be deployed to respond to a public health event or outbreak.

Laboratory

Laboratory diagnostic capacity can help in detecting emerging or reemerging pathogens in a timely manner and can support syndromic surveillance systems by adding specificity. Given the costs associated with establishing laboratory diagnostic capacity, diagnostic capability might not be feasible for all pathogens for every country. Therefore, pooling international laboratory resources through networks of local, national, regional, and international reference laboratories is encouraged. However, countries should be able to provide certain core diagnostic tests (either through their own or through network capacity) quickly and reliably to direct disease surveillance and response activities.

The metrics focus on the ability to perform 10 international reference standard tests for patients from any part of the country. The core tests and their respective indicator pathogens are selected from the IHR immediately notifiable list, the WHO Top Ten Causes of Death in low-income countries (www.who.int/mediacentre/factsheets/fs310/en/index.html), and tests and indicator pathogens selected by the country on the basis of major national public health concern (Table 2).

However, achievement of laboratory diagnostic capacity requires all major components of the laboratory network to be well integrated in the national laboratory system. Components of such a system include sample collection, specimen transport, specimen processing, quality management systems, biosafety and biosecurity (specimen storage), staff, infrastructure, cold chains, reporting, and networking peripheral and central or regional reference laboratories. Data on the capacity and ability of the country to perform and report the 10 core tests can be used to monitor the ability of a country's own laboratories or the reference laboratories to which it sends specimens to confirm and characterize these indicator pathogens and identify areas for improvement.

Response

To implement IHR 2005, countries must have adequate rapid response capacity. During a public health emergency, timely response to public health events and threats is essential to prevent excess illness and death and control further transmission, including transborder spread. The presence of well-trained and functioning rapid response teams at local and national levels in a country can ensure a rapid, well-coordinated, and organized public health response.

These rapid response units should comprise a multidisciplinary team of trained public health professionals—medical epidemiologists, veterinarians, laboratory scientists, clinicians, chemical experts, and radiologic experts—as appropriate for the event who routinely deploy within 24 hours after a reported event. Rapid response units enhance a country's ability to respond to outbreaks in a timely and effective manner.

These teams should undergo regular exercises for responding to public health emergency events, including ≥2 field outbreak investigations per year. They also should be trained in the 10 basic steps for outbreak investigations (*10*).

To meet the goal of adequate response capacity for public health emergencies, we propose a target of ≥1 functioning rapid response team per major administrative unit (district, province, or state). Larger administrative areas might need >1 team. Data and after-action reports from outbreak responses collected annually will enable the countries to monitor their progress, identify gaps, and improve performance.

Conclusions

Implementation of IHR, required of all WHO member states, was not completed by the June 2012 deadline. The aim is for all countries to develop or enhance the ability to detect and respond to public health emergencies. Additionally, possible public health emergencies of international concern also need to be reported to prevent the spread of disease around the globe. Countries need concrete

Table 2. Core laboratory tests and indicator pathogens in the International Health Regulations

Core test	Indicator pathogen	Turnaround time from receipt in the laboratory
PCR	Influenza virus*	Within 24 h
Virus culture	Poliovirus*	Within 14 d
Serology	HIV†	Within 5 d
Microscopy	*Mycobacterium tuberculosis*†	Within 3 d
Rapid diagnostic test	*Plasmodium* spp.†	Within 2 h
Bacterial culture	*Salmonella enteritidis* serotype Typhi‡	Within 3 d
Local priority test	Local priority test§	Local priority test
Local priority test	Local priority test§	Local priority test
Local priority test	Local priority test§	Local priority test
Local priority test	Local priority test§	Local priority test

*Selected from the International Health Regulations immediately notifiable list.
†Selected from WHO Top Ten Causes of Death in low-income countries (www.who.int/mediacentre/factsheets/fs310/en/index.html).
‡Selected from WHO Global Foodborne Infections Network (www.who.int/gfn/en).
§Indicator pathogens selected by the country on the basis of major national public health concern.

and well-defined goals and indicators to monitor their progress toward implementation of IHR core capacities. Even though we described metrics for 4 of the 8 IHR core capacities, we emphasize that full IHR compliance requires implementation of all 8 capacities. Goals and progress indicators also might be useful for the other 4 capacities. Without explicit goals and targets, the promise of international consensus around IHR might be wasted, but with them there is hope that what gets measured will eventually get done.

Acknowledgments

The development of these metrics owes much to Richard Hatchett for steady leadership and to Jennifer R. Bae, Beth Cameron, Jose Fernandez, Dave Franz, Catherine Hill-Herndon, Matthew Johns, Rebecca Katz, Daniel Miller, Sumi Paranjape, Murray Trostle, Anne Yu, and many others for their intellectual contributions, policy insights, public health expertise, and international experience in the many revisions and refinements. We also acknowledge our partners at the Centers for Disease Control and Prevention who are engaged in IHR, i.e., Division of Public Health Systems and Workforce Development and Division of Global Migration and Quarantine.

All authors played a major role in preparing the manuscript, including writing (K.I., R.R.A., S.F.D.), review and feedback (K.I., E.K., R.R.A., F.J.A., S.F.D.), and overall conceptualization of the manuscript (K.I., E.K., R.R.A., F.J.A., S.F.D.).

Dr Ijaz is the deputy director for the Division of Global Disease Detection and Emergency Response, Center for Global Health, Centers for Disease Control and Prevention, and oversees the science and programmatic aspects in the division. The division is also the WHO Collaborating Center for IHR Implementation of National Surveillance and Response Capacity. His research interests include tuberculosis and detection of infectious disease outbreaks and response.

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Address for correspondence: Kashef Ijaz, Centers for Disease Control and Prevention, Mailstop D68, 1600 Clifton Rd NE, Atlanta, GA 30333, USA; email: kijaz@cdc.gov

Lessons Learned from Influenza A(H1N1)pdm09 Pandemic Response in Thailand

Kumnuan Ungchusak, Pathom Sawanpanyalert, Wanna Hanchoworakul, Narumol Sawanpanyalert, Susan A. Maloney, Richard Clive Brown, Maureen Elizabeth Birmingham, and Supamit Chusuttiwat

In 2009, Thailand experienced rapid spread of the pandemic influenza A(H1N1)pdm09 virus. The national response came under intense public scrutiny as the number of confirmed cases and associated deaths increased. Thus, during July–December 2009, the Ministry of Public Health and the World Health Organization jointly reviewed the response efforts. The review found that the actions taken were largely appropriate and proportionate to need. However, areas needing improvement were surveillance, laboratory capacity, hospital infection control and surge capacity, coordination and monitoring of guidelines for clinical management and nonpharmaceutical interventions, risk communications, and addressing vulnerabilities of non-Thai displaced and migrant populations. The experience in Thailand may be applicable to other countries and settings, and the lessons learned may help strengthen responses to other pandemics or comparable prolonged public health emergencies.

Cases of influenza A(H1N1)pdm09 virus were first reported to the World Health Organization (WHO) by the US Centers for Disease Control and Prevention (CDC) on April 24, 2009 (*1*). On April 27, the director general of WHO raised the level of the influenza pandemic phase from 3 to 4, and 2 days later, the level was raised to 5 (*2*). In Thailand, because of experience gained during the response to an outbreak of avian influenza A (H5N1) (*3*,*4*), the Ministry of Public Health (MOPH) immediately assumed a central role in coordinating national response efforts to a possible influenza A(H1N1)pdm09 outbreak in that country.

On May 12, 2009, 2 imported cases of A(H1N1) pdm09 virus infection were detected in Thailand, and by the end of the month, 12 more cases were reported by the MOPH. In early June, indigenous outbreaks associated with entertainment centers (*5*), schools (*6*), and military barracks (*7*) were reported. By July, A(H1N1)pdm09 virus transmission was detected in all 76 Thai provinces, and 65 deaths were confirmed to be associated with the infection.

National surveillance data indicated that 2 pandemic waves occurred during the initial 12-month outbreak period. The first wave began in May 2009, peaked in July, and subsided in December; the second wave began in January 2010, peaked in early February, and subsided in April. A third pandemic wave occurred during the latter part of 2010. During 2009–2010, a total of 234,050 influenza cases were reported in Thailand. Of these, 47,433 were laboratory-confirmed to be A(H1N1)pdm09 virus infections; 347 deaths were associated with the confirmed cases (Figure 1).

WHO recommends that countries review their pandemic response and mitigation efforts immediately after a pandemic peak or pandemic phase. In mid-July 2009, the MOPH proposed that the Thai national response be reviewed. This proposal was partially in response to publicly voiced criticism that the pandemic response had not been appropriately handled. To demonstrate transparency and to garner insight from countries that could share valuable insight from their pandemic experience (e.g., Australia and Hong Kong, People's Republic of China), the Thai MOPH review team was joined by WHO staff and external technical specialists. Seven focus areas were

Author affiliations: Ministry of Public Health, Nonthaburi, Thailand (K. Ungchusak, P. Sawanpanyalert, W. Hanchoworakul, N. Sawanpanyalert, S. Chunsuttiwat); Thailand Ministry of Public Health–US Centers for Disease Control and Prevention, Nonthaburi (S,A. Maloney); World Health Organization, New Delhi, India (R.C. Brown); and World Health Organization, Nonthaburi (M.E. Birmingham)

DOI: http://dx.doi.org/10.3201/eid1807.110976

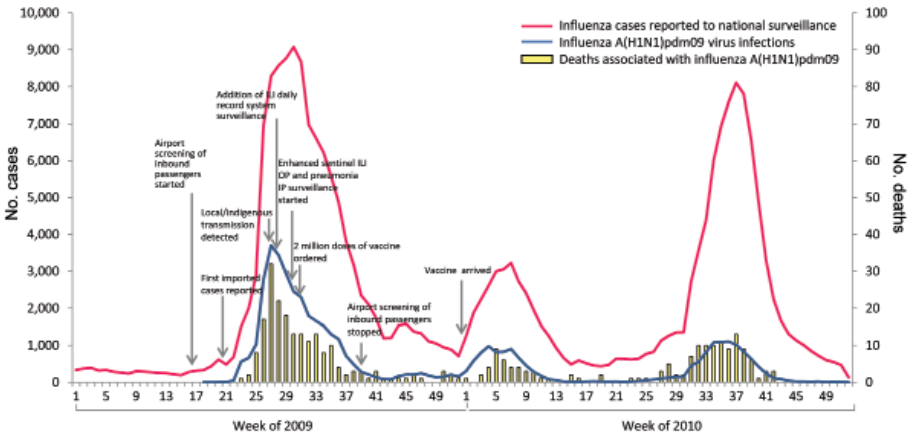

Figure 1. Reported number of influenza cases, laboratory-confirmed influenza A(H1N1)pdm09 virus infections, and deaths associated with confirmed influenza A(H1N1)pdm09 virus infections, Thailand, 2009–2010. ILI, influenza-like illness; OP, outpatient; IP, inpatient.

identified for review: 1) surveillance and epidemiology; 2) laboratory services; 3) public health interventions and control measures (including hospital infection control); 4) clinical management; 5) logistics, commodities, and operations; 6) public communications; and 7) measures to assist vulnerable non-Thai populations. The reviews were conducted during August 18–December 6, 2009. In total, 47 team members participated and contributed 271 person-days. Detailed reports and a 28-page summary of the strengths and challenges of the Thai pandemic response were submitted to the minister of health.

The formal review findings (lessons learned) as well as those from a review of the local experience in Thailand are being used to inform current and future pandemic plans in Thailand. They are also likely applicable to other countries and settings and could be used to strengthen responses to future pandemics or to comparable severe, prolonged public health emergencies. In this article, we outline some of the lessons learned during the first 12 months of the national response to the A(H1N1)pdm09 pandemic in Thailand.

Lessons Learned in Thailand

Layered Surveillance Is Critical to an Effective Pandemic Response

During the SARS outbreak, the screening of inbound passengers for fever at national/international ports of entry was a common practice by most countries. Thus, politicians and the public believed that the strategy should be included as part of any global epidemic response effort. During the A(H1N1)pdm09 pandemic, this belief created an environment in which it became difficult for the Thai MOPH to target screening activities toward identifying and testing only symptomatic persons arriving from affected countries. At the same time, the MOPH recognized that screening for A(H1N1)pdm09 infection was different than screening for SARS. They realized that SARS-like screening might be of limited value because persons with asymptomatic A(H1N1)pdm09 virus infection could transmit the virus, and persons with symptomatic infection might not have symptoms during inbound border screening. For this reason, fever screening at ports of entry was adopted, not with the expectation of containing early local spread but with the less ambitious aim of possibly detecting infections earlier and slowing the initial spread of virus, thus providing more time to prepare for the pandemic (*8*,*9*). Screening of inbound air passengers to Thailand was implemented on April 27, 2009. Persons with suspected A(H1N1)pdm09 virus infection were treated with antiviral drugs, and close contacts of possible case-patients were given prophylaxis. By June 17, a total of 1,669,501 inbound passengers had been screened at Thailand's main international airport in Bangkok; 638 of those screened had a fever, and only 2 were confirmed to have A(H1N1)pdm09 virus infection. As the pandemic spread rapidly throughout Thailand, the value of inbound screening was increasingly questioned, and screening was eventually stopped at the end of September 2009.

As expected, despite active screening of inbound air passengers, indigenous transmission and outbreaks were soon observed in entertainment venues and schools. Thailand's routine national surveillance system includes a national passive notifiable disease surveillance system, which includes notification of pneumonia and influenza cases requiring hospitalization (defined mainly by code criteria of the International Classification of Diseases, Tenth Revision). Thailand's national influenza surveillance also includes a sentinel system focused on monitoring virus infections; the system includes 8–10 sentinel hospitals that obtain data and specimens from patients seeking medical care for influenza-like illness (ILI). In response to the 2009 pandemic, the MOPH enhanced the surveillance system in 2 ways. First, in May 2009, the MOPH established a daily ILI reporting system to measure geographic and temporal

trends for ILI in hospital outpatient departments across the country. Second, the network of sentinel influenza surveillance sites previously established to monitor influenza serotypes was supplemented by an additional 14 new sites. Sites in the expanded network collected respiratory specimens and performed influenza testing for outpatients with ILI and for hospitalized patients with pneumonia. These additions enabled monitoring of spatial-temporal trends and estimations of the prevalence of disease. Overall trends for ILI mirrored those of laboratory-confirmed cases of A(H1N1)pdm09 virus infection, supporting the usefulness of ILI data (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-0976-Techapp.pdf).

In addition to these noted strengths in Thailand's national surveillance system response, the review team also identified several areas in which improvements should be pursued. These included improving linkages between epidemiologic, laboratory, and clinical data sources; expanding private health care participation in surveillance activities; and strengthening capacity for infectious disease modeling.

Development of Guidance for Clinical Management and Antiviral Drug Use Is an Iterative Process

Before onset of the pandemic, the Thailand MOPH had established a national stockpile of 300,000 oseltamivir treatment courses, which was sufficient to treat 0.5% of the population. An additional 1 million courses were added when reported cases and deaths appeared to accelerate during the first pandemic wave. The decision to increase the stockpile was prompted in part by the results of local mathematical modeling exercises, which suggested that among the Thai population of 63 million persons, 157,000 could be hospitalized with A(H1N1)pdm09 virus infection and 1,260 could die.

The process of supplying oseltamivir to health facilities was greatly facilitated by a central, Internet-based vendor-managed inventory system, which enabled daily updating of hospital inventories. Individual health care facilities were primarily responsible for monitoring and replenishing their stocks of personal protective equipment, which were supplemented by a network of regional and provincial stockpiles.

A clinical case management and practice guideline was rapidly made available to all health care workers; the guideline was updated on 3 occasions as new information became available. Revisions focused on the medical management of patients at risk for severe disease, including the need for early administration of oseltamivir (Figure 2). However, anecdotal reports suggested that nationwide adoption of new guidelines by physicians may take up to a month, indicating a need for innovative methods to introduce and implement new guidelines.

An initial policy of screening and testing all persons at risk for A(H1N1)pdm09 virus infection proved problematic. The policy was instituted in an attempt to mitigate spread of infection; however, it led to a widespread public perception that laboratory testing was mandatory for diagnosis and treatment of A(H1N1)pdm09 virus infection. The MOPH subsequently rescinded the policy and issued guidance recommending that persons with suspected A(H1N1)pdm09 virus infection be treated on the basis of clinical rather than laboratory findings. However, patients overlooked the revised policy and continued to request laboratory confirmation of infection, and physicians felt obliged to respond to patient requests; thus, laboratory services became overburdened.

Laboratory Services Can Become Overburdened

The laboratory system in Thailand provided commendable support to national efforts for combatting A(H1N1)pdm09 virus transmission, especially during the early months of the pandemic. Vast numbers of specimens were tested, and laboratories operated 24 hours a day, 7 days a week. In most cases, laboratory reports were provided within 24 hours of specimen receipt. Laboratory support and expansion were well articulated in Thailand's national influenza preparedness plan, and surge capacity was quickly organized and implemented.

During the pandemic, the use of PCR was adopted as a standard for laboratory diagnosis of influenza. PCR technology had already been used at the National Influenza Center and 3 university teaching hospitals in Bangkok, and capacity was rapidly established in an additional 14 regional laboratories to share the burden of work and enable rapid testing. The increased capacity for laboratory testing enabled the National Influenza Center and 1 university laboratory to focus on more specialized testing, including molecular sequencing and monitoring of antiviral drug resistance.

In addition to these strengths in the national response effort, the review team also identified several weak areas in need of improvement. The need to strengthen laboratories was highlighted in the national preparedness plan and implemented at the start of the pandemic; however, as discussed above, a national strategy for rational use of laboratory services during high-demand situations was not available to clinicians and public health professionals early in the pandemic. This lack of guidance resulted in an extremely heavy demand for laboratory services. Furthermore, although a plan was in place, it did not anticipate the number of specimens for which testing was requested. During the first 3 months of the pandemic, the National Influenza Center in Thailand processed 10,796 specimens, of which 4,082 were positive for A(H1N1)pdm09 virus infection. Although additional surge capacity

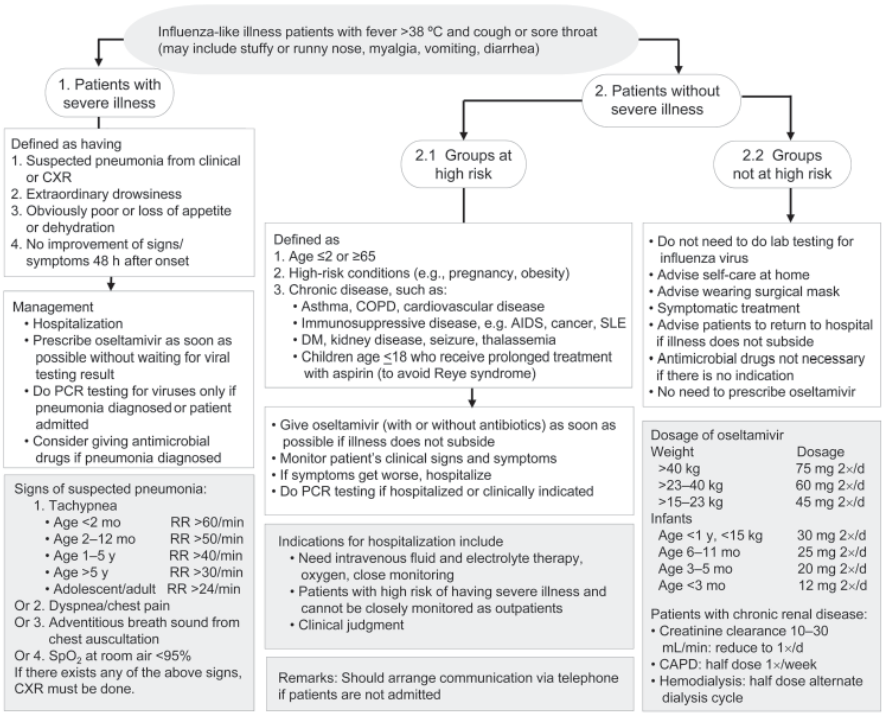

Figure 2. Third edition (July 17, 2009) of clinical practice guidelines for treatment of patients with suspected influenza A(H1N1)pdm09 virus infection in Thailand. The guidelines were prepared by the Clinical Management Taskforce, Thailand Ministry of Public Health, and experts from medical schools. The guidelines are subject to modification according to the pandemic influenza situation; updates are made available at www.moph.go.th. CXR, chest x-ray; COPD, chronic obstructive pulmonary disease; SLE, systemic lupus erythematosus; DM, diabetes mellitus; bid, twice a day; CAPD, continuous ambulatory peritoneal dialysis; RR, respiratory rate; SpO_2, saturation of peripheral oxygen.

was soon developed and implemented, the heavy demands for laboratory testing led to delays in making some results available. Recommendations for future strengthening of the laboratory system included expanding PCR capacity in provincial hospitals, which could serve as referral centers, and clarifying roles specified for each type of laboratory. Such actions would help optimize the laboratory system, maximize efficiency, and enable central laboratories to focus on more specialized functions and research activities.

Enhanced Infection Control and Surge Capacity Is Needed for Intensive Care Services

During the A(H1N1)pdm09 pandemic in Thailand, several outbreaks were reported among hospital staff and patients (*10*). Infection prevention and control practices appeared to vary at the health care facility level, although most hospitals had dedicated infection control nurses and functional infection prevention and control committees. Before the pandemic, excellent arrangements were in place for screening and triage in hospitals. The arrangements were derived from procedures established during the outbreak of influenza virus A(H5N1), However, despite those arrangements, outpatient services during the first pandemic wave were soon overwhelmed with "worried well" persons seeking information and advice. Hospitals became crowded with patients with ILI, among whom only a small proportion had moderate to severe influenza. Intensive care units in many hospitals became overburdened during the peak of the first wave. Optimal delivery of care might have been achieved through better networking among hospital intensive care units; improved networking could have enabled the sharing of caseloads, resources, and expertise.

Application and Monitoring of Nonpharmaceutical Interventions Must Be Consistent

The use of good hand hygiene practices, social distancing measures, and face masks was emphasized in national policy and prevention guidelines. These measures were widely promoted and implemented, particularly during the first pandemic wave, when awareness and anxiety levels were high. Implementation of social distancing measures varied by setting, especially in relation to school closure; the varied implementation was probably due in part to the decentralization of decision-making to the local level. The business sector, health foundations, schools, and local community authorities provided good support for public education campaigns. An initial shortage of alcohol gel and face masks was addressed by increasing local production.

An effective mechanism for oversight was not established at the outset of the pandemic, and the lack of such a mechanism presented a challenge for monitoring the effectiveness of public health interventions. Variation and "drift" in the application and implementation of national policies and guidelines were also observed at different administrative levels. Local variations in compliance with national policies and guidelines may have been related to differences in the perception of risk among health professionals and the public and to ineffective communication and feedback systems

between authorities at the central level and health providers at peripheral levels. One recommendation for addressing this challenge in the future is to establish a national public health emergency incident command center to coordinate and communicate policies, strategies, and guidance related to an emergency and to monitor their execution and facilitate feedback to concerned parties, particularly on problems related to implementation.

Availability and Uptake of Pandemic Vaccine Must Be Timely

In July 2009, the Thai government approved the procurement of 2 million doses of A(H1N1)pdm09 vaccine. This amount was determined by using existing registries and other data to estimate the number of health care workers and the number of persons considered to be at high risk for complications related to A(H1N1)pdm09 virus infection. The vaccine arrived at the end of the first wave, during the last week of December 2009, and was targeted to groups at high risk and to frontline health care workers. Vaccination campaigns began in early January 2010, but vaccination uptake was slow and less than projected. There are several possible reasons for this, including the perception of diminishing risk and safety concerns expressed by members of the public, in part related to media reports of (unrelated) fetal deaths in pregnant women who had received the vaccine. Uptake among pregnant women was only 6% (30,000) of the planned 500,000 target population. This percentage is consistent with observations by obstetricians that pregnant women in Thailand were not convinced that the potential benefits of vaccination greatly outweighed any possible risk.

At the same time that Thailand is trying to improve access to pandemic influenza vaccine, it is also trying to establish national capacity for pandemic vaccine production. Since 2008, and with the support of WHO's Global Action Plan, the Thailand MOPH has embarked on a development project to enhance national capacity for pandemic influenza vaccine development and production. This country project aims to establish capacity for producing inactivated and live-attenuated pandemic vaccines. Although this project did not produce a vaccine in time for the 2009 A(H1N1) pdm09 pandemic, it has served as an excellent platform for further development of the national and regional influenza vaccine capacity in preparation for future pandemics.

Risk Communication Requires Active Coordination and Monitoring

Public information and risk communication messages were disseminated through a variety of media, including television, radio, and extensively distributed printed materials. However, on several occasions, government officials issued contradictory statements on the status of the pandemic or conflicting health advice. A possible explanation for these shortcomings was the lack of a systematic process to ensure timely delivery of consistent and correct information to the public by politicians, officials, and partners. The communications infrastructure in Thailand is strong; however, some partners could have been better used to assist with disseminating public communications and with collecting feedback regarding the effectiveness of key messages in terms of public understanding and behavior. Furthermore, it would seem intuitive that better communication and public messaging could be used to address the challenges previously discussed in terms of vaccine uptake and health system overloads caused by the worried well. One recommendation proposed addressing these weaknesses by establishing an operational risk communications unit within MOPH.

Needs of Displaced Persons/Migrants Must Be Included in Pandemic Preparedness and Response Plans

An estimated 144,567 displaced persons live in 9 temporary shelters in Thailand, predominantly on the Thailand/Myanmar border, and ≈2 million registered and unregistered international migrants provide unskilled labor in Thailand. Displaced persons residing in temporary shelters receive basic health care services primarily from nongovernmental organizations, and a compulsory migrant health insurance scheme is in place for registered migrants in Thailand.

Surveillance for influenza and other priority communicable diseases is considered to function well and is linked with the national surveillance mechanisms. However, pandemic response plans for different displaced and migrant population settings were not always congruent, and specific national policies were not completely explicit in defining access to services (care and laboratory diagnostics) and life-saving medicines, such as oseltamivir. In many such settings, surge capacity for delivery of health care was limited, and staff and volunteer health workers were not sufficiently trained in pandemic influenza preparedness and response. Also, public health messages were sometimes not available in the language of displaced or migrant persons (*11*). However, among displaced and migrant populations during the first 12 months of the pandemic, only 1 documented A(H1N1) pdm09 outbreak occurred, with no confirmed deaths, and the review team found that, in general, services were provided on a humanitarian basis when needed.

External Evaluation of Response Efforts after a Pandemic Peak Is Useful

The team reviewing Thailand's pandemic response identified numerous strengths and several shortcomings.

Because of the timing of the review, some of the lessons learned and some of the shortcomings, particularly in the areas of health care surge capacity, surveillance, and laboratory capacity, were at least partially addressed or rectified during the second and third waves of the A(H1N1) pdm09 pandemic. In addition, the review findings and lessons learned are now being used to guide development of future pandemic preparedness plans in Thailand. The joint MOPH/WHO pandemic review required the mobilization of substantial human and financial resources at a time of already considerable demand. Therefore, consideration should be given to building a strong monitoring and evaluation component into pandemic preparedness plans, including surge capacity for handling review tasks.

Discussion

We describe lessons learned from the national response to the influenza A(H1N1)pdm09 pandemic in Thailand by reviewing the local experience and a formal MOPH/WHO report on a joint review of the response efforts (*12*). Several of the lessons learned have been identified and discussed in other reports; our work supports and enriches the published data surrounding these lessons.

A report of the WHO Review Committee on the functioning of the international health regulations in relation to the A(H1N1)pdm09 pandemic (*13*) stressed that the response requirements of health care systems needed more attention and strengthening. The report also advocated interim case-finding, treatment and management protocols and algorithms, infection control guidelines, guidance on triaging and surge capacity management, and staffing strategies. The findings in the WHO report, much like our findings, emphasized that although avian influenza had made a difference in pandemic preparedness for Asian countries, the 2009 influenza pandemic strained health care and laboratory services, and the strain would have been worse in a more severe pandemic.

Fisher et al., in a review of pandemic response lessons from 6 Asian countries (Singapore, Hong Kong, People's Republic of China, Malaysia, South Korea, and Vietnam) (*14*), noted some key health challenges similar to those in the Thailand experience: a need to strengthen health care surge capacity (especially intensive care services), an inability of containment measures to prevent cross-border entry of influenza, challenges with the adoption of recommendations for empiric use of oseltamivir, and the insufficient coordination of the dissemination of clinical management and laboratory protocols and updates and other communications. In addition, Hanvoravongchai et al. and the AsiaFluCap Project (*15*) reported results from rapid analyses of pandemic influenza preparedness in 6 Asian countries. Similar to the situation in Thailand, many of the countries were challenged by the need for greater flexibility in pandemic planning and implementation in order to accommodate changing transmission circumstances and different pandemic scenarios.

The importance of a joint review after a pandemic peak or pandemic phase cannot be overemphasized. In Thailand, the review process and reports, which clearly identified strengths and weaknesses of the pandemic response and provided concrete suggestions for how lessons learned might be used to revise plans for dealing with future events, were used to modify the national response during the second and third waves of the 2009 influenza pandemic. In addition, lessons learned from the review are serving as a helpful resource for the development of a new 5-year national strategic plan for preparedness and response to emerging diseases, which will be submitted for official government endorsement in the near future.

Acknowledgments

We thank WHO for providing the technical and financial support for this review. We greatly appreciate the contribution of the following review team members: Pasakorn Akrasewi, Michael Basso, Rajesh Bhatia, Aphaluck Bhatiasevi, Channawong Burapat, Patricia Ching, Tawee Chotipittayanond, Gilles Cimetiere, James Edward Fielding, Peter Horby, David Hui, Brian Kapella, Opas Karnkawinpong, Pensri Kerdnark, Darika Kingnate, Philip Lam, Jan-Erik Larsen, C. Raina MacIntyre, Arun Mallik, Bjorn Melgaard, Aree Moungsookjareoun, Roderico Ofrin, Kitti Pitaknitinun, John Rainford, Steven Riley, Jariya Sangsujja, Wing Hong Seto, Nahoko Shindo, Mark Simmerman, Kleete Simpson, Adelle Springer, Anupong Sujariyakul, and Chadin Tephaval.

Financial support was provided by WHO.

Dr Ungchusak is the senior expert in preventive medicine at the Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Nonthaburi, Thailand. His current work is related to avian influenza, pandemic influenza, and public health emergencies of international concern.

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Address for correspondence: Kumnuan Ungchusak, Bureau of Epidemiology, Department of Disease Control, Ministry of Public Health, Tivanond Rd, Nonthaburi, Thailand; email: kum.ungchusak@gmail.com

Seroprevalence of Schmallenberg Virus Antibodies among Dairy Cattle, the Netherlands, Winter 2011–2012

Armin R.W. Elbers, Willie L.A. Loeffen, Sjaak Quak, Els de Boer-Luijtze, Arco N. van der Spek, Ruth Bouwstra, Riks Maas, Marcel A.H. Spierenburg, Eric P. de Kluijver, Gerdien van Schaik, and Wim H.M. van der Poel

Infections with Schmallenberg virus (SBV) are associated with congenital malformations in ruminants. Because reporting of suspected cases only could underestimate the true rate of infection, we conducted a seroprevalence study in the Netherlands to detect past exposure to SBV among dairy cattle. A total of 1,123 serum samples collected from cattle during November 2011–January 2012 were tested for antibodies against SBV by using a virus neutralization test; seroprevalence was 72.5%. Seroprevalence was significantly higher in the central-eastern part of the Netherlands than in the northern and southern regions ($p<0.001$). In addition, high (70%–100%) within-herd seroprevalence was observed in 2 SBV-infected dairy herds and 2 SBV-infected sheep herds. No significant differences were found in age-specific prevalence of antibodies against SBV, which is an indication that SBV is newly arrived in the country.

During the last 2 weeks of August and the first 2 weeks of September 2011, dozens of veterinary practitioners in the Netherlands reported to a monitoring help desk (GD Veekijker) that several dairy herds with cows showed a sudden decrease in milk production, watery diarrhea, and occasional fever (*1*). The affected animals recovered, and extensive bacteriologic, virologic, and parasitologic testing of feces and blood samples of sick cows did not reveal an infectious cause for the clinical signs. Similar problems were reported at about the same time in Germany, and on November 18, 2011, the Friedrich Loeffler Institute (Greifswald, Germany) reported the detection of a novel orthobunyavirus that could be the cause of the clinical problems (*2*). Real-time reverse transcription PCR (RT-PCR), made available by the Friedrich Loeffler Institute, was used to test stored blood samples (N = 50) from the clinically diseased cattle in the Netherlands; 36% had positive test results. Since then, the virus has also been associated with congenital malformations in young animals (lambs, goat kids, and calves) (*3*).

The new virus is provisionally called Schmallenberg virus (SBV), or Shamonda-like virus. It is a RNA virus and shows 97% identity with Shamonda virus (SHAV) (small gene segment), 71% identity with Aino virus (medium gene segment), and 69% identity with Akabane virus (AKAV) (large gene segment) (*4*). All these viruses are part of the Simbu serogroup of the family *Bunyaviridae*, genus *Orthobunyavirus*. The Simbu serogroup is composed of several arthropod-borne viruses (arboviruses, including SHAV, AKAV, and Aino virus) transmitted by *Culicoides* spp. biting midges and mosquitos. SHAV was initially isolated from cattle and *Culicoides* spp. biting midges in Nigeria in the 1960s (*5*,*6*). In 2002, SHAV emerged in Japan and was isolated from *Culicoides* spp. biting midges

Author affiliations: Central Veterinary Institute, part of Wageningen UR, Lelystad, the Netherlands (A.R.W. Elbers, W.L.A.Loeffen, S. Quak, E. de Boer-Luijtze, R. Bouwstra, R. Maas, E. P. de Kluijver, W.H.M. van der Poel); Netherlands Food and Consumer Product Safety Authority, Utrecht, the Netherlands (A.N. van der Spek, M.A.H. Spierenburg); and GD Animal Health Service, Deventer, the Netherlands (G. van Schaik)

DOI: http://dx.doi.org/10.3201/eid1807.120323

and sentinel cattle (*7*). Japan has been considered an area to which several Simbu group viruses have been endemic in cattle since the 1970s (*8*).

Knowledge specifically related to SBV is limited; according to a risk assessment by the European Centre for Disease Prevention (http://ecdc.europa.eu/en/publications/Publications/231112_TER_Risk_assessement_Schmallenberg_virus.pdf), transmission of SBV to humans is considered unlikely but cannot be ruled out. Recently, serosurveys were conducted to assess zoonotic transmission of SBV in farmers and veterinarians in Germany and the Netherlands, who are likely to come in contact with the virus, but no infection was found (*9*,*10*).

In the Netherlands, reporting of suspected cases of SBV infection in animals (occurrence of arthrogryposis hydranencephaly syndrome in calves, lambs, and goat kids) is obligatory; a report is followed by confirmatory testing of brain tissue samples by RT-PCR. However, the observed suspected cases are likely an underestimation of the true rate of infection; in addition, infected livestock may give birth to healthy young animals, adding to the underestimation of the true rate of infection. Therefore, serodiagnostic studies are needed to detect past exposure to SBV in ruminant populations in the affected countries. Within weeks after the start of the SBV epidemic, a virus neutralization test (VNT) was developed at the Central Veterinary Institute (CVI). This test made it possible to quickly execute a seroprevalence study of antibodies against SBV in dairy cattle in the Netherlands.

Materials and Methods

Seroprevalence Sampling Design

To estimate the seroprevalence of SBV in the dairy cattle population in the Netherlands with considerable precision, we used the following preconditions for sample size calculation (*11*): an a priori expected prevalence of 50% (this will yield the highest possible sample size), a maximum allowable error in the prevalence estimate of ≈3%, a 95% confidence in the estimate, and a population size of ≈1.5 million head of dairy cattle (on the basis of 2012 census data of Statistics Netherlands, The Hague, the Netherlands). These conditions yielded a calculated sample size of ≥1,100 randomly selected dairy cattle.

Because dairy cattle and the premises on which they are housed are not distributed homogenously in the Netherlands, a stratified random sampling design with the 12 provinces in the Netherlands as a stratification level was set up to provide a representative sample. On the basis of census data of Statistics Netherlands (*12*), the stratified distribution of dairy cattle by province was used for setting up the sampling frame. The sampling frame comprised dairy cattle from which serum samples were collected during November 2011–January 2012 for monitoring testing of antibodies against bluetongue virus or as part of a specific surveillance investigation of 125 dairy farms to exclude introduction of notifiable animal diseases because of purchase of possibly contaminated bedding material from a third country. Serum samples from these dairy cattle were stored at CVI and were available for our study. The dairy cattle in our final sampling list (most drawn from the bluetongue monitoring set, completed with 37 randomly selected samples originating from 12 cattle herds from the surveillance investigation set) were randomly selected within each stratum (province) of the sampling frame proportional to the number of dairy cattle in each province. This process provided an accurate representation of dairy cattle of the target population in our sample.

Animals within a herd share common characteristics such as nutrition, housing, and exposure to disease pathogens (*13*). In the case of infectious diseases, common exposure to disease pathogens probably results in a common serologic status within herds. As a consequence, differences in prevalence between herds are larger than differences between animals within herds. Therefore, it is essential to sample relatively more herds and fewer animals within a herd than in a situation without clustering of disease events, as was shown for estimating the population prevalence for pseudorabies virus infection (*14*). A measure for agreement in serologic status between animals within a herd is given by the intraclass correlation coefficient μ (*15*). Because we presumed a high intraclass correlation with respect to serologic status of animals within herds (based on preliminary test results from a few infected herds), on average 2 dairy cattle (minimum 1, maximum 4) from the same dairy herd were included in the sampling list to prevent occurrence of too many cattle from the same herd. This selection procedure resulted in 1,123 samples from dairy cattle from 489 dairy herds.

In addition to estimating seroprevalence of SBV in the dairy cattle population in the Netherlands, we estimated the seroprevalence in the dairy cattle population by 3 regions in the Netherlands to determine possible regional differences in seroprevalence. These regions were the northern part of the Netherlands (465 samples), comprising Groningen, Friesland, Drenthe, and Noord-Holland Provinces; the southern part of the Netherlands (196 samples), comprising Zeeland, Zuid-Holland, Noord-Brabant, and Limburg Provinces; and the central-eastern part of the Netherlands (462 samples), comprising Overijssel, Gelderland, Flevoland, and Utrecht Provinces. A cattle density map on municipality level was created on the basis of the number of cattle per municipality as received from the "Dienst Regelingen" from the Ministry of Economic Affairs, Agriculture and Innovation. Figure 1 shows the geographic distribution of dairy herds from which we tested, on

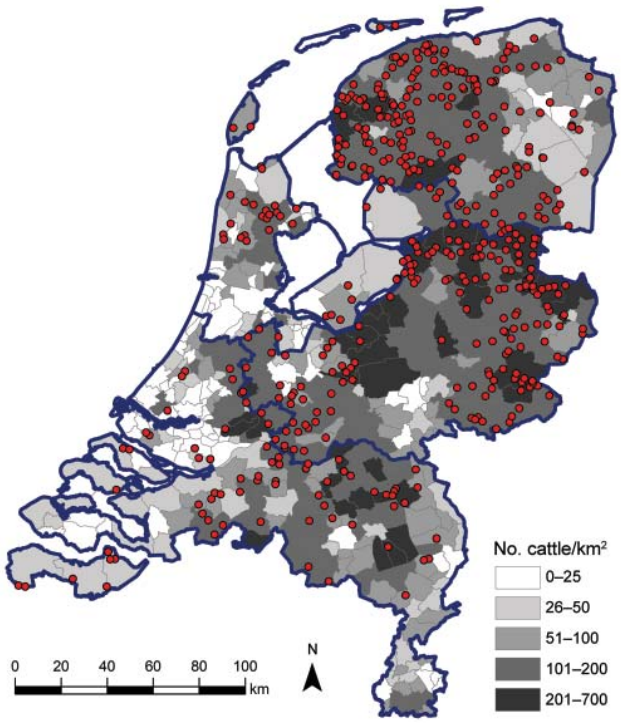

Figure 1. Geographic distribution of dairy herds from which 1–4 animals were sampled (red dots) in study of Schmallenberg virus seroprevalence, the Netherlands, 2011–2012. Cattle density is indicated by gray shading; blue outlines denote regional borders.

average, 2 dairy cattle. The data indicate that our sampling was indeed representative for the geographic distribution of cattle in the Netherlands.

The mean age of cattle tested was 23 months (range 12–79 months); 60% were 20–24 months of age. To test possible differences in age-specific antibody prevalence for cattle in the northern, central-eastern, and southern regions, we defined 3 age cohorts: <18 months, 18–24 months, and >24 months. Date of birth and date of blood sampling were available for 1,085 head of cattle, which enabled us to calculate the age of the cattle at the date of blood sampling.

Within-herd Seroprevalence

To gain insight into the within-herd seroprevalence of infected herds (based on RT-PCR test results of malformed lambs and calves that had been born), we sampled 2 cattle herds and 2 sheep flocks for comparison. We used the following preconditions for sample size calculation: an a priori expected prevalence of ≈70%, a maximum allowable error in the prevalence estimate of ≈5%, and a 95% confidence in the estimate.

Sheep flock 1 consisted of 800 ewes ≥1 year of age, 120 ewes <1 year of age, and 14 rams. The flock was located in the eastern part of the Netherlands. From the beginning of the study through December 30, 2011, a total of 41 lambs were born; 15 lambs (37%) were malformed. Clinical signs observed in the malformed lambs were arthrogryposis, ankylosis, scoliosis, torticollis, kyfosis, and hydranencephaly. From this flock, 60 ewes that had already lambed were tested.

Sheep flock 2 consisted of 81 ewes ≥1 year of age and 1 male ram. The flock was located in the southern part of the Netherlands. From the beginning of the study through February 7, 2012, a total of 30 lambs were born (15 female and 15 male); 2 lambs (13%) were malformed (1 female and 1 male). Clinical signs observed in the malformed lambs were arthrogryposis, ankylosis, torticollis, ataxia, and neurologic signs. From this flock, 35 ewes that had already lambed were tested.

Dairy herd 1 consisted of 58 dairy cattle ≥2 years of age and 40 young stock <2 years of age. The flock was located in the southwestern part of the Netherlands. From the beginning of the study through February 8, 2012, 1 calf was born malformed. Clinical signs observed in the malformed calf were arthrogryposis and ankylosis. From this herd, 34 dairy cattle were tested.

Dairy herd 2 consisted of 40 dairy cattle ≥2 years of age and 20 young stock <2 years of age. The flock was located in the northern part of the Netherlands. From the beginning of the study through January 30, 2012, 2 malformed calves (twins) were born. Clinical signs observed in the malformed calves were scoliosis and hydranencephaly. From this herd, 34 dairy cattle were tested.

Statistical Analysis

Exact 95% CIs for estimated seroprevalences were calculated according to Fleiss (*16*). Differences in mean seroprevalence of antibodies against SBV of dairy cattle populations between regions in the Netherlands were tested with the 2-sample proportion test (*17*). Differences in age-specific mean prevalence of antibodies against SBV of dairy cattle in the northern, southern, and central-eastern region were tested with the 2-sample proportion test (*17*).

An intraclass correlation coefficient μ was calculated to measure the agreement in serologic status between dairy cattle sampled within the same herd. The intraclass correlation coefficient (minimum 0, maximum 1) was estimated by using analysis of variance, with herd as independent variable and the serologic status of individual animals (seropositive or seronegative) as dependent variable (*15*).

Serologic Test

Serum samples were tested in a VNT against SBV (W.L.A. Loeffen et al., unpub. data). A virus isolate from brain tissue of a lamb, fourth passage on Vero (African green monkey kidney) cells, was used in the test, which

was performed in flat-bottomed, 96-well microtiter plates on VERO cells. The medium used for cells and dilutions was Dulbecco minimal essential medium + Glutamax (GIBCO Invitrogen, Carlsbad, CA, USA), contained with 3% fetal calf serum and 1% penicillin and streptomycin at final concentrations of 100 IU and 100 µg/mL, respectively, in the medium. Serum samples were heated for 30 min at 56°C before testing. Serum samples were diluted in the test plate, starting from 1:4, followed by 2-fold dilutions up to 1:512 in volumes of 50 µL. Subsequently, virus (500 median tissue culture infective dose per well) was added to each well, also in a volume of 50 µL. After preincubation at 37°C for 1–2 hours, 20,000 cells per well were added in a volume of 100 µL. Plates were incubated for 5 days at 37°C in 5% CO_2.

After 5 days, the plates were emptied and stained with amido black. The titer was determined as the reciprocal of the dilution in which 25%–100% of the monolayer was still intact. Titers ≥8 were considered positive on the basis of a prior validation in which a specificity and sensitivity of >99% were estimated with this cutoff. Control samples (positives and negatives) were included in each run of the test. Virus used in each run was back titrated in 24 columns of 4 dilutions each.

Results

The estimated seroprevalence of antibodies against SBV in dairy cattle, winter 2011–2012, for the Netherlands (N = 1,123) was 72.5% (95% CI 69.7%–75.1%). The agreement in serologic status between dairy cattle sampled within the same herd in our prevalence study, as measured by the intraclass correlation coefficient, was high, 0.73. This finding indicates that in any particular herd, a strong tendency exists that either most cattle in that herd will be seropositive or most will be seronegative.

We found no statistically significant ($p>0.05$) differences in age-specific mean prevalence of antibodies against SBV of cattle in the 3 regions (Table). In the southern and northern regions, we found a slight trend of increased seroprevalence from the younger to the older age cohorts; in the central-eastern region, this trend was absent.

Figure 2 shows the geographic distribution of seropositive dairy herds (≥1 cows sampled tested seropositive) and seronegative dairy herds (all cows sampled tested seronegative). These data indicate no association between cattle density and occurrence of seropositive or seronegative herds. Furthermore, the geographic distribution of seropositive and seronegative herds is random, showing no specific clusters of seropositive or seronegative herds. The estimated seroprevalence of antibodies against SBV in dairy cattle in the central-eastern part of the Netherlands (n = 462; seroprevalence 82.7%, 95% CI 78.8%–86.0%) was significantly ($p<0.001$) higher than the estimated seroprevalence of antibodies against SBV in dairy cattle in the northern (n = 465; seroprevalence 67.1%, 95% CI 62.6%–71.3%) and southern (n = 196; seroprevalence 61.2%, 95% CI 53.9%–68.0%) parts of the country. Figure 3 shows the distribution of VNT antibody titers against SBV of seropositive samples from dairy cattle; 50% of the samples showed a titer ≥512.

For testing of within-herd seroprevalence, in dairy herd 1, 25/34 cows tested seropositive (within-herd seroprevalence 73.5%, 95% CI 55%–87%); in dairy herd 2, all 34 cows tested seropositive (within-herd seroprevalence 100%, 95% CI 87%–100%). In sheep flock 1, 56/60 ewes tested seropositive (within-flock seroprevalence 93.3%, 95% CI 82%–98%); in sheep flock 2, 25/35 ewes tested seropositive (within-flock seroprevalence 71.4%, 95% CI 52%–85%).

Discussion

We found a high seroprevalence of antibodies against SBV in dairy cattle in the Netherlands in the winter of 2011–2012, which indicates widespread exposure to SBV during the biting insect seasons of spring, summer, and fall 2011. Between the start of the investigation and last day of the surveillance period (March 29, 2012), a total of 782 calves with suspected SBV infection were tested by PCR in the Netherlands (Netherlands Food and Consumer Product Safety Authority, www.vwa.nl/onderwerpen/dierziekten/dossier/schmallenbergvirus); only 14% had positive test results. Combined with our study results on within-herd seroprevalence and the fact that a certain population of infected livestock would not produce malformed calves because the dams were infected outside the critical period of pregnancy, this finding illustrates the considerable underestimation of the true rate of infection in the population when only counting suspected cases.

The seroprevalence of antibodies against SBV in dairy cattle is significantly higher in the central-eastern part of the Netherlands than in the northern and southern parts of the country. This finding likely indicates that SBV was first introduced into the eastern part of the Netherlands and is supported by the fact that the first dairy herds reporting

Table. Age-specific mean prevalence of antibodies against Schmallenberg virus among cattle, the Netherlands, 2011–2012

	No. cattle (prevalence, %)			
Age range, mo	Northern region	Central-eastern region	Southern region	Total
<18	7 (42.9)	13 (76.9)	13 (46.2)	33 (57.6)
18–24	331 (65.0)	337 (82.8)	151 (60.9)	819 (71.6)
>24	103 (72.8)	98 (80.6)	32 (68.8)	233 (75.5)

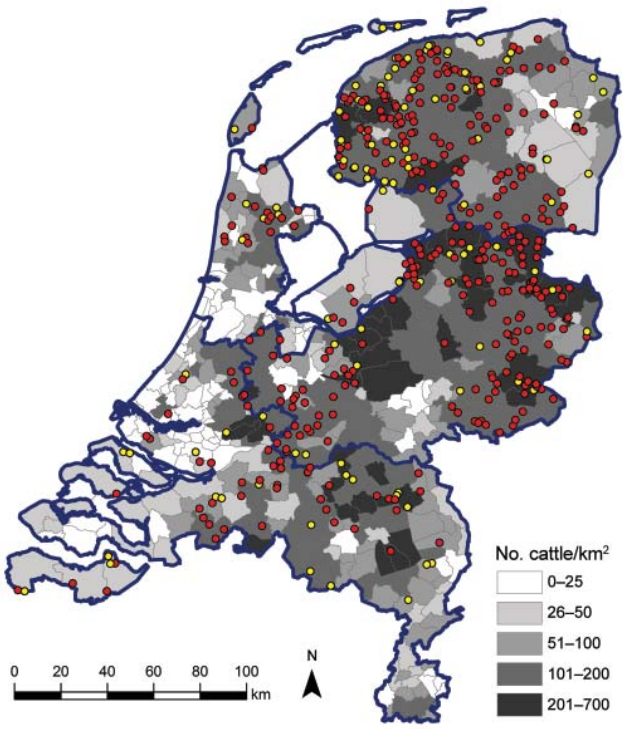

Figure 2. Geographic distribution of dairy herds sampled in study of Schmallenberg virus seroprevalence with positive results (≥1 animals sampled tested seropositive; red dots) and negative results (all animals sampled tested seronegative; yellow dots), the Netherlands, 2011–2012. Cattle density is indicated by gray shading; blue outlines denote regional borders.

cows with clinical signs of SBV infection in September 2011 were located in the same areas (*1*).

We found no significant differences in age-specific mean prevalence of antibodies against SBV of cattle in the 3 regions, which indicates that SBV is newly arrived in the area. A clear increasing age-specific prevalence would have suggested that the virus had been there for 2–3 years but unrecognized earlier on. In the southern and northern region, there was a slight trend of increased seroprevalence from the young to the older age-cohorts, which can be expected because the young age cohort is housed inside for most of the time, preventing exposure to infected vectors. In the central-eastern region, this trend was absent, which is another indication that SBV was first introduced into the eastern part of the Netherlands.

Testing of serum samples banked during other studies before 2011 is planned to determine whether evidence exists of SBV infection before 2011. We could find no comparable seroprevalence studies on SBV or SHAV activity from other countries. However, a seroprevalence study conducted at the end of the New South Wales AKAV epidemic that occurred during April–October 1974 showed 80% seroprevalence in ≈4,000 serum samples from cattle (*18*). This finding illustrates that an outbreak season with another orthobunyavirus can result in a comparable level of infection to that found in our study.

Regarding SBV within-herd seroprevalence, our preliminary results indicate that, by the end of an outbreak season, most animals within an affected herd have been infected. Previous studies investigating AKAV outbreaks showed comparably high within-herd seroprevalence of antibodies in cattle in Australia: 77% in 1964 (*19*), up to 89% in 1971 (*20*), and 99% in 1988 in New South Wales (*21*). Furthermore, serologic investigations in the Kumamoto and Kagoshima Prefectures in Japan, where 98.3% of 119 tested cattle with neurologic signs were seropositive against AKAV, showed that 74.3% of cohabitated cattle without neurologic signs in these farms were also seropositive (*22*). Monthly sampling of sentinel cattle in Australia indicated that within 1–2 months after the start of sampling, 100% of the sentinel animals within herds were seropositive for AKAV exposure (*23*,*24*).

We cannot predict the progress of SBV during the coming months in the ruminant populations in the Netherlands. While a certain level of protection against new infection may be expected for naturally infected animals, but to our knowledge, no solid information on the protective capacity of SBV antibodies exists. In addition, in a population showing a seroprevalence of 70%, it should be assumed that a considerable portion of animals remain susceptible to SBV infection. Recent reports of SBV in *Culicoides* spp. biting midges from Belgium and Denmark implicated *C. obsoletus* complex and *C. dewulfi* midges as potential vectors in the transmission and spread of SBV (*25*,*26*). From experiences with other ruminant Simbu serogroup viruses in Asia and Australia, it may be assumed that, if previously uninfected animals are infected by vectors before mating, protection would be incurred against the occurrence of congenital malformations in newborns

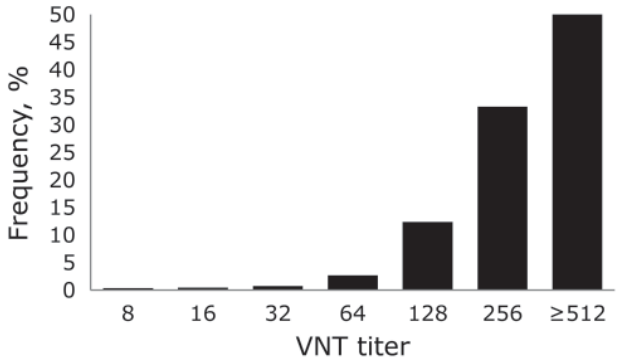

Figure 3. Frequency distribution of titers for serum samples (n = 814) positive for Schmallenberg virus antibodies by virus neutralization test (VNT) in study of Schmallenberg virus seroprevalence, the Netherlands, 2011–2012.

(*27*). Vaccination of the dams before they are mated would likely produce a similar protection; however, no vaccine for SBV is available.

Our study estimated seroprevalence at the population level and showed differences in seroprevalence among regions within the Netherlands. If an estimate of seroprevalence is desired at a more detailed regional level, a larger number of animals must be sampled and tested. This estimate will be feasible (cost- and labor-wise) when samples are tested with the VNT using a limited number of dilutions or an ELISA (less expensive and labor-intensive than the VNT) becomes available.

When designing our prevalence study, we assumed a high agreement in serologic status among dairy cattle sampled within the same herd. The observed within-herd prevalence and the high agreement in serologic status among dairy cattle sampled within the same herd in our study retrospectively indicate that sampling a relatively low number of animals within a herd and relatively more herds enables an accurate estimate of the overall seroprevalence of the dairy cattle population. These infection dynamic characteristics can be used by other research groups when designing future seroprevalence studies in the other SBV-affected countries.

Acknowledgments

We thank Hendrik-Jan Roest, Miriam Koene and Aldo Dekker for generously providing negative validation serum samples; Mieke Maris (CVI) for collecting serum samples from infected farms (positive field serum samples for validation) and culturing of the virus; Yolanda de Visser, Rick Koopman, Diana Korver, Heleen van den Heuvel and Betty Verstraten (CVI) for planning and execution of the diagnostic testing; Gert-Jan Boender (CVI) for preparing Figures 1 and 2.

This study was commissioned and funded by the Dutch Ministry of Economic Affairs, Agriculture, and Innovation (WOT programme #01).

Dr Elbers is a veterinary epidemiologist and senior scientist in the Department of Epidemiology, Crisis organisation and Diagnostics, Central Veterinary Institute, part of Wageningen UR, Lelystad. His research interests are notifiable animal diseases, and surveillance and early detection systems.

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Address for correspondence: Armin R.W. Elbers, Department of Epidemiology, Crisis Organisation and Diagnostics, Central Veterinary Institute, part of Wageningen UR, Houtribweg 39, NL-8221 RA Lelystad, the Netherlands; email: armin.elbers@wur.nl

Predicting Risk for Death from MRSA Bacteremia[1]

Mina Pastagia, Lawrence C. Kleinman, Eliesel G. Lacerda de la Cruz, and Stephen G. Jenkins

Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteremia is often fatal. To determine predictors of risk for death, we conducted a retrospective cohort study. We examined 699 episodes of MRSA bacteremia involving 603 patients admitted to an academic medical center in New York City during 2002–2007. Data came from chart reviews, hospital databases, and recultured frozen MRSA specimens. Among the 699 episodes, 55 were caused by vancomycin–intermediate resistant *S. aureus* strains, 55 by heteroresistant vancomycin-intermediate *S. aureus* strains, and 589 by non–vancomycin-resistant strains; 190 (31.5%) patients died. We used regression risk analysis to quantify the association between clinical correlates and death. We found that older age, residence in a nursing home, severe bacteremia, and organ impairment were independently associated with increased risk for death; consultation with an infectious disease specialist was associated with lower risk for death; and MRSA strain types were not associated with risk for death.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide concern; it colonizes and infects patients in the hospital and in the community (*1*). For the past 50 years in the United States, the standard therapy has been vancomycin. Recent vancomycin treatment failures have raised questions regarding optimal treatment (*2*). Although new antimicrobial drugs (e.g., linezolid, daptomycin, tigecycline) have been developed, none has been consistently superior to vancomycin for the treatment of MRSA (*3*,*4*), and MRSA resistance rapidly develops for many new drugs (*5*,*6*). Some studies have suggested MIC creep (increasing vancomycin MICs against MRSA over time), but others have not (*7*,*8*). In 2006, the upper limit of vancomycin susceptibility for *S. aureus* was redefined, lowered from 4 μg/mL to 2 μg/mL, first by the Clinical and Laboratory Standards Institute and soon thereafter by the US Food and Drug Administration and the European Committee on Antimicrobial Susceptibility (*9*).

Vancomycin treatment failures for MRSA occur even when MICs are within the range considered susceptible, especially 1–2 μg/mL (*10*–*13*). Among high-risk bacteremic patients, Sakoulas et al. documented treatment failure rates of 44% when vancomycin MICs were <0.5 μg/mL and of 90% when vancomycin MICs were 1–2 μg/mL (p = 0.01) (*10*). Hidayat et al. found that mortality rates were higher for patients infected with strains with higher vancomycin MICs (*11*)

Some apparently susceptible strains of MRSA might actually be heteroresistant vancomycin-intermediate *S. aureus* (hVISA) strains. That is, although the hVISA isolates seem to be susceptible to vancomycin according to conventional testing, the isolates contain subpopulations of colonies resistant to vancomycin. Testing for hVISA has not been standardized and is not routinely undertaken. hVISA strains are more common in strains with higher vancomycin MICs (*14*,*15*). hVISA might contribute to worse clinical outcomes, but this possibility has not been convincingly confirmed by large studies.

To determine predictors of risk for death among patients with MRSA bacteremia, we conducted a retrospective study that compared demographic and clinical characteristics of adult patients with MRSA bacteremia. MRSA strains from

Author affiliations: The Rockefeller University, New York, New York, USA (M. Pastagia); Mount Sinai School of Medicine, New York (L.C. Kleinman, E.G. Lacerda de la Cruz); and Weill Cornell School of Medicine, New York (S.G. Jenkins)

DOI: http://dx.doi.org/10.3201/eid1807.101371

[1]Parts of the data in this article were presented at the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy/Infectious Diseases Society of America Meeting, October 25–28, 2008, Washington, DC, USA; and the 19th European Congress of Clinical Microbiology and Infectious Diseases Meeting, May 16–19, 2009, Helsinki, Finland.

these patients were vancomycin susceptible, VISA, and hVISA. We analyzed a 5-year trend of vancomycin MICs among adult patients with MRSA bacteremia. We also analyzed the associations between host factors, organism factors, and death versus survival, and quantifed the marginal contribution of key factors to risk for death.

Methods

Our retrospective study was conducted in New York, New York, USA, at Mount Sinai Medical Center, a 1,171-bed tertiary-care academic center that serves a diverse ethnic and medical population. We studied 699 episodes of blood infection from 603 patients who had had MRSA bacteremia during 2002–2007. This study was approved by the institutional review board of the Mount Sinai School of Medicine.

Laboratory Specimens

At Mount Sinai Medical Center, MRSA organisms identified from blood culture are routinely stored frozen at −70°C. We retrieved frozen blood culture isolates (previously not thawed or subcultured) for all adult patients hospitalized with MRSA bacteremia from January 2002 through May 2007. We excluded episodes of polymicrobial bacteremia if MRSA was isolated in a single blood culture bottle or if the patient received inappropriate empirical treatment for the co-pathogen. We included in our analysis the first organism isolated from blood culture during any episode of MRSA bacteremia.

A computerized data system identified 748 eligible isolates, among which we were able to retrieve 699 (93.4%). These isolates had originally been tested for drug susceptibility by use of an automated instrument, the Microscan (Siemens Healthcare, Sacramento, CA, USA); for all isolates, the vancomycin MICs were ≤2 μg/mL; during 2002–2007, we used the Positive Breakpoint Combo 20 (Siemens Healthcare), which might not accurately detect VISA isolates (*16*). We sent some isolates—8 (15%) VISA strains, 88 (15%) non-VISA strains, and 10 VISA control strains (from the Network on Antimicrobial Resistance in *Staphylococcus aureus*, www.narsa.net)—to an outside laboratory for blinded testing by using the Vitek 2 (bioMérieux, Durham, NC, USA) with the AST-GP-67 card, and we sent 8 (15%) hVISA isolates to an outside laboratory for retesting by using time-killing profiles. No discrepancies were noted.

Retesting of isolates was performed with no access (blinded) to clinical data. Mueller-Hinton agar plates (study and control strains) were inoculated with 0.5 McFarland inoculum (10^8 CFU/mL), and antimicrobial drug susceptibility to vancomycin was assessed by using Etest (AB Biodisk, Solna, Sweden), which has excellent sensitivity and specificity for this purpose (*17*). Isolates for which vancomycin MICs were ≥1 μg/mL (95.1%) were tested for the presence of hVISA by using vancomycin and teicoplanin Etest strips (the Etest macromethod). In brief, study and control inocula equivalent to 2.0 McFarland turbidity standard were plated on brain–heart infusion and Mueller-Hinton agars and incubated at 35°C–37°C for 24 and 48 hours, respectively. Isolates were interpreted as being hVISA strains when vancomycin and teicoplanin MICs were ≥8 μg/mL or teicoplanin-only MIC was ≥12 μg/mL (*16*). Quality control testing was performed weekly by using American Type Culture Collection (Manassas, VA, USA) organisms 29213, 29212, 700698 (hVISA), and 700699 (VISA) per Clinical and Laboratory Standards Institute guidelines (*9*). Actual Etest values were used for MIC_{50} (the value below which 50% of MIC values for MRSA isolates tested fell), MIC_{90} (the value below which 90% of MICs fell), and geometric mean MIC calculations.

Chart Review

We abstracted electronic and paper charts for each of the 603 patients corresponding to the 699 isolates; 65 of these patients were hospitalized ≥2 times for MRSA bacteremia. Chart abstraction was performed by 2 independent reviewers with no access (blinded) to laboratory data. Each reviewer separately examined 10% of charts; the κ statistic for coding of exemplar key variables was 0.87, indicating excellent agreement (*18*). Each new hospital admission was categorized as a new episode of bacteremia; the first positive blood culture was used as the index infection. We abstracted patient information regarding demographics, concurrent illnesses, patient's residence before hospitalization (facility vs. community), bacteremia severity, and previous health care exposures.

Definitions

We categorized each MRSA infection into 1 of 3 groups according to Etest result for vancomycin MICs as follows: VISA (MIC 4–12 μg/mL), hVISA, or non–VISA/hVISA MRSA (MIC ≤2 μg/mL). We also assessed MIC_{50} and MIC_{90} for vancomycin.

We defined the number of days to clearance of bacteremia as the date of first positive MRSA culture subtracted from the date of first negative culture for all patients for whom this information was available. Episodes of bacteremia ended on the date of death or on the date of the first negative blood culture that was not followed by a positive culture within 7 days. We did not study subsequent episodes during the same hospitalization. Vancomycin trough levels were measured after 3 doses of vancomycin; if multiple levels were measured, the modal level was used and classified as either ≥15 μg/mL or <15 μg/mL. We defined prior vancomycin exposure as receipt of ≥3 doses of vancomycin at least 7 days and ≤12 months

before MRSA bacteremia. Empirically prescribed therapy was defined as appropriate if MRSA were susceptible to the antimicrobial drug used (according to in vitro susceptibility testing) and if therapy was started within 48 hours of the blood culture result. History of MRSA infection was defined as hospitalization with MRSA during the prior 12 months. We defined an episode as community associated if the patient was bacteremic within 48 hours of hospitalization and lacked health care–associated risk factors such as dialysis, nursing home residence, or history of MRSA infection. We divided health care–associated MRSA cases into community onset or hospital onset. Cases were health care–associated community onset if the patient had such risk factors and was found to be bacteremic within 48 hours of hospital admission; cases were of health care–associated hospital onset if the bacteremia occurred after 48 hours of hospitalization, consistent with the schema of Klevens et al. (*19*).

We defined renal insufficiency as serum creatinine level ≥2 mg/dL or glomerular filtration rate <50 mL/min/1.73 m^2 according to the Cockcroft-Gault equation. The source of the bacteremia was determined by a combination of positive MRSA culture growth from a site other than blood, radiologic evidence, or an attending physician's statement in the medical record. We used the Duke criteria to define endocarditis (*20*). Severity of bacteremia at onset of infection was determined by use of vasopressors, elevation of serum creatinine levels from baseline (renal insufficiency), and admission to an intensive care unit after positive MRSA blood culture result. The major patient outcome measure was 90-day all-cause mortality rate. Mortality rate was determined by calculating deaths from the date of positive MRSA blood culture result up to 90 days while hospitalized, divided by the number of patients in the study (n = 603). Data regarding death after hospital discharge were not available for analysis. MRSA-attributable deaths were not included because of the difficulty in assessing exact cause of death.

Statistical Analysis

We used SAS version 9.1 (SAS Institute, Cary, NC, USA) for statistical analyses (*21*). We used standard methods to describe univariate data and to calculate *t*-tests, and we used χ^2 for bivariate associations. We used generalized linear models (SAS Proc GLM) to assess associations between the 3 groups of MRSA infection and quantitative variables. We conducted multivariable analyses by using logistic regression (SAS Proc Logistic). We developed models for 3 variables: infection with VISA, infection with hVISA, and death. Model building was guided first by conceptual models of likely effect and informed by our bivariate analysis results. We assessed correlation coefficients between pairs of potential predictor variables by using appropriate parametric or nonparametric methods and included only 1 of any pair of variables with an r^2 of ≥0.25 in any model. Guided by the rule for stability of estimates established by Peduzzi et al., we limited the total number of predictor variables in any model (*22*). The significance of the models (Tables 1, 2) are demonstrated by likelihood ratios, Wald <0.0001. When choosing between similar or comparable models, we selected the model associated with the smallest Akaike information criterion (<582); for example, in our analysis of predictors of risk for death, we rejected variables indicating HIV, malignancy, transplant, recent surgery, and presence of a medical device because they increased the Akaike information criterion. The predictive and discriminative performance of our models is shown in Tables 1 and 2. Our model predicting death has a c-score of 0.872; among 99,198 pairs, 87.1% were concordant, 12.7% were discordant, and 0.2% had the same scores. Although we also present the more familiar adjusted odds ratios, our primary measures of impact are adjusted risk measures (adjusted risk ratio and adjusted risk difference), which we derived from regression risk analysis, an enhancement over the usual presentation of logistic regression (*23*).

Results

Bacteria Characteristics

Each year during 2002–2006, the annual number of hospital admissions in this study were 117, 77, 147, 121, and 161, respectively; through May 31, 2007, another 76 patients were hospitalized (equivalent to 184 annual hospitalizations). The original testing of strains by Microscan did not detect VISA; repeat testing using the Vitek 2 detected 2 (25%) of 8 study strains and 2 (20%) of 10 control strains. The rate at which polymicrobial bacteremia met inclusion criteria was 3% (20 episodes). The Figure demonstrates the proportion of VISA, hVISA, and non–VISA/hVISA MRSA by year and the increase in mean vancomycin MICs during the study period. The vancomycin MICs for most (87%) isolates were 1–2 μg/mL. For VISA, MICs were as high as 12 μg/mL, although for 60%, MICs were 4 μg/mL. For 94% of hVISA strains, vancomycin MICs were 1.5–2.0 μg/mL.

The geometric mean MIC of vancomycin was 1.7 μg/mL; modal MIC = 2 μg/mL. In 2002, the MIC_{50} of vancomycin was 1.5 μg/mL; by 2007, it was 2 μg/mL. The MIC_{90} was constant during the study period; vancomycin MIC_{90} was 4 μg/mL in 2002 and 2004–2007.

Patient Characteristics

Key patient characteristics are shown in Table 3. Recent medical care seemed to be associated with type of strain. Nearly 40% of infections were health care–community

Table 1. Multivariable analysis of risk factors for VISA and hVISA infections, New York, New York, USA, 2002–2007*

Risk factor and MRSA strain	Odds ratio	Adjusted risk ratio (95% CI)	Adjusted risk difference (95% CI)†
Age‡			
VISA	0.93	0.82 (0.65 to 1.12)	–0.020 (–0.080 to 0.007)
hVISA	0.93	0.96 (0.73 to 1.35)	–0.003 (–0.050 to 0.013)
Race/ethnicity			
Black			
VISA	0.90	0.76 (0.35 to 1.57)	–0.02 (–0.07 to 0.04)
hVISA	0.90	1.03 (0.44 to 2.13)	0.002 (–0.050 to 0.070)
Hispanic			
VISA	0.89	0.87 (0.36 to 1.88)	–0.01 (–0.06 to 0.05)
hVISA	0.46	0.62 (0.19 to 1.37)	–0.03 (–0.08 to 0.03)
Asian			
VISA	1.97	1.79 (0.77 to 3.34)	0.06 (–0.02 to 0.15)
hVISA	1.58	1.65 (0.66 to 3.21)	0.05 (–0.03 to 0.14)
Concurrent condition			
Diabetes			
VISA	1.42	1.44 (0.78 to 2.59)	0.03 (–0.02 to 0.08)
hVISA	0.69	0.87 (0.48 to 1.48)	–0.01 (–0.05 to 0.03)
Chronic hemodialysis			
VISA	1.25	1.23 (0.56 to 2.57)	0.02 (–0.04 to 0.09)
hVISA	1.32	1.05 (0.48 to 2.03)	0.004 (–0.040 to 0.060)
HIV			
VISA	0.50	0.45 (0.09 to 1.06)	–0.05 (–0.090 to 0.004)
hVISA	0.30	0.27 (0.06 to 1.38)	–0.060 (–0.100 to –0.008)
Liver cirrhosis			
VISA	2.38	3.43 (2.02 to 6.00)	0.14 (0.06 to 0.23)
hVISA	2.55	2.11 (1.06 to 3.87)	0.080 (0.005 to 0.170)
Malignancy			
VISA	2.02	1.96 (1.07 to 3.31)	0.070 (0.005 to 0.130)
hVISA	1.37	1.64 (0.87 to 3.07)	0.04 (–0.01 to 0.11)
Other			
Nursing home residence			
VISA	1.62	1.83 (0.88 to 3.30)	0.060 (–0.009 to 0.130)
hVISA	1.12	0.94 (0.36 to 1.80)	–0.005 (–0.060 to 0.050)
Surgical procedure§			
VISA	0.50	0.41 (0.20 to 0.76)	–0.06 (–0.10 to –0.02)
hVISA	0.79	0.62 (0.32 to 1.02)	–0.040 (–0.080 to 0.002)
Prior receipt of vancomycin			
VISA	1.87	2.09 (1.25 to 3.67)	0.06 (0.02 to 0.10)
hVISA	0.92	0.97 (0.56 to1.65)	–0.002 (–0.040 to 0.040)
Central venous catheter infection			
VISA	0.80	0.83 (0.50 to1.36)	–0.01 (–0.05 to 0.02)
hVISA	2.09	1.81 (1.13 to 3.10)	0.050 (0.009 to 0.090)

*Results from logistic regression with outcomes and covariates. VISA, vancomycin-intermediate *Staphylococcus aureus* strains, hVISA, heteroresistant vancomycin-intermediate *S. aureus* strains; MRSA, methicillin-resistant *S. aureus*.
†Adjusted risk difference refers to the absolute difference in risk; for example, an adjusted risk difference of 0.10 signifies a 10% increased risk for hVISA, VISA, given that variable.
‡Measures are adjusted odds ratio for a 10-y difference and adjusted risk difference for effect of age 50–60 y.
§Surgical procedures under general anesthesia within the past 3 months.

associated, almost all the rest were hospital associated. Average length of stay for patients with all infection types was 32.9 days. Many cases of MRSA bacteremia were in patients with renal insufficiency and/or cardiovascular disease; >40% had recently had a surgical procedure. Many (43%) cases of bacteremia were secondary to central venous catheter infections. The 90-day all-cause mortality rate was 31.5% for the 603 patients; rate was 27.2% when all 699 episodes of bacteremia were considered.

Treatment and Changes in Treatment Regimens

Among the 603 patients, 47% had been exposed to vancomycin and 60% of these had had prior MRSA infection. Prior vancomycin exposure was more likely for patients with VISA (62%, 95% CI 47.7%–65.3%) than for patients with hVISA (42%, 95% CI 29.4%–59%) or other MRSA strains (47%, 95% CI 41.3%–49.7%).

Among the 699 episodes of MRSA bacteremia, vancomycin was used to treat 566 (81%) episodes. Initial vancomycin treatment was switched to daptomycin or linezolid for 12% of MRSA (non-VISA, non-hVISA) infections and 15% of VISA infections. For the 699 episodes of bacteremia, mortality rates were 27.2% overall, 16% (95% CI 6.8%–24.8%) when antimicrobial drug treatment was changed, and 26% (95% CI 22.2%–29.8%) when not changed. Our data did not enable us to determine the extent

Table 2. Multivariable analysis of risk factors for 90-day all-cause deaths among 603 patients with MRSA bacteremia, New York, New York, USA, 2002–2007*

Risk factor	Odds ratio (95% CI)	Adjusted risk ratio (95% CI)	Adjusted risk difference† (95% CI)
Age‡	1.72 (1.29 to 2.30)	1.34 (1.12 to 1.65)	0.04 (0.03 to 0.05)
Race/ethnicity			
Black	0.71 (0.39 to 1.29)	0.85 (0.63 to 1.16)	–0.04 (–0.04 to –0.11)
Hispanic	0.85 (0.45 to 1.58)	0.93 (0.66 to 1.23)	–0.02 (–0.10 to 0.06)
Asian	1.83 (0.92 to 3.66)	1.30 (0.95 to 1.72)	0.08 (–0.01 to 0.18)
Concurrent condition			
Diabetes	0.50 (0.31 to 0.83)	0.73 (0.57 to 0.93)	–0.08 (–0.14 to –0.02)
Immunosuppressant use	0.83 (0.37 to 1.83)	0.92 (0.63 to 1.30)	–0.02 (–0.10 to 0.08)
Liver cirrhosis	2.18 (1.16 to 4.12)	1.40 (1.04 to 1.77)	0.10 (0.01 to 0.19)
Renal insufficiency	1.89 (1.18 to 3.01)	1.33 (1.05 to 1.70)	0.08 (0.01 to 0.14)
Other			
Infectious disease consultation	0.43 (0.26 to 0.69)	0.69 (0.57 to 0.86)	–0.11 (–0.16 to –0.04)
History of MRSA infection	0.77 (0.45 to 1.34)	0.89 (0.70 to 1.13)	–0.03 (–0.09 to 0.04)
Nursing home residence	3.08 (1.81 to 5.24)	1.62 (1.31 to 2.06)	0.15 (0.08 to 0.23)
Intensive care unit stay	1.71 (1.17 to 2.50)	1.29 (1.11 to 2.15)	0.07 (0.03 to 0.20)
Vasopressor use	15.44 (8.58 to 27.76)	3.67 (2.66 to 4.66)	0.48 (0.34 to 0.58)
Inappropriate antimicrobial drug therapy	1.38 (0.73 to 2.63)	1.15 (0.89 to 1.46)	0.04 (–0.03 to 0.12)
MRSA strain			
VISA	0.58 (0.24 to 1.38)	0.78 (0.49 to 1.19)	–0.06 (–0.15 to 0.05)
hVISA	1.23 (0.54 to 2.82)	1.10 (0.67 to 1.58)	0.03 (–0.09 to 0.16)
Infection source			
Pneumonia	1.77 (0.85 to 3.64)	1.28 (0.91 to 1.68)	0.07 (–0.02 to 0.17)
Vascular graft infection	0.21 (0.03 to 1.70)	0.460 (0.005 to 0.940)	–0.15 (–0.29 to –0.02)
Endocarditis	1.49 (0.80 to 2.79)	1.19 (0.87 to 1.51)	0.05 (–0.03 to 0.13)

*Results from logistic regression with outcomes and covariates. MRSA, methicillin-resistant *Staphylococcus aureus*. VISA, vancomycin-intermediate *S. aureus* strains, hVISA, heteroresistant vancomycin-intermediate *S. aureus* strains.
†Adjusted risk difference refers to the absolute difference in risk; for example, an adjusted risk difference of 0.10 signifies a 10% increased risk for all-cause deaths given that variable.
‡Measures are adjusted odds ratio for a 10-y difference and adjusted risk difference for effect of age 50–60 y.

to which switching, or not switching, antimicrobial drugs contributed to survival.

Correlates of VISA and hVISA Infections

Multivariable analyses (Table 1) demonstrate associations between key clinical characteristics and VISA or hVISA infections. The adjusted risk difference represents the absolute difference in risk for that given characteristic, all else held equal. Cirrhosis of the liver and central venous catheter infections nearly doubled the risk for hVISA infection. Cirrhosis and active malignancy increased the absolute risk for VISA by 14% and 7%, respectively. History of vancomycin exposure within 1 year increased the risk for VISA (6%) but did not increase the risk for hVISA infection.

Predictors of All-Cause Death

The effect of various clinical characteristics on risk for death within 90 days is summarized in Table 2. For patients with concomitant MRSA bacteremia, older age increased the risk of dying. Cirrhosis or renal insufficiency and having lived in a nursing home before hospitalization or having been admitted to an intensive care unit were each independently associated with death (after adjusting for covariates in the model); each increased the risk of dying by 7%–15%. Patients who required vasopressors had an absolute increase in risk for death of ≈50%, after covariates were adjusted for. Risk for death was independently associated with lower risk for death among those who had diabetes mellitus or who had had a vascular graft as the source of the infection. A consultation with an infectious diseases specialist decreased the risk for death by 11%. Neither a VISA nor hVISA strain was independently associated with all-cause death after covariates in the models were controlled for.

A subanalysis of vancomycin MICs for strains infecting patients who died in the hospital found that the mean MIC was 1.7 μg/mL. The current breakpoint of vancomycin susceptibility is 2 μg/mL.

Discussion

The idealized model for the treatment of patients with infectious diseases incorporates the triad of host, organism, and drug. Organisms and drugs are more easily classified and hence more accessible for systematic study. Our study of the 5-year experience with MRSA infections in adults at a major New York City medical center illustrates why such a dyadic approach might be insufficient. For example, the MIC, which characterizes the major intersection between organism and drug, was overshadowed by a constellation of clinical factors when predicting risk for death. Vancomycin MICs from isolates from most persons who died indicated nominal susceptibility. Several other studies have shown vancomycin MIC to not be a predictor of death (*10*,*12*,*13*).

Unlike others, who considered concurrent conditions by using scales such as the Charlson Index (*15*,*24*), we investigated the association between specific patient characteristics, organisms, drugs, and outcomes. Not all concurrent conditions were alike in either magnitude or direction of effect. Regression risk analysis enabled us to identify the independent contribution of these factors in relative and absolute terms. We identified critical prognostic factors, including concurrent conditions (cirrhosis and renal insufficiency suggested a poorer prognosis; diabetes, a better one) and source of admission (nursing home residence suggested a poorer prognosis). We learned that strain type was not an independent negative prognostic factor. As one might expect, the use of vasopressors presaged an increased risk for death (adjusted risk difference = 48%).

Our findings can help clinicians estimate the risk that a patient with MRSA bacteremia will die. For example, an elderly patient with liver cirrhosis and MRSA bacteremia who lived in a nursing home before hospital admission would have an extremely poor prognosis. Conversely, an otherwise healthy patient with diabetes mellitus might have a better prognosis that could be improved even more by consultation with an infectious disease specialist. We note paradoxically that several of the positive predictive factors (such as diabetes and vascular graft infections) represent situations in which host barriers to infections might be impaired. We speculate that host, organism, and drug factors might all interact; an impaired host might become infected by a less aggressive organism that in turn is more susceptible to drugs. In this study, ≈30% of patients with skin and soft tissue infections had diabetes mellitus with varying levels of baseline glucose control. Thus, the source of infection and spectrum of disease might also affect risk for death. This and alternate hypotheses should be explored in future research.

There is controversy regarding the value of testing for hVISA (*25*). Although this article is unlikely to resolve that controversy, we can say that patients infected with these strains in our cohort probably did not have increased risk for death from all causes. Other reports suggest that a down-regulation of virulence might be associated with increased vancomycin resistance (*12*,*26*).

We observed trends in which vancomycin MICs crept upward over the 5 years of the study. As a corollary, the likelihood of VISA infections increased. The rate of hVISA infections during 2002–2007 was steady, around 8%, similar to that described in the literature (*27*,*28*). Prior exposure to vancomycin was a contributing factor for infection with VISA strains (*29*–*31*). Although we describe a vancomycin MIC creep, the MIC_{90} of vancomycin remained relatively stable over the 5-year period, perhaps hinting at why MIC did not independently predict death.

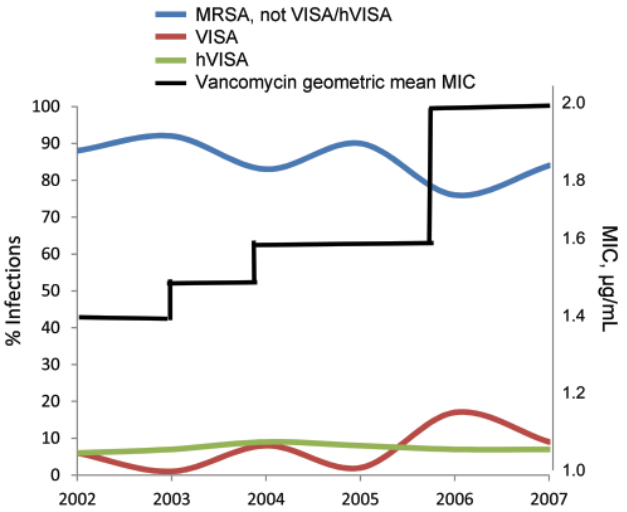

Figure. Trend of methicillin-resistant *Staphylococcus aureus* (MRSA) infection strain types, New York, New York, USA, 2002–2007. VISA, vancomycin-intermediate *S. aureus* strains, hVISA, heteroresistant vancomycin-intermediate *S. aureus* strains.

A recent study by Paul et al. found a significant increase in 30-day mortality rates for patients given incorrect therapy within 48 hours of blood culture (*32*). Schweizer et al. did not find an increase in hospital deaths among similar patients with *S. aureus* bacteremia (*33*). Our study was limited by our inability to assess deaths that occurred out of the hospital.

Consistent with mortality rates reported in the literature, ≈32% of our patients died (*34*). Considering the marginal impact of several independent risk factors, our innovative presentation of adjusted risk differences offers clinicians a quantifiable way to assess their patients' risk for death. Although the numbers were too low to analyze with multivariable models, we note the trend toward improved prognosis among those for whom antimicrobial drug therapy was switched and recognize that because switches are likely for patients who are not doing well clinically, that the most apparent bias is against such a finding. Thus, our findings hint at potential benefit for prescribing alternative drugs if patients are not improving. Two recent studies have shown that consultations with an infectious diseases specialist lower the risk for death from *S. aureus* bacteremia (*35*,*36*).

This study is limited by its retrospective design and single-center setting. MICs derived by using Etest might be higher than those derived by microdilution (*37*). hVISA strains were identified by using a method with demonstrably high sensitivity and specificity and not by using the standard method (*10*,*38*,*39*). We did not test for hVISA on the 5% of isolates for which vancomycin MIC

Table 3. Clinical characteristics for patients with MRSA bacteremia by strain type, New York, New York, USA, 2002–2007*

Characteristic	VISA, n = 55	hVISA, n = 55	non–VISA/hVISA MRSA, n = 589	p value
Age, mean y ± SD†	58.7 ± 16.5	58.7 ± 16.5	63 ± 17.3	0.04
Length of stay, mean d ± SD	33.7 ± 41.4	30.9 ± 22.7	34 ± 41.9	0.49
Days to negative culture, mean ± SD	3.7 ± 2.8	3.6 ± 3.2	4.3 ± 4.8	0.98
Male sex	29 (52.7)	28 (50.9)	340 (57.7)	0.51
Race/ethnicity				
White	19 (34.5)	21 (38.2)	238 (40.4)	0.59
Black	15 (27.3)	14 (25.5)	160 (27.2)	0.96
Hispanic	12 (21.8)	7 (12.7)	123 (20.9)	0.34
Asian	9 (16.4)	10 (18.2)	65 (11.0)	0.17
General				
Hospitalization within 1 mo of MRSA infection	29 (52.7)	17 (30.9)	295 (50.1)	0.02
Prior MRSA infection	22 (40.0)	7 (12.7)	159 (27.0)	0.006
Prior vancomycin exposure	34 (61.8)	25 (45.5)	272 (46.2)	0.08
Vancomycin trough >15 μg/mL	34 (61.8)	25 (45.5)	387 (65.7)	0.0005
Inappropriate antimicrobial drug therapy	12 (21.8)	4 (7.3)	66 (11.2)	0.09
Infectious diseases consultation	34 (61.8)	23 (41.8)	404 (68.6)	0.0003
Health care–associated hospital infection	19 (34.5)	28 (50.9)	340 (57.7)	0.003
Health care–associated community infection	31 (56.4)	25 (45.5)	241 (40.9)	0.08
Concurrent conditions				
Renal insufficiency	35 (63.6)	29 (52.7)	309 (52.5)	0.48
Chronic hemodialysis	19 (34.5)	12 (21.8)	128 (21.7)	0.09
Diabetes mellitus	27 (49.1)	18 (32.7)	209 (35.5)	0.11
HIV	5 (9.1)	2 (3.6)	60 (10.2)	0.29
Cardiovascular disease	33 (60.0)	31 (56.4)	399 (67.7)	0.14
Malignancy	14 (25.5)	17 (30.9)	117 (19.9)	0.11
Transplant	7 (12.7)	11 (20.0)	46 (7.8)	0.007
Cirrhosis	22 (40.0)	14 (25.5)	78 (13.2)	<0.0001
Steroids	17 (30.9)	12 (21.8)	193 (32.8)	0.25
Surgery <3 mo before MRSA infection	14 (25.5)	19 (34.5)	271 (46.0)	0.005
Implanted device	8 (14.5)	7 (12.7)	151 (25.6)	0.02
Intensive care unit stay	23 (41.8)	26 (47.3)	273 (46.3)	0.96
Infection source				
Central venous catheter	27 (49.1)	33 (60.0)	242 (41.1)	0.04
Pneumonia	10 (18.2)	6 (10.9)	39 (6.6)	0.62
Endocarditis	7 (12.7)	3 (5.5)	81 (13.8)	0.22
Wound/skin or soft tissue	10 (18.2)	10 (18.2)	92 (15.6)	0.80
Bone/joint	6 (10.9)	1 (1.8)	65 (11.0)	0.10
Vascular graft	2 (3.6)	1 (1.8)	17 (2.9)	0.85
Death within 90 d of MRSA infection	15 (27.3)	14 (25.5)	161 (27.3)	0.38

*Values are no. (%) with a given variable per strain type unless otherwise indicated. MRSA, methicillin-resistant *Staphylococcus aureus;* VISA, vancomycin-intermediate *S. aureus* strains, hVISA, heteroresistant vancomycin-intermediate *S. aureus* strains.
†N = 699.

was <1 μg/mL; other studies have noted few or no hVISA in this MIC group (*10*,*24*,*25*,*27*). The accuracy of timing of vancomycin trough levels was limited. Daily bacterial cultures were not always conducted, decreasing the data points for clearance of bacteremia. Misclassification error is possible because we reviewed records available at Mount Sinai Medical Center only. Almost all study patients had sufficient contact with the health care system that not many infections were classified as community acquired, limiting our ability to generalize to those infections. As noted, we were limited by our inability to link to death data outside of the hospital records.

Until now, the major focus on active MRSA infections has been on the organism and its susceptibility to the drug. Although decreased vancomycin susceptibility has resulted in prolonged bacteremia and treatment failure in several studies (*15*,*24*), our findings suggest that incorporating the context, the host, and the environment is similarly useful. Our study emphasizes that after a diagnosis of MRSA bacteremia is made, it is crucial to determine patient risk factors and not just the vancomycin MIC for the infecting strain. The consequences of MRSA bacteremia are clear—many patients will die or experience a decline from their baseline clinical condition. The adjusted risk difference enables clinicians to use a targeted approach, directed toward patients with the highest risk for death—i.e., the elderly, patients with liver cirrhosis, patients with renal insufficiency, and patients from nursing homes. These patients should be treated carefully and should possibly receive a consult from an infectious diseases specialist. What remains unclear is whether patients with increased risk for death should be treated with antimicrobial drugs other than vancomycin.

Acknowledgments

We thank Jeanette Francois for technical assistance with retesting strains.

This work was funded in part by a grant from Cubist Pharmaceuticals for reagent material and travel for presentations. S.G.J. executed the agreement, and M.P. was reimbursed for travel to the 19th European Congress of Clinical Microbiology and Infectious Diseases Meeting, Helsinki, Finland, and the 48th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy/Infectious Diseases Society of America Meeting, Washington, DC, to present part of the results.

The following isolates were obtained through the Network of Antimicrobial Resistance in *Staphylococcus aureus* program, supported under the National Institutes of Health/National Institute of Allergy and Infectious Diseases contract no. HHSN272200700055C: NRS 18, 19, 21, 22, 23, 24, 26, and 27.

Dr Pastagia is an instructor of clinical investigation in the Laboratory of Bacterial Pathology and Immunogenesis at The Rockefeller University. Her research focus is MRSA and novel treatment therapies.

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Address for correspondence: Mina Pastagia, The Rockefeller University, 1230 York Ave, Bronk Building, 8th Floor, New York, NY 10065, USA; email: minapastagia@yahoo.com

Adenoviruses in Fecal Samples from Asymptomatic Rhesus Macaques, United States

Soumitra Roy,[1] Arbansjit Sandhu, Angelica Medina, David S. Clawson, and James M. Wilson

Adenoviruses can cause infectious diarrheal disease or respiratory infections in humans; 2 recent reports have indicated probable human infection with simian adenoviruses (SAdVs). To assess the possibility of animal-to-human transmission of SAdVs, we tested fecal samples from asymptomatic rhesus macaques housed in 5 primate facilities in the United States and cultured 23 SAdV isolates. Of these, 9 were purified and completely sequenced; 3 SAdV samples from the American Type Culture Collection (SAdV-6, SAdV-18, and SAdV-20) were also completely sequenced. The sequence of SAdV-18 was closely related to that of human adenovirus F across the whole genome, and the new isolates were found to harbor 2 fiber genes similar to those of human adenovirus (HAdV) strains HAdV-40 and HAdV-41, which can cause infectious diarrhea. The high prevalence of adenoviruses in fecal samples from asymptomatic rhesus macaques and the similarity of the isolates to human strains indicates the possibility of animal-to-human transmission of SAdVs.

Adenoviruses, which can cause infectious diarrhea or respiratory infections in humans, were isolated from monkeys soon after their initial characterization by Rowe et al. (*1*). Hull et al. (*2*,*3*) identified adenoviruses as one of several classes of viruses found as outgrowths from primary cultures of monkey kidney cells that were being cultivated for propagation of poliovirus. Adenoviruses were also found in rectal swab and fecal samples taken from monkeys in captivity (*4*,*5*).

The classification of monkey adenoviruses was initially done in a manner analogous to that used for human isolates, i.e., on the basis of differences in their ability to agglutinate erythrocytes from different species. Rapoza classified monkey adenoviruses into 4 hemagglutination subgroups on the basis of their differential agglutination properties with respect to their ability to agglutinate rat, rhesus, and guinea pig erythrocytes (*6*). Most viruses tested at the time were found to belong to groups 2 and 3; groups 1 and 4 contained only 1 member each. The current classification of macaque adenoviruses by the International Committee on Taxonomy of Viruses has 1 defined species, simian adenovirus A (SAdV-A), to which SAdV-3 has been assigned. SAdV-1 and SAdV-7, while not considered human viruses, are now classified as human adenovirus G (HAdV-G) because of their similarity to a virus (HAdV-52) isolated from a human patient with gastroenteritis (*7*).

Recently, transmission of a monkey adenovirus from captive titi monkeys to a researcher was reported at the California National Primate Research Center (Davis, CA, USA); the researcher may, in turn, have transmitted it to a family member (*8*). Further, HAdV-52 (*7*) isolated from a patient with gastroenteritis has been found to be very dissimilar to all previously isolated human adenoviruses but closely related to monkey adenoviruses SAdV-1 and SAdV-7. This similarity raises the possibility that this patient was, in fact, infected with a monkey adenovirus.

We have previously found that adenovirus DNA is readily detected in fecal samples from monkeys who have no symptoms of any clinical adenoviral disease (*9*). Given the close physical proximity and intermingling of human and monkey populations in several locales in Asia, Africa, and Latin America, better characterization of monkey adenoviruses is of public health importance. To clarify the nature of adenoviruses shed in the feces of asymptomatic monkeys, we attempted to culture adenoviruses from fecal

Author affiliation: University of Pennsylvania, Philadelphia, Pennsylvania, USA

DOI: http://dx.doi.org/10.3201/eid1807.111665

[1]Current affiliation: Crucell Holland, Leiden, the Netherlands.

samples from rhesus macaques (*Macaca mulatta*) housed in 5 primate facilities in the United States.

Materials and Methods

Isolation of Novel Adenoviruses from Rhesus Macaques

Approximately 350 rhesus macaque fecal samples were processed: 150 from Covance (Vienna, VA, USA), 100 from the New England Primate Research Center (Southborough, MA, USA), and 98 from the Tulane National Primate Research Center (Covington, LA, USA). Additional samples were obtained from rhesus macaques housed at the Oregon National Primate Research Center (Beaverton, OR, USA) and the primate facility of the Gene Therapy Program at the University of Pennsylvania (Philadelphia, PA, USA).

Samples were suspended in Hanks' Balanced Salt Solution (Life Technologies, Grand Island, NY, USA), particulates were removed by centrifugation, and the supernatants were sterile filtered through 0.2-μm syringe filters. We injected 100 μL of each filtered sample into BS-C-1 and LLC-MK2 cells grown in culture medium containing 1% Penn-Strep (Mediatech, Herndon, VA, USA) and 50 μg/mL gentamicin. After ≈1–2 weeks, if cytopathic effects typical of adenovirus infection were seen, the presence of adenoviruses in the cultures was confirmed by PCR amplification of an internal 1.9 kb of the hexon, including the region encompassing the hypervariable regions, which is predominantly responsible for conferring serotype specificity. The primer pair used for PCR was 5′-CAGGATGCTTCGGAGTACCTGAG-3′ and 5′-TTGGCNGGDATDGGGTAVAGCATGTT-3′. The sequence obtained from this region was used to make an initial determination of adenoviral species and novelty of the serotype. Adenovirus isolates were propagated to high titer and purified on cesium chloride gradients by using standard procedures. Viral DNA obtained from purified virus preparations was completely sequenced (QIAGEN, Hilden, Germany).

Adenoviruses and Cell Culture from ATCC

We obtained 3 adenoviruses from the American Type Culture Collection (ATCC). SAdV-6 (catalog number VR-353, originally deposited as SV-39) had been isolated from *Macaca* spp. monkeys (*6*,*10*). SAdV-18 (catalog number VR-943) (*4*,*11*) and SAdV-20 (catalog number VR-541, originally deposited as simian adenovirus V340) had been isolated from vervet monkeys (*Cercopithecus aethiops*) (*12*). BS-C-1 (*C. aethiops* monkey kidney cells, ATCC catalog number CCL-26) or LLC-MK2 (*M. mulatta* monkey kidney cells, ATCC catalog CCL-7) were purchased from ATCC and maintained in culture as recommended.

Sequence Analysis

Propagation and purification were performed for 23 isolates as shown in Table 1; 9 of the isolates were completely sequenced. In addition, we propagated, purified, and sequenced the 3 monkey adenoviruses (SAdV-6, SAdV-18, and SAdV-20) from ATCC. All analyses were conducted at the www.phylogeny.fr sequence analysis server. Nucleotide sequence alignments for each open reading frame (ORF) (after excising introns if necessary) were completed by using ClustalW version 2.0.3, and the alignments were refined by using Gblocks version 0.91b. The alignments were used to construct phylogenetic trees using PhyML version 3.0 aLRT, under the HKY85 model. The resulting trees were rendered by using Treedyn 198.3. Protein sequence alignments and phylogenetic trees were constructed by using the Vector NTI (Life Technologies) and CLC Bio (CLC Bio, Aarhus, Denmark) software packages.

Results

All the macaque adenoviruses that we sequenced were similar to previously sequenced primate adenoviruses with respect to the identity and order of identifiable ORFs organized into defined early and late transcription regions (*13*). The notable differences observed were in structures of the E3 region genes and of the fiber genes.

Phylogenetic analyses of the nucleotide sequences that encode genes of several of the adenoviral proteins showed the sequences were generally concordant with one another. As examples, the phylogenetic trees for the sequences encoding E1a, DNA polymerase, hexon, and E4 34K are shown in Figure 1. The sequences have been compared with each other and with previously sequenced macaque adenoviruses SAdV-1, SAdV-3, SAdV-7, SAdV-48, SAdV-49, SAdV-50, titi monkey adenovirus, and cynomolgus adenovirus 1 (*8*,*9*,*14*–*17*). The human isolate HAdV-52 (*7*), which is known to be closely related to SAdV-1 and SAdV-7, was also included in the analyses. Macaque adenoviruses form a distinct clade when compared with human or ape adenoviruses (*9*). However, HADV-F (HAdV-40 and HAdV-41) and HAdV-A (HAdV-12) are also included in the analyses because these species are the most closely related to macaque adenoviruses (*14*,*15*).

The phylogenetic trees (Figure 1) show that SAdV-6, obtained from ATCC and sequenced, is similar to SAdV-48, which we had previously isolated from stool samples of an apparently healthy macaque (*9*), and to SAdV-3 (*14*). These adenoviruses have been grouped together with SAdV-3 as SAdV-A. The 9 adenoviruses that we isolated from rhesus stools are closely related to one another and to 2 other macaque adenoviruses that we had previously isolated and sequenced, SAdV-49 and SAdV-50 (*9*); these isolates have been grouped as SAdV-B. The adenovirus isolated

Table 1. Characterization of adenovirus isolates from rhesus fecal samples obtained from primate facilities in the United States*

Isolate	Source†	Particle titer	Total yield	Sequence analysis
A1123	ONPRC	4.0×10^{11}	9.6×10^{11}	Hexon similar to SAdV-3
A1128	Covance	6.0×10^{11}	1.3×10^{12}	Hexon similar to SAdV-3
A1129	Covance	6.1×10^{11}	3.8×10^{13}	Hexon (A1128)
A1139	Covance	4.7×10^{12}	1.8×10^{12}	Full genome
A1161	Covance	2.0×10^{12}	4.3×10^{13}	Hexon similar to SAdV-48
A1163	Covance	5.4×10^{12}	5.4×10^{12}	Full genome
A1166	Covance	2.8×10^{12}	1.3×10^{11}	Hexon (A1161)
A1169	Covance	1.1×10^{11}	2.5×10^{13}	Hexon (A1161)
A1173	Covance	5.2×10^{12}	2.3×10^{12}	Full genome
A1179	Covance	4.6×10^{12}	1.0×10^{13}	Hexon (A1128)
A1258	GTP	1.8×10^{12}	2.6×10^{12}	Full genome
A1261	GTP	8.7×10^{11}	9.0×10^{12}	Hexon (A1285)
A1285	GTP	2.7×10^{12}	2.0×10^{13}	Full genome
A1296	Covance	5.4×10^{12}	7.3×10^{11}	Full genome
A1297	Covance	3.7×10^{11}	7.0×10^{11}	Hexon (A1163)
A1312	Covance	6.1×10^{11}	8.4×10^{11}	Full genome
A1313	TNPRC	6.5×10^{11}	1.4×10^{13}	Hexon similar to A1312
A1327	TNPRC	2.4×10^{12}	1.0×10^{12}	Full genome
A1328	NEPRC	6.7×10^{11}	2.7×10^{12}	Hexon similar to SAdV-49
A1329	TNPRC	3.7×10^{12}	9.2×10^{12}	Hexon (A1312)
A1335	NEPRC	6.0×10^{12}	2.8×10^{13}	Full genome
A1339	TNPRC	3.1×10^{12}	5.7×10^{12}	Hexon (A1163)
A1340	Covance	5.1×10^{12}	3.1×10^{13}	Hexon (A1335)

*The total yield and the particle titer obtained from a 50-plate (150-mm) preparation are shown. In each case, the hexon region was PCR amplified and sequenced. **Boldface** indicates the 9 isolates that were completely sequenced. For the other isolates, the hexon sequences that are identical or very closely related (<5% nucleotide sequence dissimilarity) are indicated in parentheses. SAdV, simian adenovirus.
† ONPRC, Oregon National Primate Research Center (Beaverton, OR, USA); Covance, Covance, (Vienna, VA, USA); GTP, primate facility of the Gene Therapy Program at the University of Pennsylvania (Philadelphia, PA, USA); TNPRC, Tulane National Primate Research Center (Covington, LA, USA); NEPRC, New England Primate Research Center (Southborough, MA, USA).

from a cynomolgus macaque for which a partial sequence (including the hexon) was reported (*16*) also belongs to this group. The adenovirus of unknown origin that recently caused an outbreak of pneumonia and hepatitis in a colony of new world titi monkeys and sickened an animal handler (*8*) is distant from these adenoviruses. SAdV-18, which had been deposited with ATCC as an isolate from a vervet monkey in the 1960s (*11*), was found to be most closely related to HAdV-F members HAdV-40 and HAdV-41 in the protein phylogenies and also for most of the length of its genome (data not shown). HAdV-F is the only human adenovirus species that harbors 2 fiber genes, a feature common in macaque adenoviruses, including HAdV-G members; however, SAdV-18 differs from HAdV-F members in possessing a single fiber gene.

All sequences have been deposited in GenBank. The accession numbers are as follows: simian adenovirus strain A1139, JN880448; strain A1163, JN880449; strain A1173, JN880450; strain A1258, JN880451; strain A1285, JN880452; strain A1296, JN880453; strain A1312, JN880454; strain A1327, JN880455; strain A1335, JN880456; SAdV-6, JQ776547; SAdV-18, FJ025931; and SAdV-20, HQ605912. The accession numbers for other adenovirus sequences used in the analyses are as follows: SAdV-1, NC_006879; SAdV-3, NC_006144; SAdV-7, DQ792570; SAdV-48, HQ241818; SAdV-49, HQ241819; SAdV-50, HQ241820; SAdV-titi, HQ913600; HAdV-2, NC_001405; HAdV-3, NC_011203; HAdV-4, NC_003266; HAdV-12, NC_001460; HAdV-17, HQ910407; HAdV-18, GU191019; HAdV-40, NC_001454; HAdV-41, DQ315364; and HAdV-52, DQ923122.

Analysis of the E3 Regions

The gene content and disposition (as discerned by the presence of ORFs) of the E3 regions of the 9 adenoviruses isolated from macaques are shown in Table 2. The 12.5K protein of unknown function, as well as the anti-apoptotic RID-α and RID-β, and the 14.7K proteins (*18*), are present in all the newly isolated viruses. They all also harbor homologs of the CR1 proteins that contain conserved domains of unknown function designated CR1 and CR2 (*19*). However we found that in 6 of the newly sequenced adenoviruses, the 2 CR1 proteins (CR1-α and CR1-β) were fused into a single ORF (Figure 2). An example of the fusion is illustrated in Figure 3, in which the fused CR1 protein (designated CR1-αβ) of the adenovirus isolate A1139 E3 region has been aligned with the CR1-α and CR1-β proteins of the E3 region of A1312. The E3 CR1 proteins possess a single putative transmembrane domain near their C-termini and are likely to have arisen by gene duplication (*20*,*21*). The putative transmembrane domain of the CR1-α protein appears to have fused to the hydrophobic (putative) secretion signal of the CR1-β protein. This fused version indicates that the CR1-α and CR1-β proteins are likely disposed on opposite sides of the membrane. One possible model for A1163 CR1-αβ fused protein (based

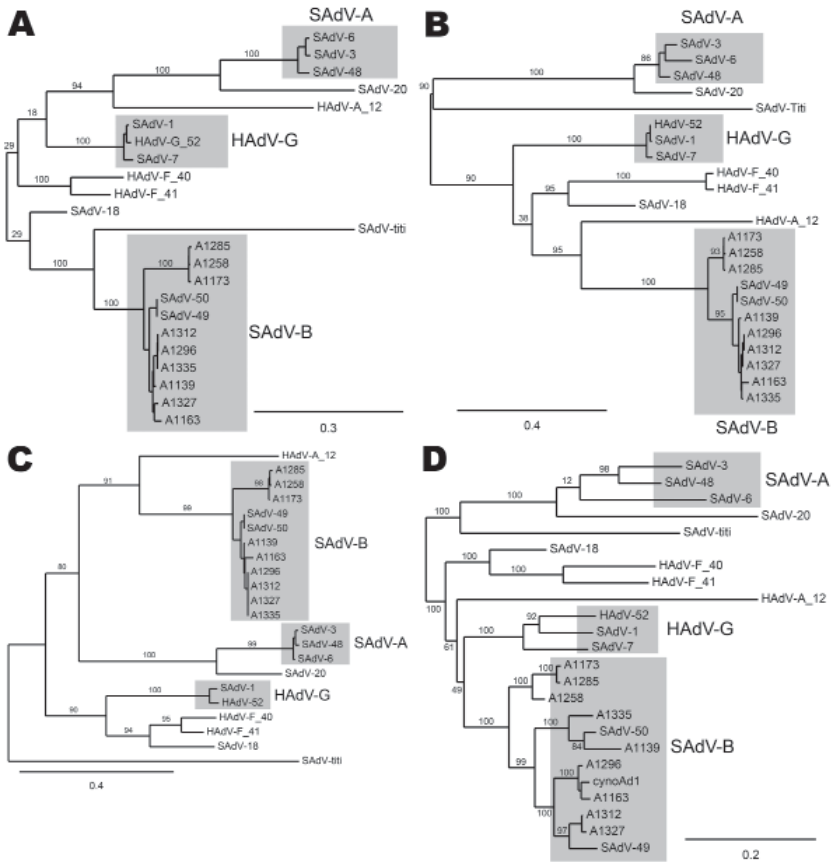

Figure 1. Phylogenetic trees of the genes coding for A) DNA polymerase, B) E4 34K, C) E1a, and D) hexons of macaque adenoviruses identified in study of prevalence of adenoviruses in fecal samples from rhesus macaques, United States. Members of the human adenovirus (HAdV) species HAdV-A (HAdV-12), HAdV-G, and HAdV-F that are thought to have the closest phylogenetic proximity to macaque adenoviruses are included for comparison. Branch support values are indicated. Simian adenoviruses (SAdV) SAdV-1 and SAdV-7 have been grouped together with HAdV-52 into HAdV-G; the other macaque adenoviruses (except for SAdV-18, SAdV-20, and the titi monkey adenovirus) have been grouped into SAdV-A and SAdV-B. SAdV-18 is seen to be closely related to HAdV-F (HAdV-40 and HAdV-41) in all the trees. Scale bars indicate number of substitutions per site.

on a prediction by TMPRED, a software program that makes a prediction of membrane-spanning regions and their orientations; www.ch.EMBnet.org) indicates that the N-terminal hydrophobic domain (residues 3–21) is oriented outside to inside (luminal to cytoplasmic), followed by the CR1-α segment on the cytoplasmic side of the membrane. This model predicts the central transmembrane domain (residues 151–170) to be oriented inside to outside with the CR1-β segment on the luminal side. The C-terminal transmembrane domain (residues 430–455) would thus be oriented outside to inside, followed by a highly basic stop transfer segment. Separately encoded CR1-α and -β proteins likely follow this topology as well.

Of the newly sequenced adenoviruses, SAdV-18 exhibited the shortest E3 region. The SAdV-18 genome encodes only one E3 protein, the homologue of the E3 12.5K protein, for which a function has not yet been determined. In this respect, SAdV-18 is similar to SAdV-7 which also has a severely truncated E3 region that encodes only the E3 12.5 K protein (*17*).

Analysis of the Fiber Genes

The fiber knob domain mediates the initial virus-cell interaction by binding to a cellular receptor. A phylogenetic tree generated on the basis of an alignment of the fiber knob domains of macaque and human adenoviruses is shown in Figure 4. It is evident that the knob domains of the long fiber (fiber 2) of the human adenoviruses belonging to HAdV-F are more similar to those of SAdV-18 than to any other human adenovirus. The SAdV-18 fiber sequence is very similar to the HAdV-F long fiber throughout its length, and the sequence similarity between the knob domains of HAdV-40 and those of SAdV-18 is >90% (Figure 5. HAdV-F species (HAdV-40, HAdV-41, and serologically related isolates) are known to be enteric adenoviruses that frequently cause diarrhea in infants. The HAdV-F long fiber knob can bind the cellular receptor CAR (*22*), and the SAdV-18 fiber knob would probably be able to do so as well. Notably, the shaft domain of SAdV-18 is 391 residues long (the longest such domain of any primate adenovirus sequenced) and harbors as many as 25 iterations of the β-spiral repeat motif (Figure 5). However, unlike HAdV-F, SAdV-18 contains a single fiber gene.

Discussion

All of the SAdV-B adenoviruses we isolated from macaque fecal samples harbored 2 fiber genes. As discussed above, the only adenoviruses readily isolated from humans

Table 2. E3 region proteins of the 11 macaque adenoviruses belonging to SAdV-B isolates from macaque fecal samples compared with the E3 regions of other macaque adenoviruses*

Adenovirus type	12.5K	CR1-α	CR1-β	RID-α, RID-β, and 14.7K
SAdV-A				
SAdV-3	Present	Present	Present	Present
SAdV-6	Present	Present	Present	Present
SAdV-48	Present	Present	Present	Present
SAdV-B				
A1173	Present	Present	Present	Present
A1285	Present	Present	Present	Present
A1312	Present	Present	Present	Present
A1139	Present	Fused†		Present
A1163	Present	Fused†		Present
A1258	Present	Fused†		Present
A1296	Present	Fused†		Present
A1327	Present	Fused†		Present
A1335	Present	Fused†		Present
SAdV-49	Present	Fused†		Present
SAdV-50	Present	Fused†		Present
HAdV-G				
SAdV-1	Present	Present	Present	Present
HAdV-52	Present	Present	Present	Present
SAdV-7	Present	Not present	Not present	Not present
Not yet classified				
SAdV-18	Present	Not present	Not present	Not present
SAdV-20	Present	Present	Present	Present

*SAdV, simian adenovirus; HAdV, human adenovirus.
†Some members of SAdV-B were found to have a novel configuration in which 2 CR1 proteins were fused into a single open reading frame (see Figures 2, 3).

that harbor 2 fiber genes belong to HAdV-F (*23*–*25*). The 2 fibers of HAdV-F isolates differ in the lengths of the shaft domain: the first fiber ORF harbors a short shaft (187 residues comprising 12 iterations of the ≈16 amino-acid repeat motif, pfam 00608) and the second fiber gene possesses a longer shaft (330–346 residues, comprised of 21 or 22 iterations of the 16 amino-acid repeat motif) (*23*,*25*,*26*). Both fiber proteins are incorporated into mature virions, with each penton base having either a short or a long fiber protein embedded in them (*23*). Similar to members of HAdV-F, HAdV-G members (macaque adenoviruses SAdV-1 and SAdV-7 and the closely related HAdV-52) also harbor 2 fiber genes. As with HAdV-F, the first fiber gene (fiber 1) encodes the shorter shaft with 9 (SAdV-7) or 10 (SAdV-1 and HAdV-52) motif repeats, and the second fiber gene (fiber 2) encodes the longer fiber shaft, similar in length to that of HAdV-41, which harbors 22 motif repeats. The long fiber of HAdV-F members can bind CAR; a cellular receptor capable of binding the short fiber knob domain has not been identified.

One difference between the newly sequenced adenoviruses we isolated from stool samples (SAdV-B) and previously sequenced adenoviruses with 2 fiber genes (HAdV-40, HAdV-41, SAdV-1, SAdV-7, and HAdV-52) is that first fiber gene (fiber 1) encodes a shaft domain that is longer than the shaft domain of the second fiber gene (fiber 2). The shaft of fiber 1 of the SAdV-B isolates is ≈330 residues long (21 motif repeats, except for SAdV-49 and SAdV-50 which have 1 fewer motif repeat). Except for the length, however, the sequence of the fiber 1 shaft of the SAdV-B isolates is much more similar to the HAdV-F fiber 1 (short) shaft (49.7% sequence identity, 65.6% consensus match; see alignment in Figure 5) than it is to the fiber 1 (short) shaft of SAdV-1 or SAdV-7 (30.8% sequence identity, 47.7% consensus match). The sequence similarity is striking enough to suggest that the HAdV-F

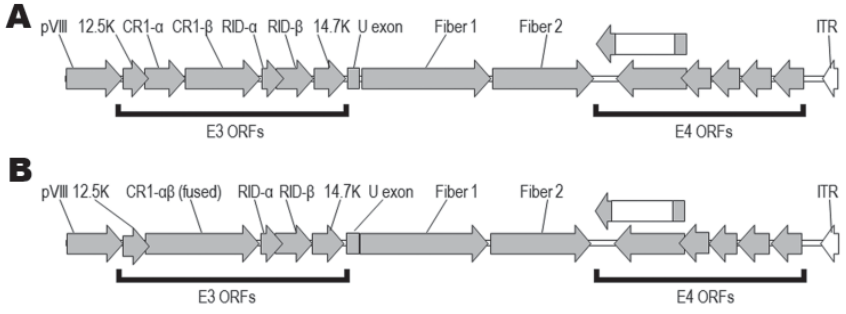

Figure 2. Open reading frames (ORFs) in the right ends of the genomes of 2 macaque adenovirus isolates identified in study of prevalence of adenoviruses in fecal samples from rhesus macaques, United States. A) Isolate A1312 right end (9,606 bp); B) isolate A1139 right end (9,591 bp). ORFs for the E3 proteins CR1-α and CR1-β are present in A1312 but have been combined into a single ORF in A1139. The 2 fiber genes, in which a long fiber protein (fiber 1) is followed by a shorter fiber protein (fiber 2), are also shown. ITR, inverted terminal repeat.

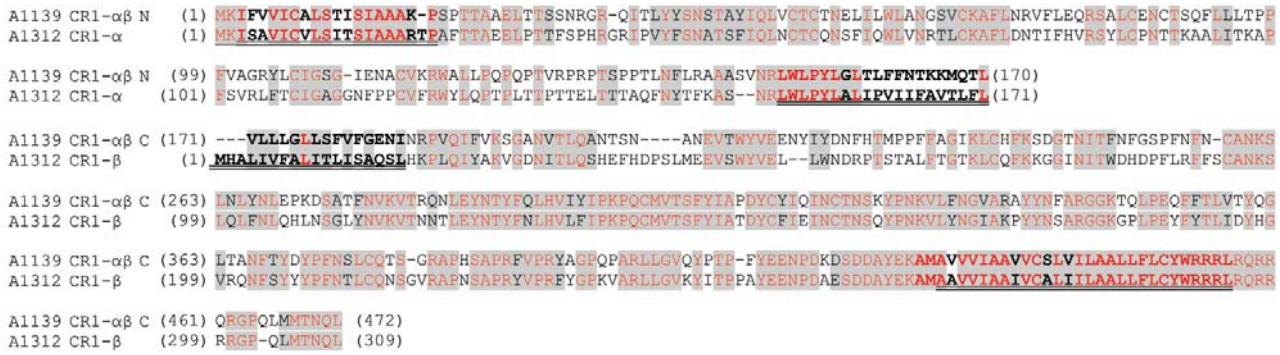

Figure 3. Sequence alignments of a subset of simian adenovirus type B (SAdV-B) isolates identified in study of prevalence of adenoviruses in fecal samples from rhesus macaques, United States. The putative fused E3 CR1-αβ protein from isolate A1139 (see Table 2) and the corresponding separately encoded CR1-α and CR1-β proteins from isolate A1312 are shown. The N-terminal (N) and C-terminal (C) sections of the fused A1139 CR1-αβ proteins have been separately aligned with the A1312 CR1-α and CR1-β proteins, respectively. Gray shading indicates homologous regions, red font indicates identical residues, and underlining indicates hydrophobic regions.

short fiber arose by deletions within the shaft domain of a SAdV-B fiber 1–like phylogenetic precursor. In contrast to HAdV-F and HAdV-G members, the second fiber gene of the SAdV-B macaque adenoviruses encodes a shaft domain that is shorter than that of the first fiber, ≈205 residues in length and harboring 14 motif repeats.

For adenoviruses that contain an integrin-binding RGD motif in the penton base proteins, an initial virus-cell interaction mediated by the fiber knob with its receptor (CAR for HAdV-A, C, E and F; CD46 for HAdV-B, and possibly sialic acid for HAdV-D), the penton base–integrin interaction has been shown to be the first step in adenovirus internalization (*27*). Because HAdV-F members do not contain an integrin that binds the RGD motif in their penton base protein, an as-yet-unidentified cellular-binding partner for the short fiber knob domain has been postulated to mediate virus internalization (*27*) in a manner analogous to the penton base–RGD interaction. However, all members of SAdV-A and SAdV-B contain the RGD motif in the penton base protein, which suggests that the short fiber (fiber 1 in HAdV-F and HAdV-G, fiber 2 in SAdV-B) may provide a function that is not necessarily analogous to that provided by the penton base–RGD motif. Moreover, because the knob domain of the SAdV-B fiber 1 gene is "elevated" above the knob on fiber 2, it may play a more critical role in the initial virus-receptor interaction than formerly suspected.

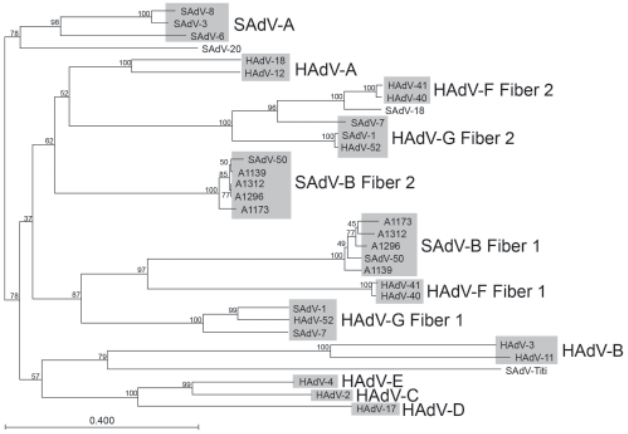

Figure 4. Neighbor-joining alignment of amino acid sequences for the fiber knob domains of macaque adenoviruses (5 representative members of simian adenovirus type B [SAdV-B]) and representative members from each human adenovirus (HAdV) species, with bootstrapping at 1,000 replicates. Alignment was performed by using CLC Bio version 6.1 software (CLC Bio, Aarhus, Denmark). Bootstrap values (percentages) are indicated on the nodes. SAdV-1 and SAdV-7 have been grouped together with HAdV-52 into HAdV-G; the other macaque adenoviruses (except for SAdV-18, SAdV-20, and the titi monkey adenovirus) have been grouped into SAdV-A and SAdV-B. Scale bar indicates number of substitutions per site.

We have previously reported the isolation and sequencing of 3 macaque adenoviruses (SAdV-48, SAdV-49 and SAdV-50) from macaque fecal samples (*9*). Even adenoviral DNA could be detected in most samples by using a sensitive, nested PCR technique, outgrowth of adenoviruses in culture in the monkey cell lines LLC-MK2 or BS-C-1 only ranged from 1% to 16% from various primate colonies. Most of these adenoviruses can be classified into a single subgroup (SAdV-B), although SAdV-A isolates (hexon sequences similar to those of SAdV-48 and SAdV-3) were also identified (Table 1). It is unclear whether these represent a common commensal of the macaque gastrointestinal tract or whether the presence of these adenoviruses in the fecal samples is an artifact of captive status, where virus spread between animals may be more common than in the wild.

The species HAdV-B, HAdV-C, and HAdV-E, which cause acute upper respiratory tract disease in humans, also

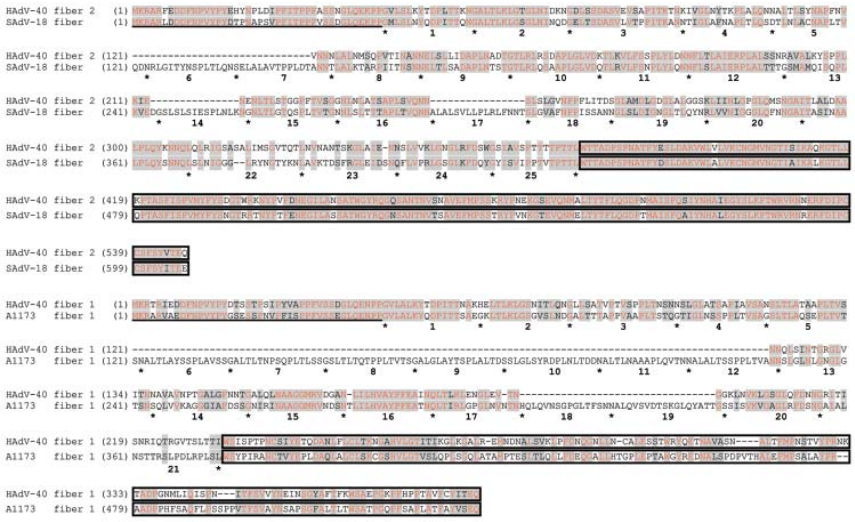

Figure 5. Sequence alignments of the amino acid sequences of human adenovirus (HAdV) 40 long fiber (fiber 2) with simian adenovirus (SAdV) 18 fiber (upper lines) and HAdV-40 short fiber (fiber 1) with macaque adenovirus isolate A1173 (lower lines). Gray shading indicates homologous regions, red font indicates identical residues, underlining indicates N-terminal 30 residues that constitute the tail, and boxes indicate C-terminal knob domains. Intervening shaft domains harboring varying numbers of the ≈16-residue β-spiral repeat sequences are separated by asterisks and numbered sequentially.

may set up chronic persistent infections in both humans and apes (*9*,*28*). In contrast, HAdV-F strains, which frequently cause infectious diarrhea in locales with poor sanitation, are only rarely detected in healthy persons (*29*). On the other hand, we have found that monkey adenoviruses belonging to SAdV-A and SAdV-B, as well as SAdV-18 (isolated from primary monkey kidney cells [*2*]), that bear a strong genetic resemblance to HAdV-F do cause chronic infections in otherwise healthy monkeys. HAdV-F may have evolved as a human intestinal pathogen after a recent cross-species transmission event and is thus less well adapted to human hosts than are HAdV-B, HAdV-C, or HAdV-E.

Macaque adenoviruses are usually not thought to infect humans, but the properties of macaque adenoviruses that constitute the species barrier are not known. Recently documented instances of human infections with macaque adenoviruses (*7*,*8*) show that these barriers can sometimes be broken. A more careful investigation of the etiology of infectious diarrhea in areas where monkeys and humans live in proximity (e.g., by PCR of fecal samples followed by sequencing of isolates) could be used to ascertain whether monkey adenoviruses do cause human infections more commonly than is currently surmised.

Acknowledgments

We thank the PennVector personnel for their help in propagating and purifying adenoviruses.

This work was sponsored by grants from the Bill and Melinda Gates Foundation.

Dr Roy was a senior research investigator at the University of Pennsylvania at the time of this study. His research interests include the characterization of nonhuman primate adenoviruses and viral adaptation to novel host environments.

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Address for correspondence: James M. Wilson, Department of Pathology and Laboratory Medicine, Division of Transfusion Medicine, University of Pennsylvania, TRL, Suite 2000, 125 S 31st St, Philadelphia, PA 19104, USA; email: wilsonjm@mail.med.upenn.edu

Spike Protein Fusion Peptide and Feline Coronavirus Virulence

Hui-Wen Chang, Herman F. Egberink, Rebecca Halpin, David J. Spiro, and Peter J.M. Rottier

Coronaviruses are well known for their potential to change their host or tissue tropism, resulting in unpredictable new diseases and changes in pathogenicity; severe acute respiratory syndrome and feline coronaviruses, respectively, are the most recognized examples. Feline coronaviruses occur as 2 pathotypes: nonvirulent feline enteric coronaviruses (FECVs), which replicate in intestinal epithelium cells, and lethal feline infectious peritonitis viruses (FIPVs), which replicate in macrophages. Evidence indicates that FIPV originates from FECV by mutation, but consistent distinguishing differences have not been established. We sequenced the full genome of 11 viruses of each pathotype and then focused on the single most distinctive site by additionally sequencing hundreds of viruses in that region. As a result, we identified 2 alternative amino acid differences in the putative fusion peptide of the spike protein that together distinguish FIPV from FECV in >95% of cases. By these and perhaps other mutations, the virus apparently acquires its macrophage tropism and spreads systemically.

Coronaviruses (subfamily *Coronavirinae*, order *Nidovirales*) are enveloped, plus-strand RNA viruses that infect mammals and birds. They are quite common and cause infections in humans and a wide variety of animals; infection typically results in respiratory or enteric disease. Severe acute respiratory syndrome coronavirus (SARS-CoV), which emerged suddenly in 2002 and caused severe acute respiratory disease in humans, is the most notorious coronavirus. SARS-CoV spread rapidly around the globe, infecting thousands and killing ≈800 persons. The virus presumably originated from bats and was transmitted to humans either directly or by using civets or raccoon dogs as intermediate hosts (*1*,*2*).

SARS-CoV best illustrates the remarkable potential for CoVs to change their tropism. Tropism switching has been implicated in the zoonotic emergence of human coronavirus OC43 from a bovine coronavirus and in turning transmissible gastroenteritis virus, an enteric porcine coronavirus, into porcine respiratory coronavirus, a respiratory pathogen (*3*,*4*). Such changes can be accompanied, although not necessarily, by cross-species transmissions; thus, the erratic occurrence and unpredictable new disease manifestations of tropism switching are a matter of public health concern.

The feline coronaviruses (FCoVs) present an example of pathogenetic change apparently associated with tropism switching. These viruses occur as 2 pathotypes with an enigmatic, even controversial, relationship: the low-virulence or nonvirulent feline enteric coronavirus (FECV) and the highly lethal feline infectious peritonitis virus (FIPV). FECV and FIPV are considered independently circulating viruses by some investigators (*5*,*6*). However, accumulating evidence supports the mutation hypothesis, which proposes that FIPV evolves from FECV by mutation in individually infected cats (*7*–*12*). A responsible mutation(s) has not been identified to back this hypothesis.

FECV is ubiquitous and spreads efficiently by the fecal-oral route; hence, seropositivity among cat populations can reach 90%, depending on the field conditions (*13*). The infection is restricted to the enteric tract, where the virus replicates in epithelial cells lining the gut mucosa. FECV infection is mild, causing transient enteritis that often passes unnoticed. The infection cannot be cleared efficiently by the immune system and thus persists, often for weeks or months, sometimes even longer

Author affiliations: Utrecht University, Utrecht, the Netherlands (H.-W. Chang, H.F. Egberink, P.J.M. Rottier); and J. Craig Venter Institute, Rockville, Maryland, USA (R. Halpin, D.J. Spiro)

DOI: http://dx.doi.org/10.3201/eid1807.120143

(*14–18*). In contrast, FIPV is rare, but the consequences of infection are devastating. FIPV infection causes a progressive systemic disease called feline infectious peritonitis. The disease affects many organs, usually inducing fatal immunopathologic disease characterized by disseminated pyogranulomas and severe inflammatory damage to serosal membranes.

In the past, sequence differences in several virus genes, including those encoding membrane and spike (S) structural proteins and the so-called group-specific proteins 3c and 7b, have been implicated in the FCoV virulence shift (*5–7,10,11,19–22*). However, none of these differences appeared to consistently correlate with disease phenotype. To establish a consistent cause for a virulence shift in FCoV, specifically the predominant serotype I FCoV, we sequenced the entire genome of several FECV and FIPV specimens and then concentrated on the most conspicuous region of consistent difference by collecting and sequencing additional FECV and FIPV samples.

Materials and Methods

Viruses and Clinical Specimens

FECV strain RM and FECV strain UCD (FECV UU2) were propagated in specific pathogen–free cats. FIPV UU3 was obtained from a lymph node of a cat infected with FECV UCD; the presence of feline infectious peritonitis in the cat was pathologically confirmed. During 2006–2011, with the assistance of veterinarians in the Netherlands, we randomly obtained field cats with suspected feline infectious peritonitis and feces samples from apparently healthy cats from all geographic areas of the Netherlands; we did not use any selection criteria, such as age, sex, or breed of cat. Cats originated from 144 different catteries and single- and multicat households. Cats were pathologically diagnosed with feline infectious peritonitis by postmortem examination at the Veterinary Pathology Department, Utrecht University; findings confirmed that the cats had feline infectious peritonitis. Ascites samples and lesions from affected organs were obtained for RNA isolation. Fecal material from apparently healthy cats was obtained from the rectum by using a cotton swab.

RNA Preparation

We suspended fecal specimens to a final concentration of 10% (wt/vol) in phosphate-buffered saline by vigorously vortexing the specimens. The supernatant was cleared (centrifugation for 10 min at 3,000 × *g*) and then used for RNA extraction. Following the manufacturer's protocols, we used the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA) to extract viral RNA from 140 μL of fecal supernatants or ascites and the QIAamp RNeasy Mini Kit to extract viral RNA from 30 mg of organ tissue homogenate.

FCoV Detection and Serotyping

We tested RNA isolated from organs or ascites of cats with feline infectious peritonitis and from feces of apparently healthy cats for the presence of FCoV RNA by using a reverse transcription nested PCR (RT-nPCR) targeting the highly conserved 3× untranslated region (*23*). Samples with results positive for FCoV were checked for the virus serotype by using an RT-nPCR targeting the S gene (*24*). Only samples positive for serotype I FCoV were included in this study.

We determined the sequence in the S gene region of interest by using an RT-nPCR. In brief, we synthesized complementary DNA by using an antisense primer (5′-CCCTCGAGTCCCGCAGAAACCATACCTA-3′) and superscript II reverse transcriptase (Promega, Madison, WI, USA) at 50°C for 1 h. We then performed the PCR by using *Taq* DNA polymerase (Promega) and specific primers (sense 5′-CAATATTACAATGGCATAATGG-3′, antisense 5′-CCCTCGAGTCCCGCAGAAACCATACCTA-3′) for the first reaction and specific primers (sense 5′-GGCATAATGGTTTTACCTGGTG-3′, antisense 5′-TAATTAAGCCTCGCCTGCACTT-3′) for the second reaction. PCR cycling conditions were 30 cycles at 94°C for 60 s, at 50°C for 30 s, and at 72°C for 1 min plus a 7-min extension at 72°C at the end of the reaction. All enzymes were used according to the manufacturer's instructions. Primer pairs were expected to generate a 598-bp product for the first PCR run and a 142-bp product for the second run.

Representative PCR products were purified by electrophoresis in 2% agarose gel followed by extraction from the gel by using a gel extraction kit (QIAGEN) according to the manufacturer's recommended instructions. Macrogen Inc. (http://dna.macrogen.com/eng/) sequenced the gel-purified DNA.

Full Genome Sequencing

Two 96-well plates of degenerate primers (online Technical Appendix, www.cdc.gov/eid-static/spreadsheets/12-0143-Techapp.xls) were designed from aligned reference genomes by using a computational PCR primer design pipeline. The pipeline was developed at the J. Craig Venter Institute (JCVI) to produce tiled amplicons with an optimal length of 550 bp, with 100-bp overlap to provide 6-fold sequence coverage of the genome. An M13 sequence tag was added to the 5× end of each degenerate primer and was used for sequencing. Primers were arranged in a 96-well plate format, and all PCRs for each sample were performed in 2 plates. The primers used in this study are listed in the online Technical Appendix.

Sequencing reactions were performed by using Big Dye Terminator (Applied Biosystems, Foster City, CA, USA) chemistry. Each amplicon was sequenced from both ends by using M13 primers, and sequencing reactions

were analyzed by using a 3730 ABI sequencer (Applied Biosystems). Raw sequence data were trimmed to remove any primer-derived and low-quality sequence; gene sequences were assembled by using a viral assembly tool (www.jcvi.org/cms/research/software). Assemblies were edited computationally and manually. When insufficient underlying sequence information was obtained, the sample was entered into the secondary sequencing pipeline and reamplified by using existing primers or primers designed from the problematic sequence assembly itself. The reamplified sample was then sequenced again.

An RNA virus genome prediction program called VIGOR (Viral Genome ORF Reader, JCVI (www.jcvi.org/vigor) can decode many classes of viruses, taking into account virus-specific features, such as alternative splicing, internal open reading frames, and ribosomal slippage. This program was used to annotate de novo assemblies of coronaviruses sequenced at JCVI and also to validate newly assembled genomes during the finishing process. Last, we performed a quality control assessment and manually inspected the gene predictions before loading them into the annotation database at JCVI, from which they were exported in formats acceptable to the National Center for Biotechnology Information.

Multiple-Sequence Alignment

The full-length and partial FCoV genomic nucleotide sequences we obtained were deposited in the National Center for Biotechnology Information database (www.ncbi.nlm.nih.gov). The sequence accession numbers of the full-length FCoV sequences used in this study are listed in Table 1. The GenBank accession numbers for the partial S gene sequences are JQ304323–JQ304518. Multiple-sequence alignments were constructed by using Clustal W (www.ebi.ac.uk/clustalw) with the Lasergene MegAlign (DNASTAR, www.dnastar.com/t-sub-products-lasergene-megalign.aspx) and MEGA4 (www.megasoftware.net) software programs. To identify key differences between FIPV and FECV, we analyzed their genomes and proteomes; for each nucleotide or amino acid position, we determined the rate at which FIPVs differed from all FECVs at that position. Phylogenetic analysis was performed by using features of the MEGA4 suite of programs. Phylogenetic trees of these sequences were obtained by using the neighbor-joining method. The bootstrap consensus tree, inferred from 1,000 replicates, was prepared; positions containing gaps and missing data were eliminated from the dataset.

Results

Full Genome Sequencing

To identify the distinguishing difference(s) between the FCoV pathotypes, we initiated a full genome sequencing program of FECVs found in the feces of apparently healthy cats and of FIPVs found in organs or ascites of cats with pathologically confirmed feline infectious peritonitis. To obtain a more extensive analysis of the coronavirus genome, this sequencing program is still ongoing; however, after the sequences of 11 genomes of each pathotype were completed, we performed a comparative FECV–FIPV analysis, screening the genomes for nucleotide differences (Table 1). This was done by counting, for every nucleotide position, the number of FIPV genomes for which the identity at that position differed from that in all FECV genomes.

Table 1. GenBank accession numbers for viruses for which the genomes were fully sequenced in a study to distinguish virulent from nonvirulent feline coronaviruses

Virus strain	Accession no.
Feline infectious peritonitis viruses	
UU3	FJ938061
UU4	FJ938054
UU5	FJ938056
UU8	FJ938055
UU9	FJ938062
UU15	FJ938057
UU16	FJ938058
UU17	HQ012367
UU21	HQ012369
UU24	HQ012370
UU30	HQ392472
Feline enteric coronaviruses	
RM	FJ938051
UU2 (UCD)	FJ938060
UU7	FJ938053
UU10	FJ938059
UU11	FJ938052
UU18	HQ012368
UU19	HQ392490
UU20	HQ392471
UU22	GU553361
UU23	GU553362
UU31	HQ012371

Our results showed that differences were scattered along the entire genome (Figure 1). At 2,963 (10%) of the 29,277 genome positions, the nucleotide identity in at least 1 of the 11 FIPVs did not occur in any of the 11 FECVs. Of these 2,963 positions, 1,187 occurred in gene 1ab, 1,246 in the S gene, 248 in gene cluster 3abc, 22 in the envelope protein gene, 42 in the membrane protein gene, 113 in the nucleocapsid protein gene, and 106 in gene cluster 7ab, showing the disproportionally large genetic variation in the S gene. The frequency with which differences occurred at different nucleotide positions across the genome showed the following distribution: a difference was detected 1× at 1,914 positions, 2× at 945 positions, 3× at 87 positions, 4× at 15 positions, and 5× at 1 position. At 1 position (23531), the nucleotide identity in 9 of the FIPVs was not found in any of the FECVs. No position(s) uniquely distinguished the 2 FCoV pathotypes.

Nucleotide identity differed the most at position 23531: it was highly conserved (100% A) in all FECV

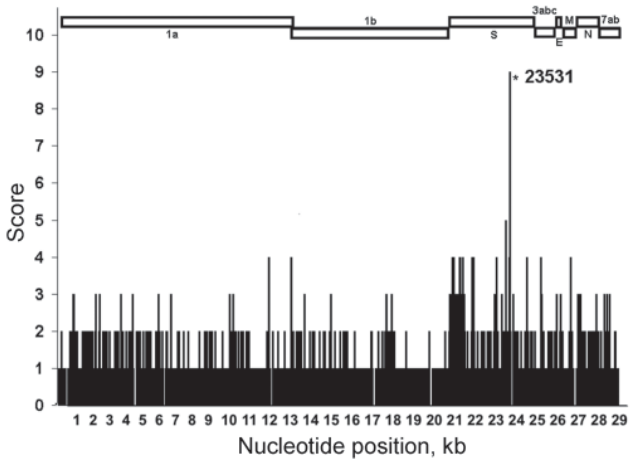

Figure 1. Comparison of full genomes of 11 lethal feline infectious peritonitis viruses (FIPVs) with full genomes of 11 nonvirulent feline enteric coronaviruses (FECVs). Nucleotide (nt) positions are shown on the x-axis; y-axis indicates number of FIPV genomes for which the identity at the nt position differed from identity at same position in all FECV genomes. FIPV strain C1Je (GenBank accession no. DQ848678) was used as the reference for nt numbering. *Highest difference score: 9 FIPVs had identities at nt position 23531 that differed from those at the same position in all FECVs. 1a, gene 1a; 1b, gene 1b; S, spike protein gene; 3abc, gene cluster 3abc; E, envelope protein gene; M, membrane protein gene; N, nucleocapsid protein gene; 7ab, gene cluster 7ab.

genomes, and it was C or T in 9 of the 11 FIPV genomes. This difference occurs in the S gene and results in an amino acid difference in the predicted S protein. Thus, although all FECV S proteins have a methionine at position 1058, a leucine is encoded in the 9 FIPVs, irrespective of the identity of the genetic difference (C or T).

Sequencing of the S gene

To further investigate the single most prominent region of difference between FECVs and FIPVs, we established an RT-nPCR method to amplify and analyze the genomic region covering nucleotides 23442–24040 for the first PCR run and nucleotides 23451–23593 for the second run, which includes deviant position 23531. Altogether, 183 FECV and 118 FIPV RNAs isolated from different cats were sequenced in this specific region. Results for the 11 entirely sequenced FECVs and FIPVs are shown in Figure 2. The A at nucleotide 23531 was 100% conserved in all 183 FECVs in our collection. Of the 118 FIPVs, 96 (81.4%) had a T and 12 (10.2%) a C at this position; in both cases, this changes the methionine occurring at position 1058 in the FECV S protein into a leucine in FIPV (i.e., mutation M1058L).

Phylogenetic Analysis

Assuming that the difference observed in 108 of the 118 sequenced FIPVs may be responsible for the virulent phenotype, the remaining 10 viruses should be expected to carry alternative differences. In search of those differences, we performed a phylogenetic analysis of the partial nucleotide sequences accumulated by the RT-nPCR procedure, but the results did not enable further differentiation (data not shown). When carrying out a phylogenetic analysis of the translated partial amino acid sequences, we observed a small but distinct second cluster B in addition to the major cluster A constituted by the FIPVs having leucine at position 1058 in their S protein (Figure 3). This smaller cluster, formed by 5 (4.2%) of the sequenced FIPVs, appeared to be characterized by the occurrence of an alanine at position 1060 (i.e., mutation S1060A), just 2 residues downstream of the M^{1058} that is changed in most FIPVs. All other FIPVs and all sequenced FECVs consistently had a serine at this position. The difference is brought about in all 5 cases by a T→G change at nucleotide 23537. Overall, we have detected characteristic differences with FECV for 113 (95.8%) of the 118 sequenced FIPVs. These differences were observed in both pathologic forms (i.e., wet and dry forms) of feline infectious peritonitis (Table 2).

Discussion

Our findings show differences in 2 alternative codons of the FCoV S gene that correlate with the feline infectious peritonitis disease phenotype in >95% of cases. Besides providing a realistic basis for diagnostic discrimination of the 2 FCoV pathotypes, our findings also support the

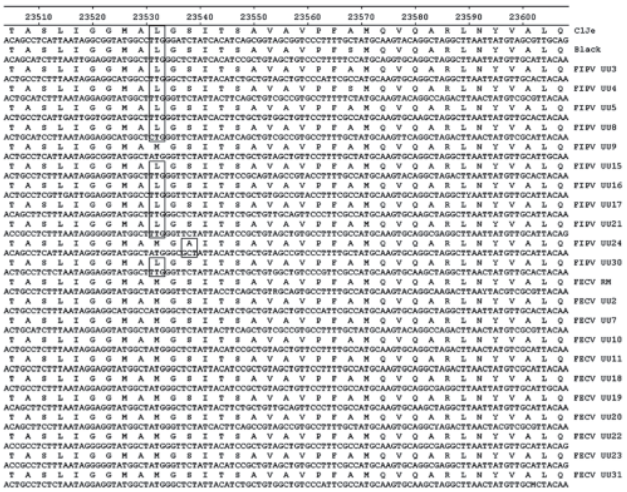

Figure 2. Alignment of partial nucleotide sequences and translated amino acid sequences in the spike protein of 11 strains each of 2 feline coronavirus pathotypes: FIPVs (lethal) and FECVs (nonvirulent). The viruses were sequenced in a study to distinguish virulent from nonvirulent feline coronaviruses (see Table 1). FIPV strain C1Je (GenBank accession no. DQ848678) was used as the reference for numbering. Sequence positions are shown along the top; virus strains are shown on the right. Specific differences between the pathotypes are boxed. FIPVs, feline infectious peritonitis viruses; FECVs, feline enteric coronaviruses.

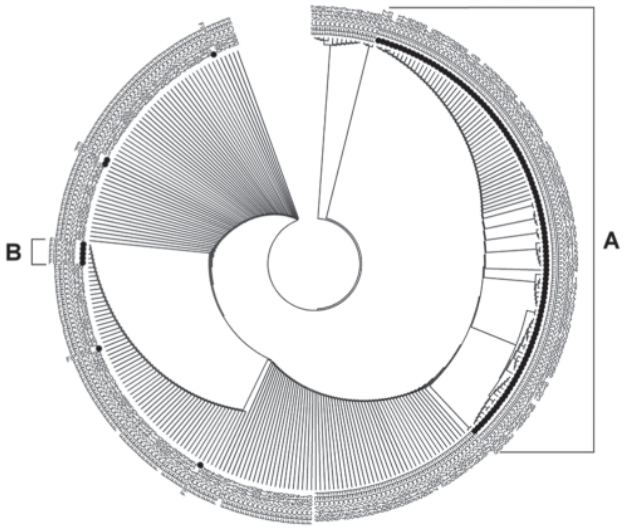

Figure 3. Phylogenetic tree based on partial amino acid sequences (aa 1056–1069) of the spike proteins of 118 feline infectious peritonitis viruses (FIPVs) and 183 feline enteric coronaviruses (FECVs) obtained by using reverse transcription nested PCR and sequencing of the distinguishing genomic region. A circular rooted neighbor-joining tree was constructed by using the bootstrap method and applying 1,000 replicates. Black dots indicate FIPVs. Clade A comprises FIPVs containing the M1058L mutation; clade B comprises FIPVs containing the S1060A mutation.

mutation hypothesis. Thus, we propose that alternative mutations in the S protein of FECV give rise to a tropism change that allows the virus to escape from the intestine into body tissues, where it causes feline infectious peritonitis. Proof of this hypothesis will require introduction of these mutations into the FECV genome and demonstration of the virulence switch by infection of cats. However, this is a formidable challenge in the absence of a reverse genetics system and a proper cell culture system to generate and propagate these viruses.

Our findings relating the S protein to FCoV pathogenicity are not surprising, given earlier explorations into the involvement of various genes (7a, 7b, M, and 3c) (*5*–*7*,*10*,*11*,*19*–*22*,*24*,*25*). One of the most notable consequences of the presumed mutation in FECV is the acquisition of monocyte/macrophage tropism by the resulting virus (*26*). Thus, whereas replication of FECV is restricted to the epithelial cells lining the gut, the virulence mutation enables FIPV to efficiently infect and replicate in macrophages and spread the infection systemically (*26*). Such tropism change corresponds most logically with a modification in the S protein. An earlier study, using serotype II FCoVs, indicated a virulence role for the S protein (*21*); however, identification of the mutation(s) was not pursued because of the controversial nature of the FECV strain used in the study (*14*).

As for the serotype II viruses, the putative virulence mutations detected in the serotype I FCoV spike occur in the membrane-proximal domain of the protein. In coronaviruses, the S protein functions in cell entry; it is responsible for receptor attachment and membrane fusion. While the receptor binding site is located in the N terminal part of the protein, fusion is mediated by its membrane-proximal part. Coronavirus S proteins are class I fusion proteins, which typically contain domains instrumental for this process: 2 heptad repeat regions and a fusion peptide (*27*). The fusion peptide is located just upstream of the membrane-distal heptad repeat region, but it remains to be proven that it functions as a fusion peptide. The 2 putative virulence mutations identified in our study, M1058L and S1060A, map to this characteristic hydrophobic domain. Both changes are subtle and do not give clues as to their functional consequences. We assume, however, that these alternative mutations have a similar effect, and we speculate that the mutations in the remaining 4% of cases might also occur in the fusion peptide of the S protein.

If these mutations are all that is needed to convert a nonvirulent FECV into a lethal FIPV, the question arises as to why feline infectious peritonitis occurs so infrequently. For example, simple calculations based on a 10^{-4} frequency and a stochastic occurrence of RNA polymerase errors across the genome (*28*) predict that the M1058L mutation, for which 2 alternative substitutions of A^{23531} (to T or G) occur, would statistically arise once in every 1.5×10^4 genomes produced. In experimental FECV infection of kittens, we showed that up to 10^8 genome equivalents of the virus are shed per microliter of feces (*18*); thus, typical FECV infections would be expected to generate thousands of progeny carrying 1 of the critical mutations. However, the virulence phenotype supposedly associated with the mutation is not observed to any proportional extent. We can only speculate as to the reasons.

One likely possibility is that additional mutations (1 or more, perhaps alternative mutations) are required to generate the virulent pathotype. Such mutations would most probably involve the accessory gene 3c, which is intact in FECVs but severely affected in about two thirds of FIPVs (*7*,*10*–*12*). The 3c protein apparently is

Table 2. Prevalence of alternative mutations in feline infectious peritonitis virus spike protein of cats with wet and dry forms of FIP*

	Pathologic form of FIP	
Mutation	Wet	Dry
M1058L	71	46
S1060A	5	1
No mutation	3	3
Total	79	50

*Cats were diagnosed with the dry or wet form of FIP (feline infectious peritonitis) during postmortem examination by a veterinary pathologist. The occurrence and nature of the mutation in the spike gene was established by sequencing.

essential for replication of FECV in the gut but becomes nonessential once virulence mutation(s) elsewhere in the genome (e.g., in the S gene) enable the virus to infect macrophages and spread systemically. As we suggested earlier, loss of 3c function may not only be tolerated, it may even enhance the fitness of the mutant virus in its new biotope and, as a consequence, hamper its return to the gut. If the mutant virus is absent in the gut, it will not be shed in feces, providing an explanation for the seemingly rare incidence of feline infectious peritonitis outbreaks. Our discoveries of the critical differences between FECVs and FIPVs are clearly only a small step toward understanding the pathogenetic phenomena of feline coronavirus infections.

Acknowledgment

We thank Niels Pedersen for providing FECV strain RM and FECV strain UCD.

This project was funded in part by the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services (contract no. HHSN272200900007C).

Dr Chang is a veterinary postdoctoral researcher in the Virology Division, Department of Infectious Diseases and Immunology, Veterinary Faculty, Utrecht University, the Netherlands. Her research interests include viral pathogenesis and veterinary virology, pathology, and immunology.

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Address for correspondence: Peter J.M. Rottier, Virology Division, Department of Infectious Diseases and Immunology, Veterinary Faculty, Utrecht University, Yalelaan 1, 3584 CL Utrecht, the Netherlands; email: p.rottier@uu.nl

Enterococcus faecalis Clones in Poultry and in Humans with Urinary Tract Infections, Vietnam

Louise Ladefoged Poulsen, Magne Bisgaard, Nguyen Thai Son, Nguyen Vu Trung, Hoang Manh An, and Anders Dalsgaard

Enterococcus spp. as pathogens have increased, but the sources of infection often remain unclear. To investigate whether poultry might be a reservoir for *E. faecalis*–associated urinary tract infections (UTIs) in humans, we characterized *E. faecalis* isolates from patients in Vietnam with UTIs during January 2008–January 2010 and poultry living in close contact with them by multilocus sequence typing (MLST), pulsed-field gel electrophoresis, analysis of antimicrobial drug susceptibility patterns, and sequencing of virulence genes. In 7 (23%) of 31 UTI cases, we detected identical MLST, indistinguishable or closely related pulsed-field gel electrophoresis patterns, and similar antimicrobial drug susceptibility patterns. Isolates from urine and poultry showed identical virulence gene profiles, except for 1 variation, and individual genes showed identical sequences. The homology of isolates from urine and poultry further indicates the zoonotic potential and global spread of *E. faecalis* sequence type 16, which recently was reported in humans with endocarditis and in pigs in Denmark.

Enterococci are commensals of the human and animal gastrointestinal tract and opportunistic pathogens that cause urinary tract infections (UTIs), endocarditis, and sepsis (*1*). Nosocomial infections caused by enterococci have increased; these pathogens are now the third most common at hospitals after *Escherichia coli* and *Staphylococcus aureus* (*2*); and enterococci are frequently recorded as the cause of UTIs, wound infections, bacteremia, and endocarditis (*3*–*6*).

The sources of enterococcal infections in humans are not clear, but animal reservoirs have been suggested (*2*,*4*,*7*–*9*). A study comparing enterococcal isolates from 4 European countries and the United States demonstrated that *E. faecalis* isolated from pigs in Portugal had pulsed-field gel electrophoresis (PFGE) patterns identical to those of multidrug-resistant isolates at hospitals in Spain, Italy, and Portugal, all of which were shown by multilocus sequence typing (MLST) to belong to sequence type (ST) 6 (*7*). In Denmark, high-level gentamicin-resistant *E. faecalis* of ST16 with an identical PFGE pattern was isolated from pigs and from humans with endocarditis (*9*). Identical and closely related PFGE patterns were demonstrated by isolates from humans and from pork and chicken meat in the United States, all of which contained high-level gentamicin-resistant genes (*4*). Our objective was to characterize epidemiologically related *E. faecalis* isolated from humans with UTIs and from poultry living in the same households in Vietnam to evaluate the zoonotic potential of *E. faecalis*.

Author affiliations: University of Copenhagen, Copenhagen, Denmark (L.L. Poulsen, M. Bisgaard, A. Dalsgaard); Military Medical University, Ha Dong, Hanoi, Vietnam (N.T. Son, H.M. An); and Hanoi Medical University, Hanoi (N.V. Trung)

DOI: http://dx.doi.org/10.3201/eid1807.111754

Materials and Methods

Recruitment of Patients, Urine Collection, and Bacterial Culture of Urine

Urine samples were collected during January 2008–January 2010 at the Military Medical University, Hospital 103, in Ha Dong, Hanoi. Patients with clinical symptoms of UTI (i.e., ≥1 of the following symptoms: frequent urination; painful urination; hematuria; cloudy urine; or pain in pelvic area, flank, or low back) were referred from nearby pharmacies and informed about the project. A midstream urine sample was collected at the hospital under supervision of a nurse. Only patients with uncomplicated UTIs were included; patients reporting underlying diseases, such as hematologic disorders, respiratory infections, diarrhea, diabetes, cancer, HIV/AIDS, liver cirrhosis, alcoholism, anatomic malformations of urinary tract, nephrolithiasis, or

urolithiasis were excluded, as were patients with hospital-acquired UTIs. The urine was cultured immediately after collection. Thirty-one UTI patients met the study criteria of having *E. faecalis* CFU $>10^3$/mL isolated from a urine sample in pure culture and were raising poultry in their households.

The urine samples were cultured on Flexicult agar plates (Statens Serum Institut, Copenhagen, Denmark), where *E. faecalis* grows as small green/blue-green colonies and *E. faecium* as small green colonies (*10*). Three colonies were isolated from each UTI patient. All 31 participants were interviewed when urine samples were collected. Personal information recorded included age, sex, and underlying diseases. The following clinical symptoms were recorded: frequent urination, painful urination, cloudy urine, blood in urine, pain in pelvic area, flank pain, pain in low back, and fever. In addition, information about duration of symptoms; previous UTIs; and medical treatment before arrival at the hospital, including type of antimicrobial drug used, was recorded.

Species identification of all 31 presumptive *E. faecalis* isolates from urine and 83 isolates from poultry were confirmed by species-specific PCR as described by Dutka-Malen et al. (*11*). Only isolates identified as *E. faecalis* by PCR were further characterized.

All study participants were informed orally and in writing about the study and provided written consent. The ethics committee at Army Hospital 103 approved the study protocols.

Collection of Cloacal Swabs from Poultry

When a urine sample was positive for *E. faecalis*, the patient's household was visited within 1 week, and cloacal swabs were taken from 2–4 chickens in the household. Fecal samples were taken with a sterile cotton swab and immediately placed in Cary-Blair media (Oxoid, Basingstoke, Hampshire, UK) for transportation to the laboratory. Samples were then streaked on Slanetz and Bartley agar medium (Merck, Darmstadt, Germany) the same day and incubated for 24–48 h at 37°C. Subsequently, 2 individual colonies were randomly selected and subcultured on nonselective LB-agar, Lennox plates (Difco, Becton Dickinson, Sparks, MD, USA), which were incubated overnight at 37°C to obtain pure cultures. Colonies were then grown in brain–heart infusion broth (Oxoid) overnight at 37°C and stored for further characterization at –80°C in cryotubes containing 30% glycerol.

MLST and PFGE

To investigate whether isolates of *E. faecalis* from urine and poultry belonged to identical STs, we characterized isolates from urine and poultry by MLST. Urine isolates were characterized by sequencing of all 7 housekeeping genes used in the MLST scheme: *gdh*, *gyd*, *pstS*, *gki*, *aroE*, *xpt*, and *yqiI*. To confirm that the UTIs were caused by a single strain, 1 additional colony from 9 (29%) of 31 urine samples was characterized by sequencing the *gki* and *yqiI* genes. Two isolates from each chicken were characterized by sequencing the *gki* and *yqiI* genes. When sequences of both genes in 2 isolates corresponded to the sequence of the same genes in the urine isolate, which occurred in 11 cases, 1 of the 2 isolates from poultry was randomly selected and further characterized. When gene sequences in only 1 isolate from poultry were identical to the isolate from urine, the isolate was further characterized. Primers and PCR conditions are described on the *E. faecalis* MLST website (http://efaecalis.mlst.net/). Amplicons were sequenced in both directions by Macrogen (Seoul, South Korea). DNA sequences obtained were assembled using CLC Main Workbench 5.2 software (CLC bio, Aarhus, Denmark) and compared with published alleles, and an ST was assigned to each strain (http://efaecalis.mlst.net/). PFGE was performed as described (*12*) by using the restriction enzyme *sma*I (New England BioLabs, Ipswich, MA, USA).

Virulence Genes

The presence and sequence of the following 6 virulence genes were used to further characterize the isolates from urine and poultry: *asa*1, *CylA*, *efaA*, *Esp*, *gelE*, and EF0591 (*13*). After detecting the virulence genes by PCR (*13*), we sequenced the genes in both directions using Macrogen. DNA sequences were compared, and possible nucleotide differences were calculated by using Smith-Waterman local alignment (EMBOSS) available online from the European Bioinformatics Institute: (www.ebi.ac.uk/).

Antimicrobial Drug Susceptibility Testing

MICs were determined for 16 antimicrobial drugs for comparison analyses by using the Sensititer system (Trek Diagnostics Systems, East Grindstead, UK) according to the manufacturer's guidelines. These drugs were ampicillin (2–32 μg/mL), avilamycin (4–32 μg/mL), chloramphenicol (2–64 μg/mL), daptomycin (0.25–16 μg/mL), erythromycin (0.5–32 μg/mL), gentamicin (16–1,024 μg/mL), kanamicin (128–2,048 μg/mL), linezolid (0.5–8 μg/mL), moxifloxacin (0.25–8 μg/mL), penicillin (2–32 μg/mL), salinomycin (2–16 μg/mL), streptomycin (64–2,048 μg/mL), quinupristin-dalfopristin (0.25–16 μg/mL), tetracycline (1–32 μg/mL), tigecycline (0.015–2 μg/mL), and vancomycin (1–32 μg/mL).

Results

In 7 (23%) of 31 UTI cases, *E. faecalis* isolated from patient urine and poultry demonstrated identical STs and an indistinguishable (4 pairs) or closely related PFGE pattern (3 pairs, defined as showing ≤3 fragment difference) (Figure).

In addition, antimicrobial drug susceptibility patterns were similar, and only 1 variation was found in the virulence gene profiles (Tables 1, 2). Five of these 7 patients reportedly had a profession where they worked with poultry. A total of 22 patients who did not share a clone of *E. faecalis* found in poultry in their household reported working with poultry.

MLST

Sequencing the 7 housekeeping genes in the 31 *E. faecalis* strains showed the following 14 STs: 4, 16, 17, 93, 116, 136, 141, 314, 410, 411, 412, 413, 415, and 417, with ST16 shown by 16 (51.6%) isolates. Three isolates belonged to ST4, and each of the remaining STs was represented by only 1 isolate. In 7 of 31 households, the same ST was obtained from poultry and urine (Table 1). In 3 households, ST16 was isolated from urine and poultry. In the remaining 4 households, STs 93, 141, 413, and 415 were identified (Table 1). Because each pair of isolates from all selected patients (28%) showed identical *gki* and *yqil* gene sequences, we concluded that the UTI cases were associated with 1 *E. faecalis* strain.

PFGE

We detected 6 PFGE patterns (A1–A6). Of these, 4 pairs from urine and poultry from the same households showed indistinguishable patterns (Table 1; Figure).

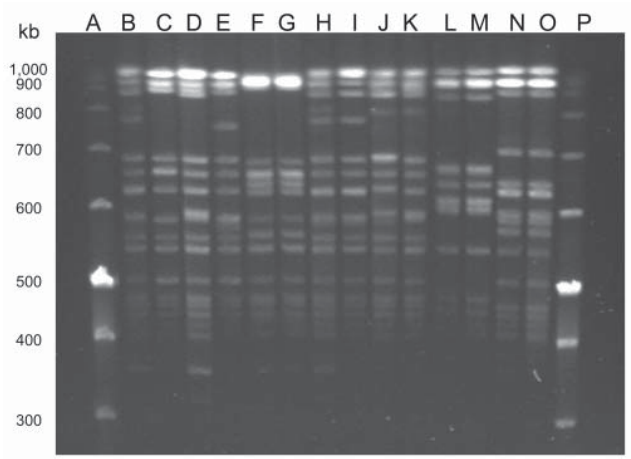

Figure. Pulsed-field gel electrophoresis of *Sma*I-digested *Enterococcus faecalis* isolated from humans with urinary tract infections and from poultry in the same houselhold, Vietnam, January 2008–January 2010. Lanes A and P are molecular weight markers. Lane B, isolate 90U; lane C, isolate 90P; lane D, 122U; lane E, 122P; lane F, 186U; lane G, 186P; lane H, 191U; lane I, 191P; lane J, 204U; lane K, 204P; lane L, 217U; lane M, 217P; lane N, 221U; and lane O, 221P.

Antimicrobial Drug Susceptibility Testing

When we compared isolates from urine and poultry from individual households, we detected similar MICs of each tested antimicrobial drug, showing a 1-dilution factor deviation (Table 2). For several isolates, an MIC could not be established because the MIC fell outside the test intervals. We detected different MICs for 7 antimicrobial drugs when we compared strains 204U and 204P. All isolates were fully susceptible (lowest or second lowest MIC tested) to ampicillin, avilamycin, linezolid, penicillin, salinomycin, tigecycline, and vancomycin (results not shown in Table 2).

Virulence Genes

PCR for the 6 virulence genes showed that the isolates from urine and poultry from an individual household contained identical virulence genes that varied from 1 to 5 genes, except for 1 household in which the isolate from urine (90U) did not contain the *asa*1 gene (Table 1). When we compared the DNA sequences from the epidemiologically related urine and poultry strains, we found that all 23 sequenced gene pairs showed 100% similarity.

Discussion

We document isolation of the same clone of *E. faecalis* in urine and poultry from the same households in which patients had close contact with the poultry. The potential for zoonotic transmission of *E. faecalis* has been suggested, but to our knowledge, only epidemiologically unrelated isolates have been investigated (*3*,*4*,*7*–*9*,*14*).

Most of the isolates in our study belonged to ST16, which has been isolated from animals and humans, including clinical and nonclinical isolates (*14*). ST93 was isolated from a patient with an ulcer in Poland and from an unknown source in the United States, and ST141 was isolated from chickens in Denmark and from a blood sample of a hospitalized person in Poland (http://efaecalis.mlst.net/).

When we interpreted PFGE patterns for their relatedness using criteria suggested by Tenover et al. (*15*), we found 4 pairs of *E. faecalis* strains with indistinguishable band patterns that could be "considered to represent the same strain" (*15*). From 3 individual households, isolates from urine and poultry showed PFGE patterns with 1 or 2 band differences and thus can be considered closely related (*15*). These identical or closely related PFGE patterns, together with the supporting findings by MLST and virulence gene profiling, suggest that *E. faecalis* might be transmitted from poultry to humans, causing UTIs. However, the finding of similar isolates from humans and poultry also could result from sharing a common clone of *E. faecalis*. ST16 has been reported from various epidemiologically unrelated human and animal sources (*14*), which could indicate a common clone in humans and animals. Because no data about ST16 in the environment are available, an environmental reservoir cannot be ruled out.

Table 1. MLST, PFGE, and virulence gene profiles for *Enterococcus faecalis* isolated from humans with urinary tract infections and poultry from the same households, Vietnam, January 2008–January 2010*

Strain†	Source	MLST type	PFGE pattern	Virulence genes						Duration of symptoms, mo
				asa1	*CylA*	*efaA*	*Esp*	*gelE*	EF0591	
90U	Urine	16	A1	–	+	+	+	–	+	1
90P	Poultry	16	A2	+	+	+	+	–	+	NA
122U	Urine	16	A2	+	+	+	+	–	+	7
122P	Poultry	16	A1	+	+	+	+	–	+	NA
186U	Urine	93	A3	–	–	+	–	–	–	24
186P	Poultry	93	A3	–	–	+	–	–	–	NA
191U	Urine	413	A3	–	+	+	+	–	+	24
191P	Poultry	413	A1	–	+	+	+	–	+	NA
204U	Urine	16	A1	–	+	+	+	–	+	2
204P	Poultry	16	A1	–	+	+	+	–	+	NA
217U	Urine	415	A3	+	–	+	–	+	–	0.5
217P	Poultry	415	A3	+	–	+	–	+	–	NA
221U	Urine	141	A6	–	–	+	–	+	–	120
221P	Poultry	141	A6	–	–	+	–	+	–	NA

*MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; +, positive; –, negative; NA, not applicable.
†All isolates except 221U were recovered from female patients. Strains isolated from patients with urinary tract infections are designated as U and strains isolated from poultry as P.

Because 27 of the 31 patients reported having contact with poultry through their work, contact with poultry outside the household environment cannot be excluded as the source of *E. faecalis*. Epidemiologic risk factor studies are needed to document actual transmission routes.

The variation found in resistance patterns might have resulted from exposure to different antimicrobial drugs, resulting in different selection pressure on *E. faecalis* in the human and poultry hosts. The 7 patients studied had UTI symptoms for an average of 514 days (range 5 days–10 years), which is unusually long for UTI (Table 1). Although self-medication is well established to be a common practice in Vietnam (*16*), only 2 of the 7 patients acknowledged use of antimicrobial drugs to treat their UTI symptoms before they participated in the study (data not shown). Over time, patients tend to forget what kind of medication they received. Furthermore, the questionnaire asked only whether antimicrobial drugs were used against UTI, not whether they were used to treat other diseases. In addition, poultry might have been exposed to antimicrobial drugs through growth promoters added in the feedstuff and during therapeutic or preventive treatments, but information about such use was not available.

In most Western countries, contact with poultry occurs mainly through handling and consumption of poultry meat. However, the risk for zoonotic transmission of *E. faecalis* from poultry meat remains to be investigated. Thus, similar studies and risk factor studies should be conducted in more countries to evaluate the effect on zoonotic transmission of differences in human habits of poultry consumption and contact with poultry. In addition, animals other than pigs and poultry should be investigated as sources of zoonotic *E. faecalis* transmission. Finally, we cannot exclude the possibility that *E. faecalis* pathotypes found in poultry might represent transmission from humans, e.g., in this study, from UTI patients. However, poultry as carriers of

Table 2. Antimicrobial drug susceptibility (MIC) testing of *Enterococcus faecalis* isolated from humans with urinary tract infections and poultry in the same household, Vietnam, January 2008–January 2010*

Strain	Antimicrobial drug and test interval, µg/mL								
	CHL, 2–64	DAP, 0.25–16	ERY, 0.5–32	GEN, 16–1,024	KAN, 128–2,048	MXF, 0.25–8	STR, 64–2,048	Q-D, 0.25–16	TET, 1–32
90U	64	4†	>32	>1,024	>2,048	≤0.25	>2,048†	16	>32
90P	>64	>16†	32	512	>2,048	≤0.25	128†	16	>32
122U	64	4	>32	64†	>2,048	≤0.25	128†	16	>32
122P	>64	4	>32	1,024†	>2,048	≤0.25	>2,048†	16	>32
186U	4	4	>32†	≤16	≤128	≤0.25	≤64	16	>32†
186P	4	8	≤0.5†	≤16	≤128	≤0.25	≤64	16	≤1†
191U	32	4	>32	>1,024	>2,048	≤0.25	>2,048	16	32†
191P	64	8	>32	512	>2,048	≤0.25	>2,048	16	≤1†
204U	64†	4	>32†	>1,024†	>2,048†	≤0.25†	>2,048†	16†	32
204P	4†	8	4†	≤16†	≤128†	2†	≤64†	0.5†	32
217U	4	4	≤0.5	32	≤128	≤0.25	256	8	≤1
217P	4	4	≤0.5	≤16	≤128	≤0.25	256	8	≤1
221U	64	4	≤0.5	≤16†	≤128	≤0.25	128	8	>32
221P	64	4	1	64†	≤128	≤0.25	128	8	>32

*CHL, chloramphenicol; DAP, daptomycin; ERY, erythromycin; GEN, gentamicin; KAN, kanamicin; MXF, moxifloxacin; STR, streptomycin; Q-D, quinupristin-dalfopristin; TET, tetracycline.
†>1 dilution difference between urine and poultry strain.

ST16 has been documented (*17*), and it seems more likely that humans are exposed to poultry litter than that poultry are exposed to human feces.

We did not investigate the route of *E. faecalis* transmission, but the route could be colonization of the human intestine and subsequently ascending the urethra as reported for *E. coli* (*18*). Further studies are required to explain routes of transmission. The emergence of enterococci as causes of human infections and their resistance to some of the crucial antimicrobial drugs used for human treatment emphasizes the need to elucidate transmission routes and reservoirs for the enterococci and their resistance genes (*5*,*6*,*19*–*21*).

Acknowledgments

We thank all patients for their participation in the study; Flemming Scheutz for his involvement and advice during the development of the protocols; staff at the Army Hospital 103, Ha Dong, Hanoi; and Nina Flint and Gitte Petersen for their excellent technical and laboratory support.

This study was supported by the University of Copenhagen through an ordinary PhD stipend to L.L.P. The Danish International Development Assistance (Danida) provided financial support through the project "Chickens as a possible reservoir for urinary tract infections in humans."

Dr Poulsen is a PhD student in the Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen. Her primary research interests include zoonotic diseases and transmission routes.

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Address for correspondence. Anders Dalsgaard, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; email: ad@life.ku.dk

Loss of Household Protection from Use of Insecticide-Treated Nets against Pyrethroid-Resistant Mosquitoes, Benin

Alex Asidi, Raphael N'Guessan, Martin Akogbeto, Chris Curtis,[1] and Mark Rowland

Pyrethroid resistance is becoming widespread in *Anopheles gambiae* mosquitoes, coinciding with expanded use of insecticide-treated nets (ITNs) throughout Africa. To investigate whether nets in use are still protective, we conducted household trials in northern and southern Benin, where *An. gambiae* mosquitoes are susceptible and resistant, respectively, to pyrethroids. Rooms were fitted with window traps and monitored for mosquito biting and survival rates before and after the nets were treated with pyrethroid. Sleeping under an ITN in the location with resistant mosquitoes was no more protective than sleeping under an untreated net, regardless of its physical condition. By contrast, sleeping under an ITN in the location with susceptible mosquitoes decreased the odds of biting by 66%. ITNs provide little or no protection once the mosquitoes become resistant and the netting acquires holes. Resistance seriously threatens malaria control strategies based on ITN.

Insecticide-treated nets (ITNs) and long-lasting insecticidal nets (LLINs) are the primary interventions for preventing malaria in sub-Saharan Africa (*1*,*2*). Nets accumulate holes through wear and tear during the course of everyday use, but the pyrethroid treatment continues to provide personal protection and to reduce vector capacity through excito-repellency and the killing of mosquitoes that contact the net (*3*,*4*). During the last decade, pyrethroid resistance in *Anopheles gambiae* mosquitoes became widespread in western Africa and spread to or developed in eastern Africa (*5*–*9*). As coverage of LLINs expands across the continent under programs supported by the President's Malaria Initiative and Global Fund (*10*), resistance will inevitably increase (*11*–*13*).

Although resistance is perceived as a serious threat to the future of malaria control, the current distribution of resistance is patchy, and its severity seems to differ from 1 location to another. In the western African country of Benin, pyrethroid resistance has evolved in the M (Mopti) molecular form of *An. gambiae* mosquitoes that appears to combine the knockdown resistance (*kdr*) gene with oxidase mechanisms (*14*,*15*). Carriers of this resistance were not controlled by pyrethroid treatments in experimental hut trials of ITNs or the leading brands of LLINs, PermaNet 2.0 (Vestergaard Frandsen SA, Aarhus, Denmark) and Olyset (Sumitomo Chemicals, Osaka, Japan) (*16*,*17*). However, further west in Côte d'Ivoire, the *kdr* in *An. gambiae* S (Savannah) form mosquitoes conferred only limited resistance, and trials of ITNs continued to protect against mosquito blood feeding (biting) and malaria transmission by this species (*18*–*20*).

Results from experimental hut trials in Benin raise an alarm. Of key concern is whether ITNs that are subject to wear and tear under everyday household conditions fail to protect ITN users now that *An. gambiae* mosquitoes are becoming resistant. Modern mosquito nets lack physical durability, and household nets can accrue an average of 12–20 holes during 1–2 years of use (*21*). Net replacement schemes struggle to meet demand at this level of deterioration and attrition. To assess protection conferred

Author affiliations: London School of Hygiene & Tropical Medicine, London, UK (A. Asidi, R. N'Guessan, C. Curtis, M. Rowland); and Centre de Recherche Entomologique de Cotonou, Cotonou, Benin (A. Asidi, R. N'Guessan, M. Akogbeto)

DOI: http://dx.doi.org/10.3201/eid1807.120218

[1]Deceased.

by in-use polyester nets, we compared nets in households of northern Benin, where *An. gambiae* mosquitoes are mostly susceptible to pyrethroids, with nets in households of southern Benin, where *An. gambiae* mosquitoes are mostly resistant (*7*,*16*,*22*). Residents of the selected households were all regular users of nets.

Materials and Methods

Study Sites

Three suburbs (Ladji, Fifadji, and Abomey Calavi) of Cotonou in southern Benin support breeding of mosquitoes of *An. gambiae* M form that is mostly pyrethroid resistant with a high frequency of *kdr* (>90%) (*14*,*22*). Malanville, 800 km north of Cotonou, is situated in an area in which mosquitoes are mainly pyrethroid susceptible, where *An. gambiae* M form mosquitoes show a *kdr* frequency of <0.05 (*7*,*14*).

Selection of Households and Torn Nets

We selected 3–5 households from each site. The criteria for selection were that each house contain a sleeping room with a close-fitting door and a window suitable for fitting a mosquito exit trap and in which occupants possessed ≥1 worn nets under regular use. The points of entry for mosquitoes were through open doors or eave gaps between walls and roofs. Nets were made of polyester, cotton, or nylon and contained holes of various sizes and number. Before inclusion, the nets were subjected to World Health Organization cone bioassays by using a laboratory-susceptible strain of *An. gambiae* to detect pyrethroid residue. Only untreated nets or nets that had lost their insecticide through washing were retained for the study.

Household members gave informed consent to participate in the study and were provided with chemoprophylaxis throughout. The London School of Hygiene & Tropical Medicine and the Benin national ethics committees granted ethics approval.

Mosquito Exit Window Traps

Unidirectional window traps were fixed to window frames for collecting exiting mosquitoes. Each trap consisted of a 30-cm–sided metal frame covered with polyester netting, with 1 side drawn into a funnel to direct mosquitoes into the trap (*23*). The trap was fixed to a plywood sheet that could be fitted to window frames of differing sizes. The traps were placed before dusk and emptied of mosquitoes at 7 AM.

Treatment of Mosquito Nets

Nets were treated with a microencapsulated formulation of lambdacyhalothrin (Icon 10 CS, Syngenta, Basel, Switzerland). The standard rate of 18 mg/m^2 was used.

Mosquito Collection

We conducted the trials during May and June 2008 at the southern sites and during July and August 2008 at the northern site. Rooms of selected houses containing untreated nets were fitted with traps and monitored for 5 consecutive nights to assess baseline mosquito density and blood-feeding and death rates. Nets were then treated with lambdacyhalothrin and monitored for 5 additional nights. Houses that attracted too few mosquitoes during baseline monitoring were dropped. Each morning, mosquitoes were collected from the window traps by mouth aspirator and transferred to paper cups and provided with sugar solution. Indoor resting mosquitoes were then collected from white floor sheets after the windows were sealed off and the rooms were sprayed with a nonresidual pyrethroid. Mosquitoes were identified to species and recorded as blood fed or unfed by microscopy. Scoring of blood-feeding rates was pooled for window trap and room collections. Death rates of the exit trap collections were determined after a 24-hour holding period. *An. gambiae* mosquitoes were identified to species and molecular form by using the method of Favia et al. (*24*) and genotyped for *kdr* by using the method of Martinez-Torres et al. (*25*).

Data Analysis

We assessed the effect of pyrethroid-treated nets on the proportions of *An. gambiae* blood-feeding or killed mosquitoes using a random effects generalized linear mixed model, recording the proportions of female mosquitoes before treatment as the baseline (control) group and the proportions after treatment as the test group. The model comprised 4 independent variables: treatment, number of holes per net, total area of all holes in the net under test, and number of persons in the household. Random effects in the model also accounted for repeated sampling over several days and the number of persons sleeping in the room. Regional differences in the condition of nets and in household size between the sites with resistant and susceptible mosquitoes were analyzed by using the Wilcoxon rank sum test. All statistical analyses were conducted by using STATA 9 software (STATA Corp., College Station, TX, USA).

Results

Baseline Characteristics of Mosquito Nets and Sleepers

Eleven households at the southern sites (where mosquitoes are resistant) and 5 households at the northern site (where mosquitoes are susceptible) participated in the study. Each household contributed 1 sleeping room and 1 net to the study. Numbers of holes per net recorded at the southern and northern sites did not differ ($p = 0.41$) (Table 1). The area of holes per net was significantly

Table 1. Baseline characteristics showing condition of selected mosquito nets in households in northern sites, where mosquitoes are pyrethroid susceptible, vs. southern sites, where mosquitoes are pyrethroid resistant, Benin, 2008*

Variable	Northern site	Southern sites	Difference (95% CI)	p value
Households, no.	5	11	NA	NA
Household members, average no. (range)	2.2 (1–3)	5.1 (2–7)	2.9 (1.4–4.4)	0.025
Holes in nets				
Average no. (range)	10.2 (5–13)	9.5 (5–25)	0.65 (–5.3 to 6.6)	0.41
Average size, cm^2 (range)	28 (11–49)	11 (5–20)	15 (7–21)	0.0013

*NA, not applicable.

smaller for nets from the south (p = 0.0013) (Table 2). Household size in the south was twice that in the north (p = 0.025).

Efficacy of Mosquito Nets Before and After Treatments

During the 2-month trial, 692 *An. gambiae* mosquitoes; 2,271 *Culex quinquefasciatus* mosquitoes; and small numbers of *Mansonia uniformis*, *An. pharoensis*, and *Aedes aegypti* mosquitoes were collected at the southern sites. At the northern site, 1,856 *An. gambiae* mosquitoes, 1,051 *Mansonia* spp. mosquitoes, and small numbers of *An. funestus* and *Ae. aegypti* mosquitoes were collected. Only the malaria vector *An. gambiae* was analyzed further.

The blood-feeding rate of *An. gambiae* mosquitoes under untreated nets was higher in the north (46%) than in the south (20%) (Table 2), probably because of the larger size of holes in nets in the north. At the northern site (susceptible mosquitoes), the odds of blood feeding were lower after treatment than before treatment with or without adjustment for other covariates (adjusted odds ratio 0.34; 95% CI 0.26–0.44; p<0.001) (Table 2). The overall protective effect of treatment was 66% (95% CI 56%–74%). The OR for nets with smaller and larger areas of holes indicated that ITNs provided similar levels of protection against the susceptible mosquito population regardless of the condition of the nets (Table 2).

At the southern sites, where mosquitoes are resistant, we found no evidence that sleeping under a treated net was more protective than sleeping under an untreated net (adjusted odds ratio 1.14; 95% CI 0.73–1.76; p = 0.566) (Table 2). There was no difference in blood feeding rates between nets that had a higher number and nets that had a lower number of holes. Nor was there any difference between nets that had a higher surface area or lower surface area of holes. These findings indicated that regardless of physical condition, treated nets provided no additional protection over that of untreated nets.

Mosquito mortality rates in the exit traps at the northern site (susceptible mosquitoes) were 8% before insecticide treatment of the nets and 70% after treatment. Mosquito mortality rates at the sites where they are pyrethroid resistant were similar before and after treatment of the nets and did not exceed 12% (Figure).

Table 2. Protection against *Anopheles gambiae* s.l. mosquitoes for persons sleeping under in-use mosquito nets before and after treatment with 18 mg/m^2 lambdacyalothrin in houses in northern vs. southern sites, Benin, 2008*

Area, net condition	Treatment of nets	Blood fed, no. (%)	OR (95% CI)	p value	aOR (95% CI)	p value
Northern (pyrethroid-susceptible mosquitoes)	Before	810 (46)	1		1	
	After	1,041 (16)	0.22 (0.18–0.28)	<0.001	0.34 (0.26–0.44)	<0.001
No. holes						
≤10	Before	503 (45)	1		1	
	After	850 (14)	0.20 (0.16–0.27)	<0.001	0.26 (0.20–0.34)	<0.001
>10	Before	307 (48)	1		1	
	After	191 (24)	0.34 (0.23–0.51)	<0.001	0.37 (0.27–0.64)	<0.001
Size of holes, cm^2						
≤15	Before	59 (36)	1		1	
	After	217 (17)	0.38 (0.20–0.73)	0.003	0.38 (0.20–0.73)	0.003
>15	Before	751 (47)	1		1	
	After	824 (16)	0.21 (0.17–0.27)	<0.001	0.21 (0.17–0.27)	<0.001
Southern (pyrethroid-resistant mosquitoes)	Before	268 (20)	1		1	
	After	424 (23)	1.19 (0.81–1.73)	0.37	1.14 (0.73–1.76)	0.57
No. holes						
≤10	Before	111 (18)	1		1	
	After	200 (21)	1.15 (0.55–1.67)	0.28	1.17 (0.62–1.81)	0.31
>10	Before	165 (27)	1		1	
	After	224 (25)	0.89 (0.56–1.42)	0.64	0.89 (0.56–1.41)	0.63
Size of holes, cm^2						
≤15	Before	115 (19)	1		1	
	After	189 (23)	1.31 (0.74–2.36)	0.35	2.59 (1.26–5.37)	0.01
>15	Before	153 (21)	1		1	
	After	235 (23)	1.10 (0.67–1.8)	0.70	1.09 (0.18–1.80)	0.713

*OR, odds ratio; aOR, OR adjusted for condition of nets and household size.

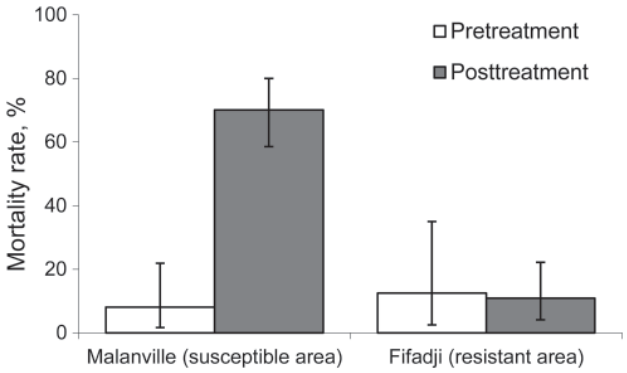

Figure. Death rates of *Anopheles gambiae* s.l. mosquitoes collected in exit traps at Mallanville (where mosquitoes are pyrethroid susceptible) in northern Benin and Fifadji (where mosquitoes are pyrethroid resistant) in southern Benin, 2008. Error bars indicate 95% CIs.

Species, Molecular, and *kdr* Genotyping

PCR identified *An. gambiae* s.s. as the main sibling species at southern and northern sites (Table 3). *An. arabiensis* mosquitoes were present as a minor sibling species at the northern site. The M form of *An. gambiae* mosquitoes predominated at all sites (83%). The frequency of *kdr* was 0.80 in the south and 0.10 in the north.

Discussion

In this comparative study in areas of contrasting pyrethroid resistance and susceptibility, we used vector blood feeding as a surrogate for malaria risk and demonstrated that ITNs lose their capacity to provide protection once *An. gambiae* M form develops pyrethroid resistance of the type found in southern Benin (*16*). These findings clearly show that ITNs in local use fail to protect against *An. gambiae* populations that contain *kdr* resistance at high frequency. The mechanisms of resistance in southern Benin are complex, and metabolic resistance appears to contribute (*14*–*16*). The demonstration of contrasting blood-feeding and survival rates between resistant and susceptible *An. gambiae* mosquitoes in the household trial corroborates findings and predictions from earlier experimental hut studies in southern and northern Benin and confirms the veracity of experimental huts as a tool for predicting protection or control in the home (*16*,*22*).

We chose to use ordinary household nets rather than new or intact nets. Intact nets might have provided barrier protection against resistant and susceptible mosquitoes, but such a trial would not have reflected local reality. Household nets are inevitably subject to wear and tear, and several studies have documented the association between naturally damaged ITNs and mosquito blood-feeding rates. Before the advent of ITNs, Port and Boreham (*26*), in an experimental hut study of bed nets previously used by local Gambians, found a strong correlation between blood feeding and the number and size of holes. More recently, Irish et al. (*27*), in an experimental hut trial of treated nets against pyrethroid-resistant *Cx. quinquefasciatus* mosquitoes, found an association between the proportion of mosquitoes blood feeding and the number of holes in the ITN. Cross-sectional parasite prevalence surveys in Equatorial Guinea showed that children sleeping under intact ITNs were protected against infection with *Plasmodium falciparum* but that the level of protection progressively decreased as the nets' condition deteriorated (*28*). Our study also stratified nets according to condition, and the analysis showed that persons sleeping under ITNs with holes in areas with pyrethroid-resistant mosquitoes had the same risk from mosquitoes as did persons using untreated nets, whereas in areas of pyrethroid susceptibility, the ITNs remained protective regardless of physical condition. As nets inevitably acquire holes over time, the loss of the nets' integrity will be felt most strongly in areas with resistant mosquitoes, and the community will be put at greater risk for malaria.

Campaigns of universal LLIN coverage aim to protect the families least able to afford nets (*29*). With the loss of net integrity over time, malaria transmission will continue across all age groups. Our results predict that mass distribution campaigns of LLINs would benefit populations in areas of pyrethroid susceptibility but are unlikely to control malaria in areas of high resistance. In villages of rural Benin, where pyrethroid resistance in *An. gambiae* mosquitoes is moderate (*kdr* frequency averaging 40%), the regular use of LLINs has had some effect on the prevalence of malaria among children <5 years of age (*30*). We anticipate that in villages with *kdr* frequency >80% that are subject to high rates of malaria transmission, as in the southern provinces (*22*,*31*), the effects on the community of LLINs on malaria would be compromised among families who have poor-quality ITNs.

Sustained protection by any LLIN depends on 2 factors: the rate of loss of insecticide residue from the fibers and the retention of textile integrity. Our research shows

Table 3. Characteristics of *Anopheles gambiae* s.l. mosquitoes collected from study households in southern and northern Benin, 2008*

Location, mosquito resistance	Species		Molecular form of *An. gambiae*		*kdr* genotype			*kdr* frequency
	An. arabiensis	*An. gambiae*	M	S	SS	RS	RR	
Northern, susceptible	5	30	27	3	28	5	1	0.10
Southern, resistance	0	60	56	4	10	3	45	0.80

*Values are no. mosquitoes except as indicated.

that the emphasis placed by the World Health Organization and net manufacturers on developing nets that retain insecticide after recurrent washing is overlooking the role of net durability on effectiveness. A net that retains insecticide after multiple washes or 3 years of use is of no benefit if, before this period, the physical condition of the net and the holes that accumulate mean that in locations with high levels of resistance the net has lost the capacity to protect. During household use, polyester- and polyethylene-based LLINs acquiring holes within the first year and are starting to be discarded after 2 years (*21*,*28*,*32*). LLIN manufacturers need to create new types of fiber or increase the tensile strength to give better resilience against tearing or acquiring holes. Any such product should have a strong commercial advantage.

Resistance capable of undermining the effective use of LLINs is not confined to southern Benin. With the growing coverage of LLINs, the continuing selection of resistance in mosquitoes and its spread to mosquitoes in neighboring provinces and countries is inevitable. Restoring protection of LLINs requires innovation that combines pyrethroids and novel insecticides to which this form of *An. gambiae* mosquitoes shows no resistance.

Acknowledgments

We thank Renaud Govoetchen and Nazaire Aizoun for technical assistance.

All authors shared in the design of the study, which was co-initiated by C.C. before his sudden death in May 2008 and by M.R. A.A. organized and managed the trial and drafted the manuscript. R.N. supervised the study, analyzed the data and co-wrote the manuscript. M.A. revised the manuscript. M.R .supervised the study, analyzed and interpreted the results, and redrafted the final manuscript. All authors read and approved the final manuscript.

The study was funded by the London School of Hygiene & Tropical Medicine (LSHTM) and through a personal grant from C.C. (Chris Curtis Foundation). LSHTM and the Centre de Recherche Entomologique de Cotonou are members of the Pan African Malaria Vector Research Consortium (www.pamverc.org). A.A., R.N'G., and M.R. are supported by the Malaria Centre of LSHTM.

Dr Asidi is a postdoctoral scientist based at the Centre de Recherche Entomologique de Cotonou, Benin. His primary research interest is novel types of insecticide for use on ITNs.

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Address for correspondence: Mark Rowland, Department of Disease Control, London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT, UK; email: mark.rowland@lshtm.ac.uk

Retrospective Evaluation of Control Measures for Contacts of Patient with Marburg Hemorrhagic Fever

Aura Timen, Leslie D. Isken, Patricia Willemse, Franchette van den Berkmortel, Marion P.G. Koopmans, Danielle E.C. van Oudheusden, Chantal P. Bleeker-Rovers, Annemarie E. Brouwer, Richard P.T.M. Grol, Marlies E.J.L. Hulscher, and Jaap T. van Dissel

After an imported case of Marburg hemorrhagic fever was reported in 2008 in the Netherlands, control measures to prevent transmission were implemented. To evaluate consequences of these measures, we administered a structured questionnaire to 130 contacts classified as either having high-risk or low-risk exposure to body fluids of the case-patient; 77 (59.2%) of 130 contacts responded. A total of 67 (87.0%) of 77 respondents agreed that temperature monitoring and reporting was necessary, significantly more often among high-risk than low-risk contacts (p<0.001). Strict compliance with daily temperature monitoring decreased from 80.5% (62/77) during week 1 to 66.2% (51/77) during week 3. Contacts expressed concern about development of Marburg hemorrhagic fever (58.4%, 45/77) and infecting a family member (40.2%, 31/77). High-risk contacts had significantly higher scores on psychological impact scales (p<0.001) during and after the monitoring period. Public health authorities should specifically address consequences of control measures on the daily life of contacts.

In July 2008 in the Netherlands, an imported case of Marburg hemorrhagic fever (MHF) (*1*) was diagnosed in a person after possible exposure in a bat cave in Uganda. MHF is caused by Marburg virus, which belongs to the family *Filoviridae* (*2*,*3*). The main route of transmission is by direct contact with blood or body fluids (*4*). The virus was discovered in 1967 during a laboratory outbreak in Marburg, Germany (*5*,*6*). Apart from this person, since the outbreak in Marburg, MHF has been diagnosed only once outside Africa (*7*).

Because of the high case-fatality rate and propensity for further transmission, a case of MHF is considered to be a public health emergency of international concern and requires prompt intervention to isolate the case-patient and trace and monitor all contacts for early signs of disease. Persons at risk for contracting MHF caused by prior or ongoing contact with an infected person were identified by means of a public health investigation conducted by public health services. The national outbreak response team issued guidelines for classification of these contacts and control measures, including restrictions on leaving the country.

Imported cases of hemorrhagic fever and other severe diseases with the potential to spread among health care workers and the general population have a small, yet realistic chance of occurring in the Western world, as was the situation with Ebola fever and Lassa fever (*8*–*12*). Outbreaks can also originate from other sources, as was the case with Ebola-Reston virus (*13*). Because there are no alternative interventions, such as vaccination or prophylactic treatment, to protect contacts from acquiring MHF, control measures are aimed at early identification of possible case-patients and isolating them from the rest of the population. However, we do not know how persons exposed to MHF respond when confronted with control measures. To date, the consequences of measures to control outbreaks (e.g., monitoring, quarantine) have only been partially investigated for diseases that are not comparable to MHF from the point of view of routes and risk of transmission, e.g., severe acute respiratory syndrome or

Author affiliations: National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (A. Timen, L.D. Isken, M.P.G. Koopmans); Elkerliek Hospital, Helmond, the Netherlands (P. Willemse); Atrium Medical Centre, Heerlen, the Netherlands (F. van den Berkmortel); Public Health Service Brabant-Zuidoost, Helmond (D.E.C. van Oudheusden); Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands (C.P. Bleeker-Rovers, R.P.T.M. Grol, M.E.J.L. Hulscher); Elisabeth Hospital, Tilburg, the Netherlands (A.E. Brouwer); and Leiden University Medical Centre, Leiden, the Netherlands (J.T. van Dissel)

DOI: http://dx.doi.org/10.3201/eid1807.101638

influenza (*14*–*17*). Evidence is needed to determine the effectiveness of follow-up procedures for MHF contacts.

To evaluate the consequences and the psychological effect of control measures on contacts' daily life, a retrospective cohort study (including serologic testing) was undertaken among 130 contacts of the person in the Netherlands in 2008 who acquired MHF. Contacts were categorized as high-risk or low-risk on the basis of their exposure history. We describe criteria to optimize the effect of control measures and provide proper care to contacts exposed to a person-to-person transmissible virus with the potential to cause severe disease.

Methods

This study was determined to be part of the public health response to the imported case of MHF and follow-up of contacts. Therefore, explicit ethical evaluation was not necessary.

Case-Patient Description

On July 2, 2008, after returning from a visit to Uganda from June 5 through June 28, a 41-year-old woman showed development of chills and high fever. She was admitted to hospital A on July 5. Initially, hemorrhagic fever was not included in the differential diagnosis and she was placed in a general ward among other patients, without specific contact precautions. Because she later showed clinical deterioration, liver failure, and tendency to hemorrhage, hemorrhagic fever was suspected and she was transferred to hospital B on July 7. In hospital B, she was placed in strict isolation in accordance with guidelines prescribed for pathogens belonging to Hazard Group 4 (*18*). We placed the patient in a single room with negative air pressure ventilation and an anteroom for 2 reasons. First, although evidence for airborne transmission of MHF in humans has not been documented, transmission by aerosols has been demonstrated in animal models (*7*). Second, the patient was likely to undergo aerosol-generating procedures (e.g., endotracheal intubation) while in the latter stages of illness when viral loads in body fluids were expected to be high.

On July 10, the diagnosis of MHF was confirmed; the next day, the patient died. A complete case history and the public health response have been reported (*1*).

Control Measures

The outbreak response team formulated measures for follow-up of contacts considered to be at risk for exposure. Measures were based on preexisting national and international guidelines on management of hemorrhagic fever caused by filoviruses (*18*–*20*). The patient was considered to be potentially infectious from the onset of fever (July 2) until death (July 11). The period of monitoring contacts was set at 21 days after the last contact with the patient or patient body fluids. The public health service traced the contacts in the community. The hospital hygienist and occupational physician, and attending physicians were responsible for in-hospital contacts. Contacts were provided with written instructions (Table 1).

Contacts classified as at high risk for contracting the disease (i.e., had unprotected contact with the patient or her body fluids) were asked to measure their temperature twice a day and report it to the health care provider. Furthermore, they were prohibited from leaving the country and were told to report any intention to leave to the public health authority. Contacts were asked to notify the assigned health care provider immediately if they had fever (body temperature ≥38°C measured twice at least 12 hours apart) or any abnormal symptoms (e.g., vomiting, headache, abdominal pain, diarrhea, jaundice). Contacts who had handled the patient or her body fluids while carrying out strict isolation measures were perceived to be at low risk for exposure. They were asked to measure their temperature twice a day, but only to report a temperature ≥38°C. This group was strongly encouraged not to travel abroad.

Study Population

A retrospective cohort study involving the 130 contacts was conducted by using an online questionnaire. High-risk contacts (n = 64) included household contacts, health care providers at the general practice, those involved in patient care at hospital A (nurses, physicians, and laboratory workers), patients sharing the same hospital ward in hospital A, and cleaning staff. Low-risk contacts (n = 66) included health care and laboratory workers at hospital B who had all taken appropriate personal protective measures. Data were collected from December 2008 through February 2009, which was 5–7 months after possible exposure. At the same time, serologic testing was conducted to assess asymptomatic transmission.

Variables and Instruments

Questionnaire

We recorded personal characteristics of respondents and any symptoms during the monitoring period. The questionnaire (62 questions) addressed understanding of control measures, clarity of instructions, reported compliance with measures, and perceived interference with daily life (e.g., restrictions on social life, difficulties with control measures, extra costs incurred, and fear of becoming infected). To develop questions regarding interference and reported compliance, we drew from the current literature on the effect of restrictive measures during outbreaks (*14*,*15*,*21*).

Questions regarding clarity of instructions and understanding measures were based on parts of the

Table 1. Instructions for contacts of a person with Marburg hemorrhagic fever, by risk contact group, the Netherlands, 2008*

Characteristic	Instruction
You have been assigned to the high-risk category	You have shared the household (or the ward) with the patient. You have cared for the patient in the hospital without wearing PPE. You had or might have had unprotected contact (without PPE) with the blood or body fluids of the patient.
The following restrictions have been imposed on you	Remain in the neighborhood of your home address during the monitoring period of 3 weeks after last possible contact (date). Stay in contact with the health care provider you have been assigned (the public health service, the hospital hygiene specialist, or the occupational medicine specialist). Do not leave the country. Cancel or postpone a holiday trip abroad.
Control measures during monitoring period	Inform your health care provider if you use temperature-lowering medication. Measure your temperature in the morning and evening. Use your own thermometer (one that is not to be used by others) and write down your temperature accurately. Disinfect the thermometer with 70% alcohol after every use and wash your hands with soap and water. Contact your health care provider daily and provide him or her with information about your health and temperature. If you have a fever (2 consecutive temperature measurements ≥38°C 12 h apart), vomiting, headache, stomach ache, diarrhea, jaundice, or cough, immediately contact your care provider. Stay at home and restrict all contacts with others until further instructions from your health care provider. Only use your own toilet.
You have been assigned to the low-risk category	You cared for the patient (using adequate PPE) while she was admitted to the hospital and in accordance with a strict isolation protocol. You had contact with blood or body fluids of the patient while using effective PPE.
Control measures during monitoring period	Inform your health care provider if you use temperature-lowering medications. You are strongly advised not to leave the country during the monitoring period of 3 weeks after the last possible contact with the patient or patient body fluids (date). Measure your temperature daily in the morning and evening. Use your own thermometer (one that is not to be used by others) and write down your temperature accurately. Disinfect the thermometer with 70% alcohol after every use and wash your hands with soap and water. If you have a fever (2 consecutive temperature measurements ≥38°C 12 h apart), immediately contact the health care provider you have been assigned (public health service, the hospital hygiene specialist, or the occupational medicine specialist). Stay at home and restrict all contacts with others until further instructions from your care provider. Only use your own toilet.

*PPE, personal protective equipment.

Consumer Quality Index Instrument of the Netherlands Institute for Health Services Research (Rotterdam, the Netherlands), which was derived from the Consumer Assessment of Healthcare Providers and Systems Survey developed and funded by the US Agency for Health Care Research and Quality (Rockville, MD, USA) (*22*). Depending on the type of question, respondents were asked to answer either yes or no questions, or to choose an option on Likert scales of 1–5 (1, completely disagree; 2, disagree; 3, neutral; 4, agree; and 5, strongly agree or 1, never; 2, seldom; 3, sometimes; 4, often; 5, always).

Impact of Event Scale

To evaluate stress levels, we used the Revised Impact of Event Scale (IES-R; Dutch version), a 22-item international instrument designed to measure situations in life that are perceived as stressful (*23*). The instrument was divided into 3 subscales: intrusion (e.g., recurrent thoughts); avoidance (e.g., avoiding reminders of the event); and hyperarousal (e.g., concentration and sleeping problems). Each subscale contained questions derived from the Diagnostic and Statistical Manual of Mental Disorders criteria (4th edition) for posttraumatic stress disorder (*24*). Items were scored on a Likert scale of 0–4 (0, not at all; 1, a little bit; 3, quite a bit; 4, extremely), which added up to a maximum score of 88. A score ≥20 was considered an indicator of posttraumatic stress disorder (*14*,*15*). We assessed recalled stress during the monitoring period (IES during) and persisting stress during the 7 days before the completion of the questionnaire (IES after).

Statistical Analysis

Data were analyzed by using SPSS version 18 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated. Means and SDs were calculated for answers given on the Likert scale. Results were stratified by type of exposure risk (i.e., high or low risk for disease transmission). Differences in proportions were calculated by using the χ^2 test. Differences in means were calculating by using the Student *t* test. A p value <0.05 was considered significant.

We constructed overall scales. The compliance with measures scale (Cronbach α 0.71) included 4 questions regarding temperature monitoring, temperature reporting, and prohibition on travel. The interference scale (Cronbach

α 0.64) included 11 questions on perceived restrictions on social life, difficulties with control measures, extra costs, and anxiety of contacts and their families. The clarity of instructions scale (Cronbach α 0.82) included 5 questions on explicitness, completeness, unambiguity, confusion, and redundancy of the provided instructions. The overall scale for understanding of measures (items regarding awareness of the measures and their rationale) proved invalid and was not used in further analysis.

To determine which variables influenced stress levels during the monitoring period, we developed a linear regression model by using the IES during monitoring as a dependent variable and personal characteristics (sex, education, age), risk level, clarity of instructions scale, compliance scale, and interference scale as independent variables. The same model was constructed by using the IES after the monitoring period as the dependent variable.

Serologic Testing

Serologic testing was performed by using an immunofluorescence antibody (IFA) assay with blood samples collected from contacts 5–7 months after the monitoring period. IFA test slides were prepared at the Bernhard Nocht Institute for Tropical Medicine (Hamburg, Germany) by using the patient strain. Laboratory methods have been reported (*1*).

Results

Response

Of 130 eligible participants, 78 (60.0%) completed the questionnaire. One person provided systematically inconsistent answers and was excluded, which left 77 respondents (59.2%) for statistical analysis. The response rate was 70.3% (45/64) in the high-risk group and 48.5% (32/66) in the low-risk group (Table 2). Of the 77 respondents, 46 (59.7%) were female, 44 (57.1%) did not have children in the household, and 11 (14.3%) lived alone. Mean age was 38 years in the high-risk group and 43 years in the low-risk group (this difference was not significant). The high-risk group had more women (33/45, 73.3%) than the low-risk group (13/32, 40.6%) (p = 0.004) and had a lower education level (Table 2). Respondents were comparable to nonrespondents with respect to sex (M:F ratio 40.0%:60.0% vs. 42.0%:58.0%). During the monitoring period, nonspecific symptoms (those commonly occurring during the prodromal phase of hemorrhagic fever syndromes, e.g., headache, malaise, fatigue) (*2*) developed occasionally in some contacts, but no significant differences were observed between the risk groups.

Table 2. Characteristics and self-reported signs and symptoms of persons who had contact with a Marburg hemorrhagic fever patient, the Netherlands, 2008*

Characteristic	High-risk contact, n = 45, no. (%)	Low-risk contact, n = 32, no. (%)	p value†
Sex			**0.004**
F	33 (73.0)	13 (40.0)	
M	12 (37.0)	19 (60.0)	
Age, y			0.127
<25	13 (29.0)	2 (6.2)	
26–35	9 (21.0)	8 (25.2)	
36–45	10 (22.0)	10 (31.2)	
46–55	10 (22.0)	11 (34.3)	
56–65	3 (6.0)	1 (3.1)	
Education			**0.002**
Secondary	8 (18.0)	1 (3.0)	
Vocational	17 (37.0)	4 (12.0)	
Higher professional	15 (34.0)	22 (70.0)	
University	5 (11.0)	5 (15.0)	
Health status			**0.047**
Excellent	11 (24.0)	15 (47.0)	
Very good	19 (42.0)	6 (18.7)	
Good	15 (34.0)	11 (34.3)	
Reported conditions during monitoring period			
Temperature ≥38°C	2 (4.4)	2 (6.2)	0.901
Headache	17 (37.7)	8 (25.0)	0.064
Myalgia	5 (11.1)	1 (3.1)	0.407
Malaise	13 (29.0)	10 (31.2)	0.513
Nausea	6 (13.3)	2 (6.2)	0.429
Abdominal pain	6 (13.3)	3 (9.3)	0.482
Fatigue	11 (24.0)	6 (18.5)	0.222
Vomiting	1 (4.5)	0	0.399
Diarrhea	6 (13.3)	1 (3.1)	0.247

*High-risk contact, unprotected contact with the patient or her body fluids; low-risk contact, contact with the patient or her body fluids while following strict isolation measures. **Boldface** indicates statistical significance (p<0.05).
†By χ^2 test.

Understanding Measures

Of the 77 respondents, 60 (77.9%) were aware of the request not to leave the country, and 17 (22.1%) chose the neutral option. Of 60 contacts aware of the request, 54 (90.0%) in the high-risk group agreed with this request (p = 0.04). Reasons given for negative answers were no direct contact with the patient and thus no risk (n = 2), imperative reasons to leave the country such as illness in the family (n = 2), travel restrictions were meant for others, not for me (n = 1), and borders do not stop diseases (n = 1). All respondents were aware of the need to measure temperature twice a day, 67 (87.0%) agreed with the necessity of measuring and reporting temperature, and 7 (9.1%) had no opinion. Not feeling ill was the reason for disagreement in the remaining 3 (3.8%) respondents. Only 58 (75.3%) of the 77 respondents correctly identified the rationale of temperature monitoring. Respondents in the high-risk group agreed more often than their low-risk counterparts with the necessity of daily measuring and reporting for temperature (mean ± SD of agreement in the high-risk group 4.7 ± 1.0 vs. 1.6 ± 1.3 in the low-risk group) (p<0.001). Written instructions with detailed information on the control measures were received by 61 (79.2%) of 77 respondents. Of these 61 respondents, 45 (73.7%) found this information to be clear, 40 (65.6%) complete, 37 (60.6%) unequivocal, 4 (6.5%) confusing, and 3 (5.0%) redundant. There were no significant differences between the risk groups.

Compliance with Measures

Of 45 respondents who were prohibited from traveling (high-risk group), 17 (37.7%) had planned a holiday trip abroad, of whom 12 (70.6%) cancelled the holiday trip during the surveillance period, and 1 (5.9%) postponed the trip. Two (11.8%) high-risk contacts were already abroad when the diagnosis in the index case was made and 2 (11.8%) other contacts left the country a few days before the end of the surveillance period, despite prohibition on travel. Risk management in these persons, including communication with government authorities in countries to which they traveled, has been reported (*1*). Temperature was monitored twice a day by 62 (80.5%) of 77 persons, and 75 (97.4%) of 77 had a thermometer at their immediate disposal. Strict compliance with daily temperature monitoring decreased from 80.5% (62/77) in week 1 to 66.2% (51/77) in week 3. Differences per risk group per week in reported compliance with temperature monitoring and reporting are shown in Table 3.

Interference of Measures with Daily Life

The prohibition on leaving the country was perceived as difficult by 18 (23.4%) of 77 respondents; 11 (14.3%) perceived serious restrictions on their social life, and 24 (31.2%) reported feeling stressed on a regular basis during monitoring. Daily temperature monitoring was believed to be troublesome by 25 (32.5%) of 77. Extra costs were involved for 19 (24.6%) of the contacts. The questionnaire did not ask for specification of costs incurred. Of 47 health care workers among respondents, 13 (27.6%) intensified adherence to infection prevention guidelines at work during the monitoring period (mean ± SD intensified adherence in the high-risk group 1.3 ± 0.4 vs. 1.03 ± 0.2 in the low-risk group) (p = 0.006). Sustained and intensified adherence to infection prevention at work after the monitoring period ended was reported by 10 (21.3%) of 47.

Being identified as a contact caused anxiety in respondents, as reflected in the high percentage who were afraid of contracting MHF (45/77, 58.4%), those who were concerned about infecting other members of their household (31/77, 40.3%), or those who were afraid that a colleague might have been unknowingly infected by the index patient (35/77, 45.5%). Of 77 respondents, 28 (36.4%) reported that their family members were disturbed by control measures. Furthermore, 31 (40.3%) of 77 reported that their family members expressed anxiety about becoming infected, and 25 (32.5%) of 77 reported that their partners were disturbed by restrictive control measures. The results on the overall interference scale were compared and showed that control measures caused more interference with daily life (lower

Table 3. Compliance with temperature monitoring and reporting in persons who had contact with a person with Marburg hemorrhagic fever, the Netherlands, 2008*

Variable	High-risk group score, mean (SD)	Low-risk group score, mean (SD)	p value†
Temperature monitoring week			
1	4.87 (0.63)	4.25 (1.16)	0.004
2	4.87 (0.63)	3.84 (1.30)	<0.0001
3	4.82 (0.68)	3.34 (1.54)	<0.0001
Temperature reporting week			
1	4.73 (1.01)	1.56 (1.37)	<0.0001
2	4.73 (1.01)	1.50 (1.34)	<0.0001
3	4.71 (1.01)	1.50 (1.34)	<0.0001

*Answers were given on a 5-point Likert scale, according to the following categories: 1, never; 2, seldom; 3, sometimes; 4, often; 5, always. High-risk contact, unprotected contact with the patient or her body fluids; low-risk contact, contact with the patient or her body fluids while following strict isolation measures.

†By 2-sample Student *t* test assuming unequal variances. Because of the relatively large sample sizes of 45 and 32 and data that consisted of scores from 1 through 5 with the indicated SDs, this test is justified by the central limit theorem and because the t distribution with 75 = 45 + 32 – 2 df is almost identical with that of a normal distribution. All p values were statistically significant (p<0.05).

mean ± SD) in the high-risk group (mean ± SD 1.5 ± 0.3 vs. 1.8 ± 0.2; p<0.001) than in the low-risk group.

Impact of Event Scale

Means were calculated on the basis of the answers on the IES and subscale domains (intrusion, avoidance, and hyperarousal). Overall psychological distress was measured by the mean ± SD score on the IES during monitoring (2.8 ± 2.6, range 0.2–10) and 5–7 months after monitoring 0.9 (0.9 ± 1.7, range 0–9.5; p<0.001). Despite the low overall mean score, high individual scores were observed (Figure). On the subscale domains, the mean scores were higher during the monitoring period than after it, and the highest scores were reported in the high-risk group.

In the linear regression model, the score on the interference scales was a predictor of the score of the IES during monitoring, which measured the psychological effect of this event (p<0.001). After monitoring ended, the remaining psychological effect measured by the IES score was influenced by the degree of interference (p<0.001) and level of education (p = 0.015); persons with a lower education level reported more psychological consequences (Table 4).

Serologic Testing

IFA serologic testing was conducted in 85 (65.4%) of 130 participants, which represented 50 (78.1%) of 64 in the high-risk group and 35 (53.0%) of 66 in the low-risk group. All serum samples were negative for IgG and IgM against Marburg virus.

Discussion

Being at risk for acquiring MHF had a measurable psychological effect and personal consequences (such as restrictions on social life) on respondents in both risk contact groups. We will discuss implications for persons who have to adhere to control measures, discuss the psychological effect of these measures, and reflect on the prerequisites for applying control measures.

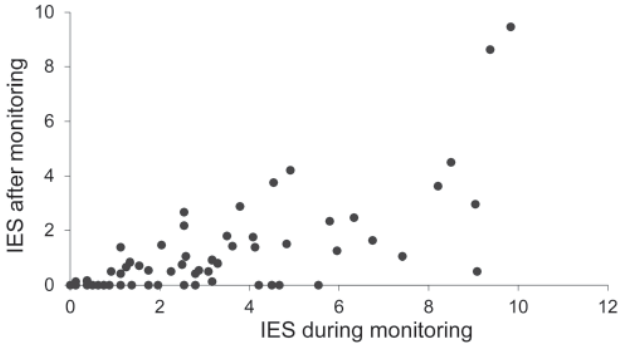

Figure. Distribution of individual scores on the impact of event scale (IES) during and after (a 7-day period before completion of a questionnaire) the monitoring period among contacts of the person with Marburg hemorrhagic fever, the Netherlands, 2008. Each circle indicates 1 person. A higher score indicates a higher level of stress.

Compliance with control measures was reported as difficult to include in daily life, a finding consistent with reports concerning a patient with imported Lassa hemorrhagic fever (*12*,*25*). Some practical problems need to be addressed to facilitate compliance with temperature monitoring and prohibitions on leaving the country. Problems were related to comprehensiveness of measures. Some respondents had already paid for a holiday trip and subsequently had to cancel it. Increased costs that persons incurred imply that compensation policies (e.g., insurance) should be explained or made available in preparedness plans (*26*). Practical constraints related to temperature monitoring should be averted by making use of online registration systems and modern technologies to contact persons and facilitate communication with health care providers.

Although temperature monitoring remains the primary method of detecting onset of fever and enabling follow-up of contacts during the incubation period (*6*), appropriateness of once a day vs. twice a day monitoring is a disputed issue. In accordance with guidelines in the Netherlands (*19*) and the Centers for Disease Control and Prevention Interim Guidance (*20*), we implemented twice a day temperature monitoring. In outbreak situations, once a day monitoring is usually followed. Although twice a day monitoring is not an evidence-based recommendation, we applied it to be able to discriminate between a single episode of fever and sustained fever needing follow-up. Given our experience, twice a day monitoring is appropriate for management of high-risk contacts of a single case-patient or few patients with imported cases, but might not be feasible in every outbreak situation.

Our study shows that control measures had a substantial psychological effect on contacts and members of their household. By quantifying the recalled level of stress at 2 time points, we demonstrated that stress-related complaints persisted for a longer period, thus enlarging the body of evidence built on the basis of previous outbreaks (*14*,*15*,*27*–*30*). We found the interference scale score to be the main predictor of perceived stress, suggesting that the disturbance experienced in daily life determines the magnitude of the psychological effect.

In the light of these findings, what should the prerequisites be for applying control measures? Public health authorities in charge of crisis management need to be aware of difficulties and stress-related issues experienced by persons to whom the measures apply and address them systematically in the crisis guidelines. Apart from overcoming practical barriers, public health authorities should address the psychosocial well-being of persons being monitored and their family members. Although health

Table 4. Linear regression model of scores on Impact of Event Scale during and after the monitoring period in persons who had contact with a person with Marburg hemorrhagic fever, the Netherlands*

Variable	IES score during monitoring		IES score after monitoring	
	B (SE)	p value	B (SE)	p value
Constant	4.73 (0.75)		2.62 (0.59)	
Sex	0.11 (0.14)	0.446	0.10 (0.11)	0.349
Education	–0.14 (0.08)	0.103	–0.17 (0.06)	**0.015**
Age	0.02 (0.05)	0.714	0.04 (0.04)	0.372
Risk category	–0.06 (0.16)	0.742	–0.06 (0.13)	0.663
Clarity of instructions scale†	–0.02 (0.08)	0.864	–0.03 (0.06)	0.678
Compliance scale‡	0.10 (0.08)	0.296	–0.05 (0.06)	0.469
Interference scale§	–2.19 (0.25)	**<0.001**	–1.08 (0.19)	**<0.001**
R^2	0.601		0.437	

*IES, impact of event scale; B, estimate of regression coefficient (a negative estimate indicates a negative association of the independent variable with the dependent variable); R^2, proportion of variance in the dependent variable accounted for by the model. **Boldface** indicates statistical significance ($p<0.05$).
†Explicitness, completeness, unambiguity, confusion, and redundancy.
‡Twice a day temperature monitoring, temperature reporting, canceling trip, and postponing trip.
§Difficulties with monitoring, extra costs, fear of becoming infected, fear of infecting household members, household members fearing infection, fear that colleagues might be infected, restrictions in social life, tensions caused by the measures, tensions experienced by partners, and tensions experienced by children in the household.

care in contacts is focused mostly on early identification of somatic symptoms, concerns, anxiety, and stress experienced by those involved are also crucial. Guidelines should emphasize the need for individual support to assess and manage stress and to address questions about personal risks (*29*).

Finally, occasional incidents of noncompliance with the prohibition on leaving the country have policy implications, which make intergovernmental collaboration necessary. Restricting freedom to travel during the incubation period of persons with high-risk exposure to a transmissible pathogen obliged by international health regulations to be reported requires effective legislation, an issue that should ideally be dealt with before such an incident occurs. Although voluntary compliance, based on effective communication and trust in the authorities, is the most appropriate approach (*31*), should compulsory means be necessary in the Netherlands, emergency legal provisions are now in place to impose travel restrictions and to monitor the health status of contacts who have been exposed to certain pathogens.

Despite being sent reminders, the response rate of participants was only 60.0%. This limits interpretation of results because motives for noncompliance remain unknown. As in most retrospective studies, we also need to acknowledge the chance of recall bias, which might influence recollection of experiences. Another limitation was inherent to the study design, which required answers only from contacts. Thus, consequences of control measures on members of the household could only be assessed indirectly. Despite the limitations of IFA (*32*), serologic findings strongly suggest that none of the contacts who provided a blood sample had become asymptomatically infected.

The strengths of this study are 1) systematic evaluation of compliance with measures and interference of measures with daily life, and 2) quantification of psychological effects of these measures during and after this major event, which had received much attention in the media. We reinforce the hypothesis that psychological symptoms may persist longer. Public health authorities need to be aware of the immediate and long-term effects that measures can have on persons being monitored.

Acknowledgments

We thank H.P.J. Daemen, P.H.S. Brabant-Zuidoost, and P. Grijpink for contributions to the study; M. van der Velde and J. Ferreira for statistical analysis; J.E. van Steenbergen for critical comments on the manuscript; and J. Box and D. Lees for editorial assistance.

This study was partially supported by a grant from the Dutch Research Foundation (ZonMW).

Dr Timen is head of the Department of Preparedness and Response at the Centre for Infectious Diseases of the National Institute of Public Health and the Environment (RIVM), Bilthoven, the Netherlands. Her main research interest is the public health response to outbreaks and threats.

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Address for correspondence: Aura Timen, Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), PO Box 1, 3720 Bilthoven, the Netherlands; email: aura.timen@rivm.nl

Validity of International Health Regulations in Reporting Emerging Infectious Diseases

Michael Edelstein, David L. Heymann, Johan Giesecke, and Julius Weinberg

Understanding which emerging infectious diseases are of international public health concern is vital. The International Health Regulations include a decision instrument to help countries determine which public health events are of international concern and require reporting to the World Health Organization (WHO) on the basis of seriousness, unusualness, international spread and trade, or need for travel restrictions. This study examined the validity of the International Health Regulations decision instrument in reporting emerging infectious disease to WHO by calculating its sensitivity, specificity, and positive predictive value. It found a sensitivity of 95.6%, a specificity of 38%, and a positive predictive value of 35.5%. These findings are acceptable if the notification volume to WHO remains low. Validity could be improved by setting more prescriptive criteria of seriousness and unusualness and training persons responsible for notification. However, the criteria should be balanced with the need for the instrument to adapt to future unknown threats.

The great influenza pandemic of 1918 and the increase in HIV/AIDS are 2 striking examples of the devastation and profound effect on human societies caused by emerging infectious diseases (EIDs) (*1*). The Institute of Medicine defines EIDs as "new, re-emerging, or drug-resistant infections whose incidence in humans has increased or whose incidence threatens to increase in the near future" (*2*). EIDs are a global phenomenon, with hotspots from which EIDs are more likely to appear, concentrated in low-latitude developing countries (*3*). EIDs are probably underreported, particularly in areas which have hotspots and also weak surveillance systems (*4*). A study in 2008 by Jones et al. reported 335 EIDs during 1940–2004 (*3*).

The purpose of the 2005 International Health Regulations (IHR) is to "help the international community prevent and respond to acute public health risks that have the potential to cross borders and threaten people worldwide" (*5*). This purpose includes development of an international reporting system, under which member states have a duty to report to the World Health Organization (WHO) "all events which may constitute a public health emergency of international concern" (*5*). These events are not limited to communicable diseases and can include contaminated food, chemical contamination of products or the environment, release of radionuclear material, or other toxic release (*6*). Events are reported to WHO by designated national focal points (NFPs) in each member state. WHO has designed a decision instrument contained in Annex 2 of the 2005 IHR (*7*) to assist with the notification process on the basis of an algorithm comprising 4 main criteria: the event has a serious public health effect, the event is unusual or unexpected, there is a major risk for international spread, and there is a major risk for international travel or trade restrictions. At least 2 of the criteria must be satisfied for an event to be notifiable.

An IHR expert committee suggested regular evaluations of the notification process (*8*). However, the only published evaluation of the Annex 2 decision instrument is a reliability study that analyzed NFPs notification concordance (*9*). This study also reported a sensitivity of 80% (on the basis of 5 events) and a specificity of 50% (on the basis of 4 events). Although the study reported a high reliability, the number of events was too low to adequately assess the sensitivity and specificity of the decision instrument. A

Author affiliations: London School of Hygiene and Tropical Medicine, London, UK (M. Edelstein, D.L. Heymann); Chatham House, London (D.L. Heymann); European Centre for Disease Control, Stockholm, Sweden (J. Giesecke); and Kingston University, London (J. Weinberg)

DOI: http://dx.doi.org/10.3201/eid1807.111608

2008 WHO technical report on Annex 2 (*10*) mentions a 2006 workshop assessing the decision instrument validity and finding a sensitivity of 100% and a specificity of 55% on the basis of 10 events. There were no details on the methods used and the study results were not published.

The aim of this study was to evaluate the predictive validity of the Annex 2 decision instrument. We focused on EIDs by applying screening test evaluation methods to the IHR Annex 2 decision instrument and estimated its sensitivity, specificity, and positive predictive value (PPV).

Methods

The sensitivity, specificity, and PPV of the Annex 2 decision instrument were calculated by asking an investigator to decide whether each event in a series of historical EID events would have been reported to WHO by using the criteria of the instrument. A panel of 3 internationally recognized EID and IHR experts, who were independent of the notifying investigator, was then asked whether each event was truly of international public health concern. The sensitivity, specificity, and PPV of the decision instrument were then calculated by cross-tabulating the outcome of the notification process and the true outcome of each event (taken as the expert panel consensus decision) in a 2 × 2 table.

The EID events used were sampled systematically from the list of 335 EID events identified by Jones et al. (*3*), starting from the most recent and going back until the required sample size was reached. The study required 160 events to have CIs that did not exceed 10% on each side of the point estimate of sensitivity and specificity if sensitivity was 90%, specificity was 55%, and 40% of events were truly of international public health concern. These values were chosen on the basis of the best available information (*9*,*10*). The IHR Annex 2 decision instrument was used to decide whether each EID event fulfilled the notification criteria. The decision was based on the information available in the references for each EID event provided in the original report by Jones et al. (*3*).

To emulate real-life conditions, the investigator used only information available at the time of event occurrence. Each criterion was answered by yes or no, and ≥2 positive answers classed the EID event as notifiable, according to WHO guidance. To establish the true outcome for every EID event, each expert had to give an opinion on 4 statements: the public health effect of the event was serious; the event was unusual or unexpected; the event spread internationally; and the event led to travel or trade restrictions. The 4 statements were derived from the IHR Annex 2 criteria, but were retrospective and ascertained the a posteriori outcome of each EID event. A Likert scale was used to score each statement with scores from 1 (strongly disagree) to 5 (strongly agree).

Experts based their decisions on their opinion and knowledge and on a supplied information sheet for each event. They were blinded to the notification outcome of each EID event and assessed each event independently. The opinion on each statement of each event for each expert was converted to a numerical score from −2 to +2 (Table 1), which was then summed to give an overall value for each statement and 4 values per EID event. For each statement, an overall positive score was considered a consensus agreement with the statement, and an overall negative score was considered a consensus disagreement with the statement. A null score was considered a failure to agree on that criterion. Events with ≥2 agreed statements were considered to be of international public health concern. Events with ≤1 agreed statement and ≥1 disagreed statement were considered to be of no international public health concern. Events for which there was 1 agreed statement and for which no agreement could be reached on 3 statements were not used in the study.

Statistical analysis was performed by using Stata version 11 (StataCorp LP, College Station, TX, USA). A description was made of the distribution of events according to WHO region, type of pathogen, and type of event. We calculated the notification rate; the prevalence of EID events of international public health concern according to the expert panel; and the distribution of these events by type of pathogen, WHO region, and type of event. Sensitivity, specificity, PPV, and CIs of the decision instrument were then calculated. Concordance and its association with type of event, type of pathogen, and WHO region were calculated by using logistic regression. Concordance for each of the 4 criteria was also calculated. An intraclass correlation coefficient (*11*) was calculated for the combined score allocated by each expert (aggregated scores of all 4 criteria for each event, which provided a measure of overall concern; possible score of 20) to each EID event.

The appropriateness of the consensus-building method was tested by translating the judgment of each expert panel member into a binary scoring system, in which for each criteria, a score of 4 or 5 would translate to "I agree" and a score of 1, 2, or 3 would translate to "I disagree." This process enabled identification of which EID events experts individually considered to be of public health concern. EID events with ≥2 criteria agreed with signifying international public health concern. Agreement levels between individual experts and the consensus were then calculated.

Results

Of 204 identified EID events, 13 were not eligible because they did not fit the definition of an EID or were duplicates. Sixteen events were discarded because of insufficient information, leaving 175 (92%) of 191 eligible events to be analyzed. Their characteristics are summarized

Table 1. Values allocated to each opinion for consensus building for emerging infectious diseases reported to WHO*

Opinion	Value allocated
Strongly disagree	−2
Somewhat disagree	−1
Neither agree nor disagree	0
Somewhat agree	+1
Strongly agree	+2

*WHO, World Health Organization.

in Table 2. A total of 124 (70.9%) of 175 events fulfilled ≥2 of the 4 decision instrument criteria according to the notifying investigator and should have been reported to WHO, according to the Annex 2 decision instrument. No EID event was withdrawn from the study because of failure of the expert panel to agree. Of the 175 EID events assessed by the expert panel, 46 (26.3%) were deemed to be of international public health concern. Characteristics of these 46 events are shown in Table 3.

Of 46 EID events of international public health concern, 44 would have been reported by using the Annex 2 decision instrument (sensitivity 95.6%; 95% CI 89.8%–100%). Of the 129 EID events that were not of international public health concern, 80 would still have been reported by using the Annex 2 decision instrument (specificity 38%, 95% CI 29.6%–46.3%; PPV 35.8%; 95% CI 27.1%–43.9%).

The overall concordance rate between notification decision and international public health concern was 53.1% (95% CI 45.7%–60.5%). The concordance rates for the 4 criteria of seriousness, unusualness, international spread, and travel and trade restrictions were 49.7% (95% CI 42.3%–57.1%), 58.3% (95% CI 51.0%–65.6%), 81.1% (95% CI 75.3%–86.9%) and 96% (95% CI 93.1–98.9), respectively. There was no strong evidence that the type of pathogen, type of event, or WHO region was associated with concordance (Table 4).

The intraclass correlation coefficient for assessing the agreement level for overall public health concern for each event, by using an aggregated score of 20, was 0.68 (95% CI 0.60–0.74). After simplifying the scores to obtain a judgment for each EID event for each expert, the agreement levels for each panel member compared with those of the consensus were 76.5%, 84.6%, and 85.7%, respectively.

Discussion

The IHR Annex 2 decision instrument has a high sensitivity (95.6%; 95% CI 89.8%–100%) but a low specificity (38%; 95% CI 29.6%–46.3%). These figures are consistent with previous anecdotal evidence (*9*,*10*). In this situation, trading specificity for high sensitivity is desirable because missing events of international public health concern would have serious consequences and would outweigh benefits of a lower volume of false-positive results. A low specificity is not a major concern as long as the volume of notification is low (*9*), and currently there is "little evidence that Annex 2 is being frequently or routinely used by State Parties in the assessment of events" (*12*). A low specificity could become problematic if the volume of events reported through Annex 2 increased. The low specificity would result in an increase in false-positives results and increased costs associated with the notification process and determination of serious events.

The low specificity is reflected in a PPV of 35.8%. The calculated PPV could be underestimated for 2 main reasons. First, the prevalence of events identified as being of international public health concern might not reflect the prevalence of events truly reported to WHO. Second, in the current study, all EID events selected were submitted to the decision instrument, regardless of personal judgment. In real life conditions, events least likely to be of international public health concern would have been excluded even before being submitted to the decision instrument, which would increase the prevalence of events of international public health concern in events submitted to the decision instrument and consequently the PPV.

The specificity estimate was lower than that in 2 other evaluations (*9*,*10*) (38% vs. 50% and 55%, respectively). Although our estimate could be a more accurate reflection of the instrument specificity, it could also be an underestimate. Because instrument criteria are quite flexible and subject to interpretation, it is possible to reach a decision to report an event in which the likelihood of it becoming of international public health concern is small. In addition, courtesy bias, in which the assessor believes that that erring on the side of caution is more acceptable than not reporting that an event, could have occurred.

Table 2. Characteristics of 175 emerging infectious disease events reported to WHO*

Characteristic	No. (%)
Type of pathogen	
Bacterium	89 (50.9)
Virus	39 (22.3)
Rickettsia	12 (6.9)
Protozoan	20 (11.4)
Helminth	2 (1.1)
Fungus	12 (6.9)
Prion	1 (0.6)
WHO region	
AMRO	77 (44.0)
EURO	49 (28.0)
EMRO	7 (4.0)
SEARO	10 (5.7)
WPRO	24 (13.7)
AFRO	8 (4.6)
Type of event	
New pathogen	70 (40.0)
Increased incidence	44 (25.1)
Antimicrobial drug resistance	54 (30.9)
New clinical manifestation	7 (4.0)

*WHO, World Health Organization; AMRO, Americas; EURO, Europe; EMRO, Eastern Mediterranean; SEARO, Southeast Asia; WPRO, Western Pacific; AFRO, Africa.

Table 3. Characteristics of 46 emerging infectious disease events of international public health concern reported to WHO*

Characteristic	No. (%)
Type of pathogen	
Bacterium	21 (45.6)
Virus	18 (39.1)
Rickettsia	2 (4.3)
Protozoan	3 (6.5)
Other†	2 (4.3)
WHO region	
AMRO	17 (37.0)
EURO	12 (26.1)
EMRO	3 (6.5)
SEARO	2 (4.3)
WPRO	7(15.2)
AFRO	5 (10.9)
Type of event	
New pathogen	19 (41.3)
Increased incidence	18 (39.1)
Antimicrobial drug resistance	8 (17.4)
New clinical manifestation	1 (2.2)

*WHO, World Health Organization; AMRO, Americas; EURO, Europe; EMRO, Eastern Mediterranean; SEARO, Southeast Asia; WPRO, Western Pacific; AFRO, Africa.
†Helminth, prion, or fungus.

The current study strictly applied the criteria described in the Annex 2 guidance without using the context of the event or personal judgment. The decision instrument criteria are designed to take context and personal judgment into account to be adaptable to current and future unknown threats (*13*). Use of personal judgment rather than strictly applying the decision instrument criteria leads to a lower notification rate (*9*).

Two events of international public health concern were missed despite the high sensitivity of the instrument, which reflected challenges of predicting evolution of an event as it occurs and potential for human error. Although a sensitivity of 100% would be difficult to attain, maintaining the number of missed events at an absolute minimum should be a priority when the instrument is revised or evaluated.

Prediction of seriousness and unusualness of events were least accurate and showed concordance rates of 49.7% and 58.3%, respectively. This finding reflects the subjectivity and broad spectrum of the seriousness and unusualness criteria. Although these findings might lead to overreporting, criteria flexibility is also "a major strength that makes the IHR future-proof against new and unforeseeable threats" (*9*). The other 2 criteria of international spread and restriction to travel and trade have higher concordance rates of 81.1% and 96%, respectively. Should there be a need to increase the specificity of the instrument, the focus should be on tightening the first 2 criteria and one should be more specific about what makes an event serious or unusual. Training staff at NFPs could also increase the specificity of the instrument (by perfecting their use of the decision instrument) and its PPV (by prefiltering which events to submit to the decision instrument). Staff of NFPs have been trained in the past by using online tools and workshops (*10*,*14*), and both approaches could be used.

Sensitivity and specificity of the decision instrument did not depend on event type, pathogen type, or WHO region of occurrence because no strong evidence of an association between concordance and these factors was found. This finding suggests the Annex 2 decision tool is adequate for reporting antimicrobial drug resistance, although it was not designed with drug resistance in mind. There have been calls to use the decision instrument for antimicrobial drug resistance events (*15*).

Although EID events were systematically, rather than randomly, sampled from the EID list compiled by Jones et al. (*3*), the distribution of events by type of pathogen was not significantly different from the distribution of events in the complete list from which the study sample is

Table 4. Association between concordance and pathogen type, by WHO region and event type*

Characteristic	No. events	No. (%) concordant events	Crude OR (95% CI)	p value†
Type of pathogen				
Bacterium	89	27 (69.2)	1	
Virus	39	46(51.7)	2.10 (0.95–4.67)	0.07
Rickettsia	12	6 (50.0)	0.93 (0.28–3.12)	0.91
Protozoan	20	7 (35.0)	0.50 (0.18–1.38)	0.18
Other‡	15	7 (46.7)	0.82 (0.27–2.45)	0.72
WHO region				
AMRO	77	40 (51.9)	1	
EURO	49	23 (46.9)	0.82 (0.40–1.68)	0.58
EMRO	7	4 (57.14)	1.23 (0.26–5.88)	0.79
SEARO	10	5 (50.0)	0.92 (0.25–3.45)	0.91
WPRO	24	15 (62.0)	1.54 (0.60–3.94)	0.37
AFRO	8	6 (75.0)	2.78 (0.53–14.6)	0.23
Type of event				
New pathogen	70	31 (44.3)	1	
Increased incidence	44	27 (61.4)	2.00 (0.93–4.31)	0.08
Antimicrobial drug resistance	54	32 (59.3)	1.83 (0.89–3.75)	0.10
New clinical manifestation	7	3 (42.9)	0.94 (0.20–4.53)	0.94

*WHO, World Health Organization; OR, odds ratio; AMRO, Americas; EURO, Europe; EMRO, Eastern Mediterranean; SEARO, Southeast Asia; WPRO, Western Pacific; AFRO, Africa.
†By Wald test.
‡Helminth, prion, or fungus.

extracted. The study sample and database from which it is extracted have a proportion of bacteria that is higher than other estimates of EID distribution (*16*,*17*). This finding can be explained by the fact that a large (43.8%) proportion of bacterial events are antimicrobial drug resistance events, which were not included as EIDs in many other studies. Jones et al. also reported a bias toward events occurring in industrialized countries, which reflect publication bias and better surveillance systems in these countries (*3*). However, these findings do not affect the internal validity of the study, and the fact that the current sample includes a wide variety of types of events can give confidence that the types of EID events truly reported to WHO are likely represented in the study sample.

The 16 events for which no information could be obtained did not statistically differ from the rest of the events, and the proportion of events without information was relatively low (8%), which made bias caused by information availability unlikely. The notifying investigator could not be blinded to EID events he or she was assessing, and it was possible to identify famous EID (such as emergence of Nipah virus) from the information, potentially introducing a bias toward reporting famous events. However, knowledge of these events is often the result of international concern, and they would have been reported regardless of these factors.

The intraclass correlation coefficient of 0.68 showed moderate-to-strong levels of agreement between expert panel members. The overall score given by each judge for each event was believed to be a good overall reflection of the role of the event. One limitation of this method was that the same score could be obtained with different opinions: e.g., if 1 expert strongly agreed that an event was serious but strongly disagreed that an event spread internationally, it would produce the same score as another expert strongly disagreeing with seriousness but strongly agreeing with international spread. However, when agreement levels were assessed for each criterion by calculating 4 intraclass correlation coefficients, there was no strong disagreement on any of the criteria, making that scenario unlikely.

The method showed agreement levels between experts and the consensus >75%. This agreement could have been improved by using a Delphi style approach (*18*), showing panel members their results compared with the mean of the whole panel and having a second round of evaluation.

This study took the approach of treating the IHR decision instrument a as a screening tool, thus enabling screening evaluation methods to be applied. One strong point of this study was the sample size: 175 real life events, a large enough sample to accurately estimate sensitivity and specificity with relatively narrow CIs. Furthermore, the fact that retrospective events were used enabled testing for predictive validity because in hindsight it was possible to evaluate the true international public health role of each event rather than just its potential for international public health concern. All panel members were internationally recognized as experts in the field. Therefore, their opinions were as reliable as can be obtained by using such a method. The fact they were blinded to whether each event would be reported and to each other's opinions, and the objective method used to decide on consensus for each EID event further strengthens the method. Increasing the size of the panel may also have added rigor to the evaluation.

The definition of an EID was wider and more encompassing than most definitions used in the literature, particularly because it included antimicrobial drug resistance. Therefore, the validity of the decision instrument was tested by using a wide variety of type of events likely to represent a range of events NFPs staff would encounter in real life.

This study attempted to replicate real-life situations by means of a theoretical exercise. The amount of information available on each event was limited, and the WHO Annex 2 decision instrument criteria described in the guidance were rigidly applied. Furthermore, political or economic considerations that could not be replicated in a study are often taken in account when reporting an event (*19*). Therefore, the study implies a degree of simplification of real-life conditions.

The sample of events was limited to EIDs in which the Annex 2 decision instrument is used for a variety of events, including radiation and chemical incidents and outbreaks of well-established pathogens. Whether the results of this study can be extrapolated to such events is not clear.

Although as much care as possible was taken to make the expert panel method objective, it still relied to some extent on individual opinion, and expert panel judgment on each event could not claim to be the definitive and universal truth. This shortcoming is inherent to the method and has been noted in other studies of the IHR Annex 2 decision instrument that used expert panels (*9*,*10*). Every attempt was made to minimize subjectivity by giving clear written guidelines to each expert, blinding the experts to the notification outcome, preventing experts from discussing the events, and deriving agreement mathematically.

The IHR Annex 2 decision instrument is a sensitive tool for reporting EIDs of international public health concern. The instrument lacks specificity mainly because of broad, nonspecific criteria that can lead to overreporting. The PPV of the instrument is also relatively low. If one considers the nature of the instrument and potential consequences of WHO not being aware of an EID event of international public health concern, sensitivity should be prioritized over specificity. In the current situation in which the volume of notification remains low, the instrument is adequate. However, if the IHR Annex 2 decision instrument is to be

used more systematically in reporting of and the volume of notification increases, there may be a need to increase the specificity and PPV of the instrument. This increase could be achieved by focusing particularly on setting more prescriptive seriousness and unusualness criteria to be more specific about what constitutes a serious or an unusual event, and by regular training of NFP staff online and through workshops to ensure that NFP staff report only relevant events, which would improve specificity without decreasing sensitivity and in turn increasing PPV. Also, focus should be placed on keeping the number of missed events to a minimum. However, instrument criteria must retain a certain level of interpretability so that the instrument can be adapted to a variety of unknown threats in the future, and not sacrifice sensitivity, which should remain the priority of the instrument. Finally, the approach taken in treating the IHR decision instrument as a screening tool and evaluating it as such has proved useful in understanding its value and limitations.

Acknowledgments

We thank David Gendy for helping retrieve articles relating to each EID event and Stephen Dorey for reanalyzing a subsample of the EID events included in this study.

Dr Edelstein is a public health specialist registrar at Haringey Public Health, London, UK. His main research interests are infectious diseases epidemiology and global health policy matters related to communicable disease.

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Address for correspondence: Michael Edelstein, Public Health Directorate, London Borough of Haringey/National Health Service North Central London, 4th Floor, River Park House, 225 High Rd, London N22 8HQ, UK; email: michael.edelstein@doctors.org.uk

Costing Framework for International Health Regulations (2005)

Rebecca Katz, Vibhuti Haté, Sarah Kornblet, and Julie E. Fischer

The revised International Health Regulations (IHR [2005]) conferred new responsibilities on member states of the World Health Organization, requiring them to develop core capacities to detect, assess, report, and respond to public health emergencies. Many countries have not yet developed these capacities, and poor understanding of the associated costs have created a barrier to effectively marshaling assistance. To help national and international decision makers understand the inputs and associated costs of implementing the IHR (2005), we developed an IHR implementation strategy to serve as a framework for making preliminary estimates of fixed and operating costs associated with developing and sustaining IHR core capacities across an entire public health system. This tool lays the groundwork for modeling the costs of strengthening public health systems from the central to the peripheral level of an integrated health system, a key step in helping national health authorities define necessary actions and investments required for IHR compliance.

In 2005, the member states of the World Health Organization (WHO) recognized the need to overhaul international public health cooperation, and they revised the International Health Regulations (IHR). The IHR (2005) focus on strengthening capabilities for confronting all potential public health emergencies of international concern when and where they occur. The 194 states parties made a commitment to develop core capacities to detect, assess, report, and respond to any public health event that might have international effects, regardless of type or origin of the event. The IHR (2005) also conferred new responsibilities on WHO and the global health community to share resources, information, and expertise to help nations prepare for and respond to public health events (*1*).

The WHO checklist and indicators for monitoring progress in the development of IHR core capacities by states parties, also known as the IHR Monitoring Framework, details 8 core capacities plus activities at points of entry that must be developed to fully implement the IHR (Table 1) (*2*). The IHR Monitoring Framework, first published in 2010, also defines country-level indicators within each core capacity. The regulations and the framework describe the core capacities needed for functional implementation of the IHR (2005) but leave flexibility for nations to determine how best to structure and develop these capacities (*3*).

The IHR also direct countries to strengthen and integrate existing systems for public health surveillance and response, rather than to create new, vertical programs. Various national approaches to IHR implementation have emerged, depending on factors such as the sophistication of preexisting systems and infrastructure, past and present objectives of health ministries and their external partners, availability of resources, architecture of health systems, and strength of regional commitments to health cooperation and coordination. Examples of the latter are the Integrated Disease Surveillance and Response strategy previously adopted by the WHO Regional Committee for Africa and shared standards developed through a Latin American subregional trade alliance (*4*–*7*). Two WHO regional offices, Southeast Asia Regional Office (SEARO) and Western Pacific Regional Office (WPRO), collaboratively developed the Asia Pacific Strategy for Emerging Diseases, providing a framework for coordinated approaches to rapid disease detection and public health emergency responses across sectors, countries, and regions (*8*).

Author affiliations: George Washington University, Washington, DC, USA (R. Katz); and Stimson Center, Washington (V. Haté, S. Kornblet, J.E. Fischer)

DOI: http://dx.doi.org/10.3201/eid1807.120191

Table 1. Summary of 2010 World Health Organization IHR Monitoring Framework*

Core capacity	Component	Country-level indicator
National legislation, policy, and financing	National legislation and policy	Laws, regulations, administrative requirements, policies, or other government instruments in place are sufficient for implementation of obligations under IHR.
	Financing	Funding is available and accessible for implementing IHR (including developing core capacities).
Coordination and NFP communications	IHR coordination, communication, and advocacy	A mechanism is established for the coordination of relevant sectors in the implementation of IHR.
		IHR National Focal Point functions and operations are in place as defined by the IHR (2005).
Surveillance	Indicator-based, or routine, surveillance (also referred to as structured surveillance, routine surveillance, and surveillance for defined conditions)	Indicator-based, routine, surveillance includes the early warning function for the early detection of public health events.
	Event based surveillance established	Event-based surveillance is established.
	Surveillance overview of information on IHR-related hazards (situation awareness)	A coordinated mechanism is in place for collecting and integrating information from sectors relevant to IHR
Response	Rapid response capacity	Public health emergency response mechanisms are established.
	Case management	Case management procedures are established for IHR-relevant hazards.
	Infection control	Infection prevention and control is established at national and hospital levels.
	Disinfection, decontamination, and vector control	A program for disinfection, decontamination, and vector control is established.
Preparedness	Public health emergency preparedness and response	Multihazard national public health emergency preparedness and response plan is developed.
	Risk and resource management for IHR preparedness	Public health risks and resources are mapped.
Risk Communication	Policy and procedures for public communications	Mechanisms for effective risk communication during a public health emergency are established.
Human Resources	Human resource capacity	Human resources are available to implement IHR core capacity requirements.
Laboratories	Laboratory diagnostic and confirmation capacity	Laboratory services are available and accessible to test for priority health threats.
		Influenza surveillance is established.
	Specimen collection and transport	System for collection, packaging, and transport of clinical specimens is established.
	Laboratory biosafety and biosecurity	Laboratory biosafety/biosecurity practices are in place.
	Laboratory-based surveillance	Laboratory data management and reporting is established.
Points of Entry	Surveillance at points of entry	Effective surveillance is established at points of entry.
	Response at points of entry	Effective response at points of entry established.

*IHR, International Health Regulations; NFP, National Focal Point. Data from (*2*).

Even with regional support, achieving the IHR core competencies is challenging for many nations at high risk for epidemic-prone or emerging infectious disease outbreaks and other public health crises. Member states initially agreed to implement IHR (2005) by June 2012, but a substantial proportion will clearly need at least one 2-year extension. Under Article 44 of the IHR, nations agreed to collaborate on developing and maintaining the public health capacities for IHR implementation by providing technical, logistical, and financial assistance to developing nations. The flexibility of the IHR framework, which enables national leaders to interpret the IHR requirements through mechanisms that are sensitive to local and regional contexts, makes it challenging to marshal such assistance effectively. The decision to measure IHR core capacity development in terms of functional outcomes rather than specific activities means that there could be 194 distinct but equally valid national approaches to fulfilling IHR (2005) obligations. Consequently, many nations that could use help with IHR implementation are still in the process of identifying opportunities for cooperative capacity building with external partners, often without information on how much it will cost to implement their national IHR action plans.

We describe steps for estimating the costs of achieving IHR (2005) implementation in countries with different economic climates by first identifying essential inputs. We identified functional pathways for implementing the 8 core capacities and actions at points of entry identified in the WHO 2010 IHR Monitoring Framework, on the basis of current and planned actions in 6 Southeast Asian case-study countries at different levels of economic and health systems development. We used this to develop a representative IHR implementation strategy to serve as a framework for a preliminary estimate of fixed and operating

costs associated with developing and sustaining IHR core capacities across an entire public health system.

Methods

Case-Study Countries

To develop an initial costing framework for IHR implementation, we sought case-study countries that could provide examples of field-tested strategies and practices in the 8 IHR core capacities and at points of entry, along with associated costs. On the basis of geographic proximity, recent responses to emerging infectious diseases of public health significance, economic development levels, and accessibility of financial and policy information, we identified 6 case-study countries in Southeast Asia: 1 low-income (Cambodia), 3 lower-middle income (Lao People's Democratic Republic, Vietnam, and Timor-Leste), and 2 upper-middle income (Malaysia and Thailand) (*9*). These 6 countries fall into the SEARO and WPRO areas, which share the Asia Pacific Strategy for Emerging Diseases capacity-building strategy (*8*).

Core Capacities Matrix

The 2010 WHO IHR Monitoring Framework identified 20 country-level indicators that states parties could use to assess IHR core capacity development (Table 1). The framework described levels of capability that could be used to evaluate progress toward each indicator, categorizing capabilities as prerequisites/foundational (level <1), inputs and processes (level 1), outputs and outcomes (level 2), and additional (level 3). Framework guidance specified that for all indicators to meet IHR requirements, countries must successfully demonstrate the attributes at levels 1 and 2.

For each country-level indicator, we identified specific activities and resources that could operationally achieve levels 1 and 2 attributes. Identification involved a 2-step process: 1) determining whether a technical standard exists for achieving each country-level indicator and 2) mapping activities and strategies among the case-study countries to the IHR Monitoring Framework.

To identify standards for building and sustaining the 8 core capacities, we reviewed guidance published by WHO and its regional offices, accrediting and professional organizations, and the US Centers for Disease Control and Prevention; consensus recommendations developed by expert working groups; and peer-reviewed publications, supplemented by additional input from subject matter experts in relevant disciplines.

To describe capabilities, activities, tools, and processes identified by decision makers in each case-study country as relevant to IHR core capacities, we reviewed published and unpublished government documents (e.g., legislation; regulations; national strategies; operational and programmatic guidance; training materials; self-assessments; proposed and enacted budgets; and plans for developing, strengthening, or maintaining IHR core capacities, pandemic preparedness, public health or emergency medical preparedness, indicator- and event-based surveillance, and laboratory systems) and materials prepared with or for development partners, technical partners, and nongovernmental stakeholders in each country. We supplemented the literature review through interviews with governmental and nongovernmental stakeholders in case-study countries. To determine requirements for diagnostic testing capabilities, we derived a priority disease list comprised of the endemic, epidemic-prone, and emerging infectious diseases specifically cited as always notifiable by the IHR (2005) Annex 2 reporting algorithm plus those appearing on ≥3 case-study country priority disease lists.

We mapped the activities and strategies identified through the reviews of technical guidance and case-study country activities to specific country-level indicators in the IHR Monitoring Framework, creating an operating core capacities matrix. To identify the practices and attributes common to some or all case-study countries for each core capacity, we compared these activities and strategies, distilling the strategies and practices into a representative Southeast Asian country, hereafter referred to as Country X, that has achieved levels 1 and 2 under each country-level indicator. Where no clear consensus emerged on strategies or practices, we selected the national approach that most closely resembled international or regional technical standards.

Costing Framework

For each activity or capability mapped to a specific country-level indicator in the Country X core capacities matrix, we extrapolated requirements for physical infrastructure (facilities, equipment, utilities), human capabilities (workforce, training, skills, and knowledge), and tools and processes (e.g., diagnostic platforms, materials, reagents, quality control and assurance, reporting systems), building on the foundations of existing public health surveillance costing platforms, such as the Integrated Disease Surveillance and Response SurvCost tool (*10*). Because of the integrated nature of the IHR core capacities, some physical infrastructure, human resources, and tools and processes might contribute to multiple core capacities. We sought to prevent overlap by including such elements only 1 time, under the most immediately relevant indicator.

To develop a preliminary cost estimate for developing and sustaining such infrastructure, human capabilities, and tools and process, we used the following: 1) costs calculated by case-study country government actors for procurement

or national budgets; 2) estimates derived with or for international partners; 3) the WHO CHOICE (CHOosing Interventions that are Cost Effective) database as a source of average salaries, per diem and travel compensation, physical infrastructure, and tradables specific to the subregions of SEARO B and WPRO B, into which the case-study countries fall; and 4) commercial price lists and supply schedules. Because the WHO CHOICE dataset expresses average costs in 2005 international dollars (defined as equivalent to $US in 2005 purchasing power parity), we likewise included or adjusted all costs in 2005 $US. We did not attempt to distinguish between the contributions of public, private, or international actors in mapping the surveillance, response, and laboratory systems for Country X. We thus assumed that the total costs of developing and sustaining each activity or capacity would be the same regardless of the payer. We did not attempt to calculate tariffs or other additional fees specific to each country.

Assumptions and Limitations

The Country X template represents a composite of demographic, political, and geographic attributes of 6 low- to middle-income case-study countries in 2 WHO sub-regions (SEARO B and WPRO B). All estimates for Country X assume a population of 60 million persons; 64 provinces with 600 functional districts; and 6 designated points of entry with a Ministry of Health responsible for public health surveillance, response, and laboratory capabilities at the national, provincial, district, and community levels (Figure 1).

Among the case-study countries, national strategies for public health surveillance depend heavily on facility-based surveillance. The reliability and timeliness of facilities-based reporting depend on population access to basic health services with trained health workers at peripheral, intermediate, and central levels. Such services are an absolute prerequisite to IHR implementation but are not explicitly included in the IHR (2005) or associated guidance. Any estimates for costs of public health surveillance and response developed through the framework described here should therefore be considered additional to the costs of developing and sustaining adequate essential health services.

Results

When we identified practices and strategies in 6 case-study Southeast Asian countries, referenced against regional/global technical standards, that could achieve the functional outcomes specified by each country-level indicator in the IHR Monitoring Framework, the resulting core capacities matrix created a detailed template for fully implementing IHR (2005) in a model Southeast Asian country. We present this matrix as a framework for determining the inputs—physical infrastructure, human capabilities, and tools and processes—required to achieve each core capacity at the peripheral, intermediate, and central levels of the Country X template and for estimating the costs associated with these inputs (Table 2).

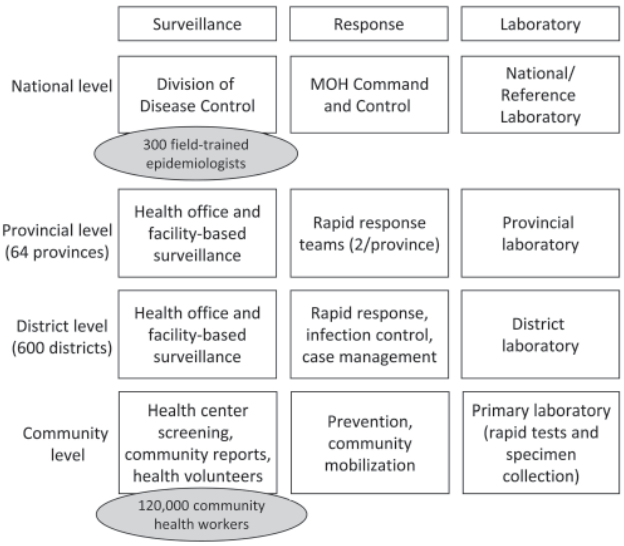

Figure 1. Overview of national public health system for model Southeast Asian country with a population of 60 million. MOH, Ministry of Health.

Core Capacity 1: National Legislation, Policy, and Financing

Country-level indicators focus on adoption of budgetary and regulatory frameworks to support IHR implementation. We identified only 1 input with cost implications—support for legal expertise (domestic or external consultants) to review and, as needed, revise national public health laws, estimated at $75,000 (in 2005 $US)—which was based on past consulting costs for revising national regulations with regard to avian and human influenza.

Core Capacity 2: Coordination and National Focal Point Communications

IHR coordination, communications, and advocacy require designation of an IHR National Focal Point and mechanisms to identify, convene, and coordinate stakeholders in public health surveillance and response across sectors. Inputs include information and communications technologies equipment and services as well as office infrastructure, transportation, and salary support for the individual or office serving as National Focal Points (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/pdfs/12-0191-Techapp.pdf).

Table 2. Summary of costs for all 8 International Health Regulations core capacities and ports of entry in Country X

Core capacity	Fixed costs, $US	Operating costs, $US
National legislation, policy, and financing	75,000	0
Coordination and National Focal Point communications	823,102	347,959–88,868
Surveillance	5,261,764	26,238,293–69,606,113
Response	20,480,332	3,981,294–5,215,857
Preparedness	2,889,166	103,726,507–103,786,408
Risk communications	4,389	1,868,869–2,141,939
Human resources	4,389	620,649–653,009
Laboratories	49,619,443	13,742,692–20,057,218
Points of entry	153,062	838,851–1,435,767
Total	79,310,647	151,365,114–203,485,179
Total cost, fixed + operating	Not applicable	230,675,761–282,795,826

Core Capacity 3: Surveillance

The IHR Monitoring Framework specifies that this core capacity encompasses indicator-based surveillance (the routine reporting of diseases or syndromes that meet specific case definitions) and event-based surveillance (the rapid detection and reporting of unusual or unexpected disease patterns, deaths, and exposure risks) (Figure 2; online Technical Appendix Table 2). All case-study countries conduct national indicator-based surveillance for priority diseases and have developed strategies for combining routine surveillance data with reports from other sources to provide early warning of emerging public health events. The resources for detecting, reporting, and managing cases of priority diseases and unusual events overlap substantially in the Country X template, particularly at the community level.

For Country X, we developed a template for surveillance staffing structure based on a combination of health systems structure and population. We did not attempt to prorate the share of office space, utilities, transportation, etc., dedicated to surveillance for epidemic-prone or emerging infections versus other goals (such as tracking nonommunicable conditions or high-risk behavior).

Indicator-based Surveillance

The template for surveillance core capacities in Country X includes activities to support indicator-based surveillance at the central, intermediate, and peripheral levels of the national health system and includes additional capabilities for collecting and analyzing urgent reports for event-based surveillance. Figure 2 provides an overview of Country X inputs; online Technical Appendix Table 2 provides a more detailed examination of the infrastructure, human capabilities, and tools and resources for supporting these inputs, including estimates intended to illustrate the approximate costs of implementing IHR core capacities according to this template.

The template uses the proposed minimum standard endorsed by the US Centers for Disease Control and Prevention: 1 field-trained epidemiologist per 200,000 population (*11*). To identify the full operating costs, the template assumes that Country X has achieved this population-based target (300 epidemiologists).

For Country X, numerous provinces with populations of ≈1 million persons serve as the hub for surveillance activities at the intermediate level, and districts (with catchment populations of ≈100,000) serve as the central hubs for surveillance activities at the peripheral level. The template for Core Capacity 3 includes dedicated personnel at the intermediate and peripheral levels to compile and report data on priority diseases and unusual events, to use and disseminate data and guidance issued from the national level, and to train local stakeholders. The template assumes that these functions are housed within existing health offices and health care facilities at the provincial and district levels. We developed the staffing model at each level on the basis of case-study country practices considered by interviewed experts to adequately serve case load.

According to experiences in the case-study countries, some mechanism is needed to extend government disease prevention and control programs to the community level. The inputs for the surveillance template include on-site training of community health center staff and for information and communications technologies equipment and services to facilitate the exchange of information between district health offices, basic health facilities, and communities. The template also includes monthly allowances for training, travel, and communications for 1 village or community health worker per 500 population (the approximate median among the case-study countries with established community health worker networks) to extend disease surveillance and prevention efforts to the household level.

Event-based Surveillance

We assume that the infrastructure, capabilities, systems, and processes developed for routine or indicator-based surveillance will also serve as the backbone for event-based surveillance, particularly at the community level where community health volunteers are likely to play a role in collecting reports of unusual disease clusters. The Country X template includes inputs for a center open 24

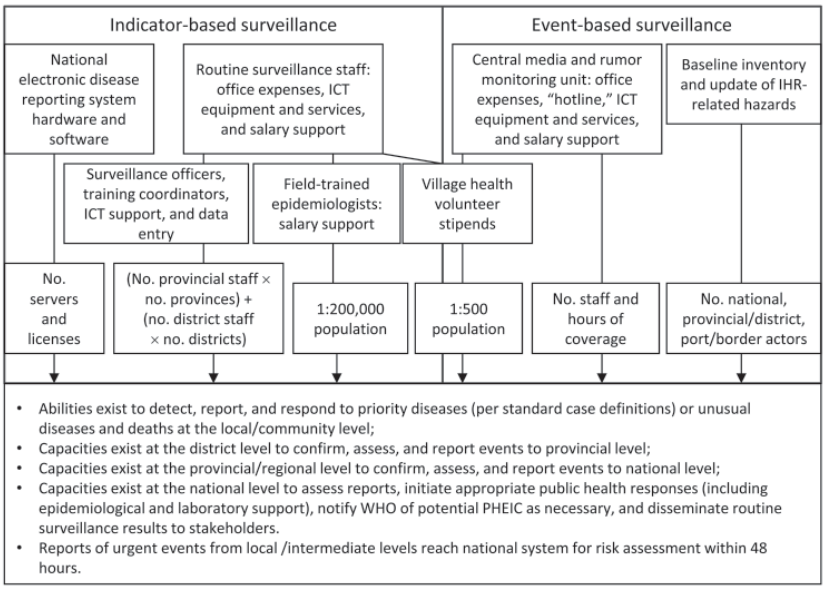

Figure 2. Inputs for Core Capacity 3 (Surveillance). IHR, International Health Regulations; ICT, information and communications technologies; WHO, World Health Organization; PoE, points of entry; PHEIC, public health emergency of international concern.

hours per day, 7 days per week, 365 days per year that would monitor and respond to urgent inquiries from health workers and the public and would collect and analyze structured and unstructured reports. To maximize the use of limited resources, the Country X event monitoring center shares physical infrastructure, information and communications technology resources, and other utilities with the Command and Control Center described in Core Capacity 4.

Hazards Mapping

The Country X template includes inputs to develop a baseline inventory of community and national health risks. It applies across all sectors through consultative workshops and field assessments.

Core Capacity 4: Response

Country X template inputs for response include a functional, dedicated command and control center with room and information and communications technology equipment and services to accommodate up to 40 personnel during an event (online Technical Appendix Table 3). Other inputs include materials, supplementary compensation, training, and travel allowances for 2 trained, 5-member multidisciplinary rapid response teams per province, plus 2 central rapid response teams, to investigate and respond to at least 1 public health event per year, with logistical and risk communications support from the provincial level. Inputs also include development and dissemination of guidance for infection control and case management, related training, and systems for isolating and transporting potentially infectious patients.

Core Capacity 5: Preparedness

The inputs for the Country X preparedness template encompass development, planning, and testing of a national public health emergency response plan (online Technical Appendix Table 4). This template is based on a comprehensive national risk assessment and on establishment of a national stockpile of materials to respond to priority events.

Core Capacity 6: Risk Communications

Inputs for risk communication include the development, printing, and dissemination of a national risk communications plan (online Technical Appendix Table 5). These activities are supported by annual training workshops and purchase of broadcast and print media at the peripheral, intermediate, and central levels.

Core Capacity 7: Human Resources

The inputs for human resources in Country X include resources for developing a coordinated national strategy for public health workforce development (online Technical Appendix Table 6). The inputs also include resources for fully supporting field epidemiology training (including travel support for field investigations) for as many as 16 trainees per year.

Core Capacity 8: Laboratories

For diagnostic testing capabilities, we identified the testing platforms, materials and reagents, and specimen collection and referral systems necessary to support detection and confirmatory testing of the Country X priority diseases as appropriate at each level of a tiered, integrated health system. Online Technical Appendix

Table 7 represents costs only of infrastructure and human capabilities associated with laboratory capacity.

Points of Entry

The inputs for the Country X template include a health office at each designated point of entry (online Technical Appendix Table 8). Each office is staffed by 4-person multidisciplinary public health response teams trained and equipped to respond to medical emergencies.

Discussion

This study was designed to help decision makers understand the demands of implementing IHR (2005) and to build the business case for strengthening global capacities to detect, assess, report, and respond to public health emergencies. The lack of standards in many areas of public health capacity-building and the many options at almost every step of investment allow for dozens of variations in each category of core capacity, with concomitant variations in costs. However, the framework described in this article illustrates the scope of IHR implementation demands and is intended to help national and international decision makers understand the inputs and associated costs of implementing the IHR (2005).

The framework includes all inputs and associated costs of building capacity for IHR-relevant public health surveillance and response rather than the marginal costs of adding new features to existing surveillance capabilities. Many countries will build the necessary capacities incrementally, and most already have capacities in place. This framework presents a way to estimate one-time capital costs, plus recurrent costs calculated on an annual basis, assuming that the total costs of national implementation depend on variables such as population, existing infrastructure, and health status. For most countries, the first step in developing a national IHR action plan is assessing the gap between current status and their ultimate strategies for implementing IHR core capacities fully.

We believe that this framework serves as a first step in helping national health authorities define to their own governments the actions and investments required to meet their IHR obligations, to protect their populations during public health emergencies, and to build a business case for potential donors. Articulating the elements of IHR core capacity-building can also help the global health community better comprehend a complex obligation that, if implemented fully, will strengthen the public health diagnostic, analytical, and information-sharing capacities that underpin effective decision making across health systems.

This research was supported by a grant from the US Department of State, Biosecurity Engagement Program.

Dr Katz is an assistant professor of health policy at George Washington University in Washington, DC. Her primary research areas are implementation of the IHR and global disease surveillance.

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Address for correspondence: Rebecca Katz, George Washington University, 2021 K St NW, Suite 800, Washington, DC 20006, USA; email: rlkatz@gwu.edu

Low Pathogenic Avian Influenza A (H7N2) Virus Infection in Immunocompromised Adult, New York, USA, 2003

Belinda Ostrowsky,[1] Ada Huang, William Terry,[2] Diane Anton, Barbara Brunagel, Lorraine Traynor, Syed Abid, Geraldine Johnson, Marilyn Kacica, Jacqueline Katz, Lindsay Edwards,[3] Stephen Lindstrom, Alexander Klimov, and Timothy M. Uyeki

In 2003, infection with low pathogenic avian influenza A (H7N2) virus was identified in an immunocompromised man with fever and community-acquired pneumonia in New York, USA. The patient recovered. Although the source of the virus was not identified, this case indicates the usefulness of virus culture for detecting novel influenza A viruses.

Limited numbers of human infections with low pathogenic avian influenza A, subtype H7, viruses have been reported and attributed to recent exposure to infected poultry (*1*–*6*). Such infections generally resulted in clinically mild illness. We report a case of low pathogenic avian influenza (LPAI) A (H7N2) virus infection in an immunocompromised adult.

The Study

On November 3, 2003, a 48-year-old man from the Caribbean sought care at an emergency department in Westchester County, New York, USA, after an episode of near syncopy; a 2–4 week history of feverishness, cough, fatigue, and myalgia; and a 10-pound weight loss over 2 months. He had lived in the United States since 1987 and had no known medical conditions. A month earlier, he had been evaluated at a clinic, and an oral antimicrobial drug was prescribed for possible pneumonia. Eight days before the emergency department admission reported here, he had sought emergency care for unilateral conjunctivitis,

Author affiliations: Westchester County Department of Health, New Rochelle, New York, USA (B. Ostrowsky, A. Huang, W. Terry); Westchester County Department of Laboratories and Research, Valhalla, New York, USA (D. Anton, B. Brunagel, L. Traynor, S. Abid); New York State Department of Health, Albany, New York, USA (G. Johnson, M. Kacica); and Centers for Disease Control and Prevention, Atlanta, Georgia, USA (J. Katz, L. Edwards, S. Lindstrom, A. Klimov, T.M. Uyeki)

DOI: http://dx.doi.org/10.3201/eid1807.111913

[1]Current affiliation: Montefiore Medical Center of, the University Hospital for The Albert Einstein College of Medicine, Bronx, New York, USA.

[2]Current affiliation: The State University of New York School of Medicine, Buffalo, New York, USA.

[3]Current affiliation: University of Colorado School of Medicine, Denver, Colorado, USA.

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Release date: June 13, 2012;
Expiration date: June 13, 2013

Learning Objectives

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

- Distinguish the usual severity of infections with LPAI
- Analyze the differential diagnosis for patients presenting with LPAI infection
- Evaluate the epidemiology of LPAI
- Assess other clinical characteristics of LPAI infection

CME Editor

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CME Author

Charles P. Vega, MD, Health Sciences Clinical Professor; Residency Director, Department of Family Medicine, University of California, Irvine. *Disclosure: Charles P. Vega, MD, has disclosed no relevant financial relationships.*

Authors

Disclosures: ***Belinda Ostrowsky, MD, MPH; William Terry, MD, MPH; Diane Anton, MS, M(ASCP); Barbara Brunagel, MS; Lorraine Traynor, BS; Syed Abid, PhD; Geraldine Johnson, MS; Marilyn Kacica, MD, MPH; Stephen Lindstrom, PhD; Alexander Klimov, PhD;*** *and* ***Timothy M. Uyeki, MD, MPH, MPP,*** *have disclosed no relevant financial relationships.* ***Ada Huang, MD,*** *has disclosed the following relevant financial relationships: owns stock, stock options, or bonds from Merck, Pfizer.* ***Jacqueline Katz, PhD,*** *has disclosed the following relevant financial relationships: received grants for clinical research from GlaxoSmithKline for research not related to the current study; received grants for preclinical research from Colby Pharmaceuticals (formerly Juvaris Bio Therapeutics) for research not related to the current study.* ***Lindsay Edwards*** *has disclosed the following relevant financial relationships: spouse owns GlaxoSmithKline stock.*

eye pain, and blurred vision; the diagnosis was corneal abrasion.

Physical examination on November 3, 2003, found that the patient was afebrile, weak, and mildly tachypneic (respiratory rate 18–26 breaths/minute, room air oxygenation saturation 98%) with bibasilar inspiratory rales. Pertinent laboratory findings included mild anemia and thrombocytopenia (hemoglobin 11.9 g/dL, platelets 107 × 10^9/L, leukocytes 8.0 × 10^9 cells/L [52% lymphocytes]), mildly elevated hepatic transaminases (aspartate aminotransferase 116 U/L, alanine aminotransferase 87 U/L), and elevated creatine kinase (1,844 U/L). A chest radiograph showed a right hilar density and left lower lobe infiltrates; computed tomographic scan of the chest and abdomen showed bilateral micronodular opacities with right perihilar infiltrates and lymphadenopathy. The patient was admitted for community-acquired pneumonia and received intravenous gatifloxicin.

A tuberculin skin test was reactive (20-mm induration). HIV ELISA/Western blot test results were positive (HIV test result from 3 years earlier was reportedly negative), and CD4 count was 300 cells/μL. Treatment was switched to rifampin, isoniazid, pyrazinamide, ethambutol, and pyridoxine. Bronchoalveolar lavage (BAL) performed on November 7 yielded influenza A virus by tissue cell culture at the Westchester County Department of Laboratories and Research and was negative for *Pneumocystis* spp., *Legionella* spp., and other bacterial or viral pathogens. A second BAL and biopsy performed later during hospitalization to evaluate adenopathy indicated inflammation without definitive pathology. The lower respiratory tract disease improved after 13 days, and the patient was empirically prescribed tuberculosis treatment (directly observed therapy) and discharged while mycobacterial culture results were pending. After 8 weeks, mycobacterial culture of the BAL specimen was negative for *Mycobacterium tuberculosis* but yielded *M. avium* complex.

The patient lived in an apartment with his wife and 4 children, none of whom were sick during his illness. He denied recent travel and had not traveled outside the United States for 4 years. He worked in a cafeteria as a dishwasher and handled food and garbage until 1 month before hospitalization. He denied any known risk factors for HIV infection.

The influenza A virus isolate was difficult to grow in culture, reacted minimally with antiserum to hemagglutinin H1, and was sent to the Centers for Disease Control and Prevention (CDC) for further characterization. On March 19, 2004, CDC reported that the influenza isolate, designated A/New York/107/2003, was an LPAI A (H7N2), not subtype H1N1, virus.

An epidemiologic investigation was initiated by the Westchester County Department of Health. During 3 interviews (with a Creole interpreter), the patient denied any exposure to live or dead poultry, wild birds, or bird feces. No live poultry markets or poultry were found on the surrounding property or in the neighborhood.

Serum samples obtained during the patient's hospitalization on November 5, 2003, and on April 4, 2004, were tested at CDC by microneutralization assay with the LPAI A (H7N2) virus from the patient. The acute-phase serum sample was negative (titer 10), but the convalescent-phase serum sample was positive (influenza [H7N2] virus neutralizing antibody titer 80), indicating seroconversion and evidence of infection with LPAI A (H7N2) virus. A confirmatory Western blot assay detected H7 hemagglutinin-specific antibody in the convalescent-phase serum sample. Testing of paired serum samples by ELISA demonstrated a 16-fold rise in H7 hemagglutinin-specific IgG. Serum samples collected from the patient's wife and 3 of the children on April 4, 2004, were seronegative for influenza A (H7N2) neutralizing antibodies.

Conclusions

In this immunocompromised man with pneumonia, the contribution of influenza A (H7N2) virus infection to his lower respiratory tract disease is unclear. The diagnosis of influenza A (H7N2) virus infection was not made until long after the patient had been discharged, and no influenza antiviral treatment was administered. The patient's history and clinical findings were consistent with HIV and community-acquired pneumonia with possible clinical response to the antimicrobial drug therapy or improvement of self-limiting viral pneumonia. In patients with HIV infection, *M. avium* complex is often detected as an indolent pathogen, especially associated with disseminated disease in patients with advanced AIDS; clinical resolution usually requires prolonged multidrug treatment (*7*). Isolation of influenza A (H7N2) virus from a BAL specimen and resolution of lower respiratory tract disease during hospitalization suggest that this infection might have contributed to the pulmonary disease. The clinical spectrum of human infection with LPAI viruses, including subtype H7, is generally mild, ranging from conjunctivitis to influenza-like illness (*1*–*6*), although hospitalization of patients with influenza A (H7N2) virus infection has been reported (*6*).

Conjunctival infection with influenza A subtype H7 viruses in persons with conjunctivitis has been confirmed by reverse transcription PCR or virus isolation (*1*–*3*,*5*). The patient initially reported ophthalmologic symptoms. Because conjunctival specimens were not available for virus testing, the role of influenza A (H7N2) virus in the conjunctivitis is unknown. However, intraocular inoculation of mice with the influenza A (H7N2) virus from the patient (A/New York/107/2003) did not result in infection, and the

virus replicated at relatively low levels in murine corneal epithelial cells ex vivo (*8*,*9*). Intranasal inoculation with the LPAI A (H7N2) virus caused respiratory symptoms, elevated mean lung titers, and cytokine increases in mice; among ferrets, the virus replicated efficiently in the upper respiratory tract and was transmissible through direct contact (*8*,*10*).

The source of the patient's infection with influenza A (H7N2) virus was not determined, although exposure to poultry was suspected. A limitation is that the investigations were conducted 5 months after the patient was hospitalized, after the influenza A isolate was identified as an LPAI A (H7N2) virus. LPAI A (H7N2) viruses have been documented among birds in live poultry markets in the northeastern United States, including New York (*11*–*13*). Such viruses have receptor-binding properties consistent with receptors found in the human upper respiratory tract (*10*).

This case of LPAI A (H7N2) virus infection was detected through influenza virus surveillance of specimens submitted from outpatients and hospitalized patients to the Westchester County Department of Laboratories and Research and illustrates the value of virus culture for detection of human infections with novel influenza A viruses, which are nationally notifiable. For the patient reported here, neither seasonal influenza nor zoonotic influenza was suspected. Whether HIV infection might have made the patient more susceptible to lower respiratory tract infection with LPAI A (H7N2) virus is unknown, but a case of pulmonary infection with LPAI A (H9N2) virus in an immunosuppressed adult has been reported (*14*). A serologically confirmed case of LPAI A (H7N2) virus infection in the United States was associated with upper respiratory tract illness (*4*). Although information about the frequency of human infection with LPAI A H7 viruses is limited, 1 study reported antibody detection in 3.8% of exposed poultry workers after an outbreak of LPAI A (H7N3) virus infection among poultry in Italy (*15*). More information is needed to clarify the risk for LPAI A H7 virus infections among immunocompetent and immunocompromised persons.

Acknowledgments

We thank the many colleagues who helped with the epidemiologic and laboratory investigations, including Ralph Bernard , who assisted with interviews and translation; the public health nursing staff who arranged for the serologic testing and directly observed therapy; the staff at the Westchester Medical Center, where the case-patient received medical care; and Perry Smith, Barbara Wallace, Bryan Cherry, and Amanda Balish.

Dr Ostrowsky was director of communicable and sexually transmitted diseases at the Westchester County Department of Health when the case reported here was investigated. She is currently director of the antimicrobial stewardship program at Montefiore Medical Center of Albert Einstein College of Medicine. Her research interests include antimicrobial drug resistance, health care–associated infections, and public health.

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Address for correspondence: Belinda Ostrowsky, Montefiore Medical Center, Albert Einstein College of Medicine, 111 E 210 St, Bronx, NY 10467, USA; email: bostrows@montefiore.org

Seroconversion to Seasonal Influenza Viruses after A(H1N1) pdm09 Virus Infection, Quebec, Canada

Mariana Baz, Jesse Papenburg, Marie-Eve Hamelin, Manale Ouakki, Danuta M. Skowronski, Gaston De Serres, and Guy Boivin

We looked for cross-reactive antibodies in 122 persons with paired serum samples collected during the 2009 pandemic of influenza virus A(H1N1)pdm09. Eight (12%) of 67 persons with A(H1N1)pdm09 infection confirmed by reverse transcription PCR and/or serology also seroconverted to the seasonal A/Brisbane/59/2007 (H1N1) virus, compared with 1 (2%) of 55 A(H1N1)pdm09-negative persons (p<0.05).

The role of seasonal 2008–09 trivalent inactivated influenza vaccines in protecting against influenza A(H1N1)pdm09 virus remains controversial (*1*). Recent reports indicated that prior infections with seasonal influenza A viruses protected against A(H1N1)pdm09 virus infection, suggesting the presence of cross-reactive antibodies (*2*). Several studies have proposed that humoral immunity and conserved B- and T-cell epitopes contribute to heterosubtypic protection (*3*,*4*). Our objective was to determine whether A(H1N1)pdm09 infection induced cross-reactive antibodies against seasonal influenza A (H1N1) and A (H3N2) viruses.

The Study

This investigation was part of a trial evaluating A(H1N1)pdm09 transmission among household contacts, conducted during the first wave of the 2009 pandemic (May–July 2009) in Quebec City, Quebec, Canada (*5*). Clinical data and samples were serially obtained from index case-patients and their contacts in 42 households. Nasopharyngeal secretions were collected from all participants during the first visit and tested by 2 different assays: a conventional reverse transcription PCR (RT-PCR) targeting the hemagglutinin gene of A(H1N1)pdm09 virus (*6*) and a universal RT-PCR targeting the matrix gene of all influenza A viruses (*7*). Blood was collected from persons ≥7 years of age at their initial visit (acute-phase sample) and 3–4 weeks later (convalescent-phase sample). Serum was tested by microneutralization assay according to World Health Organization standard protocols with minor modifications (*8*).

This serologic study comprised 122 persons from the 42 households. Twenty-four persons were RT-PCR–confirmed index case-patients (median age 15 years, range 7–56 years), and 98 were household contacts (median age 30.5 years, range 7–61 years), of whom 34 also were positive for A(H1N1)pdm09 virus by RT-PCR. For 67 patients (median age 20 years, range 7–61 years), A(H1N1)pdm09 was confirmed by RT-PCR and/or microneutralization assay: 10 (15%) by RT-PCR alone, 9 (13%) by microneutralization assay alone, and 48 (72%) by RT-PCR and microneutralization assay. Of the 67 A(H1N1)pdm09-infected persons, 8 (12%) seroconverted to A/Brisbane/59/2007 (A[H1N1] vaccine strain for 2008–09) (online Appendix Table, wwwnc.cdc.gov/EID/article/18/7/11-1680-TA1.htm). Seven A/Brisbane/59/2007 seroconverters were RT-PCR positive and A(H1N1)pdm09 seroconverters, and 1 was RT-PCR positive and a A(H1N1)pdm09 nonseroconverter. In comparison, 1 (2%) of the 55 A(H1N1)pdm09-negative patients seroconverted to A/Brisbane/59/2007 (Fisher exact test, p<0.05). Seasonal influenza viruses were not circulating in the province of Quebec at the time of this study. Only 1 of 9 A/Brisbane/59/2007 seroconverters had previously received the inactivated 2008–09 seasonal influenza vaccines. No participants were vaccinated against A(H1N1)pdm09 virus, and none received antiviral therapy or prophylaxis.

We then assessed whether this cross-reactivity was limited to the A/Brisbane/59/2007 strain, the most recent seasonal A (H1N1) virus to have circulated before A(H1N1)pdm09 virus, or whether it was broader. To this end, we tested all paired serum samples against an older seasonal A (H1N1) influenza virus, i.e., A/New Caledonia/20/1999 (H1N1 vaccine strain used during the 2000–01 through 2006–07 seasons), and a past A(H3N2) virus, i.e., A/Panama/7/2004 (H3N2 vaccine component used during the 2000–01 through 2003–04 seasons). Seven (10%) A(H1N1)pdm09 virus–positive persons also seroconverted to A/New Caledonia/20/1999(H1N1), all of

Author affiliations: Centre Hospitalier Universitaire de Quebec, Quebec City, Quebec, Canada (M. Baz, J. Papenburg, M.-E. Hamelin, G. Boivin); Laval University, Quebec City (M. Baz, J. Papenburg, M.-E. Hamelin, G. Boivin); Institut National de Santé Publique du Quebec, Quebec (M. Ouakki, G. De Serres); and British Columbia Centre for Disease Control, Vancouver, British Columbia, Canada (D.M. Skowronski)

DOI: http://dx.doi.org/10.3201/eid1807.111680

whom were RT-PCR-positive and A(H1N1)pdm09 virus seroconverters, whereas none of the A(H1N1)pdm09 virus–negative persons seroconverted to this older strain ($p<0.05$). On the other hand, seroconversion rates for A/Panama/7/2004(H3N2) did not differ significantly between A(H1N1)pdm09 virus-positive (9%) and -negative (5%) patients. In addition, we identified 4 (6%) persons with laboratory-confirmed A(H1N1)pdm09 virus infections who seroconverted to both seasonal (H1N1) viruses and 2 (3%) who seroconverted to A/Brisbane/59/2007(H1N1) and A/Panama/2007/99(H3N2) (Table). Participant 44C, a household contact of a confirmed case-patient with a negative RT-PCR for A(H1N1)pdm09 and low antibody titers in the convalescent-phase serum, showed cross-neutralizing antibodies meeting 4-fold seroconversion criteria for A/Brisbane/59/2007(H1N1) and A/Panama/7/2004(H3N2).

Conclusions

During this study, the only influenza virus detected in the province of Quebec was A(H1N1)pdm09 virus. Yet, 8 (12%) of 67 A(H1N1)pdm09 virus–infected persons in our study had a concomitant significant increase in microneutralization antibody titers against the most recent A/Brisbane/59/2007(H1N1) strain, of whom 5 persons had 4–8-fold, 2 had 16-fold, and 1 had 32-fold rises. In addition, 4 of these 8 persons also seroconverted to an older A/New Caledonia/20/1999(H1N1) virus, of whom 3 persons had 4-fold and 1 had 16-fold rises between acute-phase and convalescent-phase serum. The cross-reactivity observed in the study population does not seem to be completely subtype specific because some persons also showed rising titers against an old influenza A (H3N2) strain (A/Panama/2007/99), although in this case, seroconversion rates did not differ significantly between A(H1N1)pdm09 virus–positive and –negative persons.

A recent study in Hong Kong of 28 paired serum samples showed that infection with the pandemic virus could broaden cross-reactive immunity to other recent subtype H1 swine viruses. In contrast to our study, perhaps because of the small number of participants or older age of A(H1N1)pdm09 virus–positive case-patients (30.5 vs. 20 years), no cross-reactive response was shown against the more recent seasonal influenza virus A/HK/400599/2008(H1N1) (*9*).

We could not determine the extent to which past seasonal influenza vaccinations and/or natural infections contributed to the generation of cross-neutralizing antibodies to A(H1N1)pdm09 and the seasonal influenza strains. Our next step will be to investigate potential cross-neutralizing determinants between these seasonal and pandemic viruses. Neutralizing antibodies that bind to the stalk region of HA2 have been shown to confer broad cross-neutralizing activity against several subtypes of viruses across clades and to provide protection in animal models (*10*,*11*). Six of the 8 persons who seroconverted to A/Brisbane/59/2007(H1N1) by microneutralization assay did not meet the 4-fold criteria by hemagglutinin inhibition assay (data not shown), suggesting that the cross-reactivity might result from conserved epitopes in the stalk region of HA2 or in other proteins. Greenbaum et al. recently showed that, overall, 49% of the epitopes reported in recently circulating seasonal A (H1N1) strains were conserved in the A(H1N1)pdm09 virus (*12*). Specifically, 31%, 41%, and 69% of the B-cell, $CD4^+$ and $CD8^+$ T-cell epitopes, respectively, were conserved. Natural infection with A(H1N1)pdm09 virus also could have elicited cross-reactive responses against internal components of older viral strains (*13*). We find intriguing the elaboration of

Table. Clinical features and microneutralization antibody titers against influenza A(H1N1)pdm09 and seasonal influenza A viruses of persons who seroconverted to ≥2 influenza viruses, Quebec City, Quebec, Canada, 2009*

Participant no./age, y	RT-PCR for A(H1N1) pdm09	Symptoms		Titers, acute phase/convalescent phase (-fold increase)			
		ARI	ILI	A/Quebec/147023/ 2009; A(H1N1)pdm09	A/Brisbane/59/ 2007 (H1N1)	A/New Caledonia/ 99 (H1N1)	A/Panama/2007/99 (H3N2)
39A/7	+	+	+	<10/160 (16)	<10/320 (32)	<10/160 (16)	320/160 (0)
39C/11	+	+	+	<10/40 (4)	10/160 (16)	<10/40 (4)	2,560/2,560 (0)
49A/28	+	+	+	<10/160 (16)	40/160 (4)	<10/20 (2)	320/320 (0)
49B/23	+	+	+	<10/640 (64)	<10/160 (16)	40/160 (4)	40/40 (0)
55F/7	+	+	+	<10/40 (4)	10/40 (4)	20/80 (4)	10/10 (0)
16C/7	+	+	+	<10/80 (8)	160/320 (2)	40/160 (4)	80/80 (0)
56A/12	+	+	+	<10/40 (4)	80/320 (4)	40/80 (2)	2,560/2,560 (0)
65B/40	+	+	+	<10/320 (32)	<10/20 (2)	10/40 (4)	320/320 (0)
03E/17	+	+	+	<10/80 (8)	40/80 (2)	160/160 (0)	640/5,120 (8)
10B/14	+	+	+	<10/80 (8)	20/20 (0)	80/320 (4)	640/640 (0)
11C/61†	+	+	+	<10/1,280 (128)	40/160 (4)	160/160 (0)	320/1,280 (4)
44B/9	+	+	+	<10/<80 (8)	1,280/640 (0)	1,280/1,280 (0)	160/640 (4)
44C/44	–	+	–	<10/20 (2)	10/80 (8)	80/80 (0)	160/640 (4)
58A/13	+	+	+	<10/80 (8)	<10/20 (2)	10/20 (2)	1,280/5,120 (4)
58B/43	+	+	–	<10/160 (16)	<10/10 (0)	<10/<10 (0)	20/160 (8)

*RT-PCR, reverse transcription PCR; ARI, acute respiratory illness (i.e., presence of ≥2 of the following signs/symptoms: fever [≥37.8°C] or feverishness, cough, sore throat, or rhinorrhea); ILI, influenza-like illness (i.e., fever and cough and/or sore throat); +, positive; –, negative.
†Received seasonal vaccine in 2008–09.

cross-reactive neutralizing antibodies to more recently circulating influenza A (H1N1) strains as a result of novel A(H1N1)pdm09 virus infection, whereas the reverse has not generally been evident in serosurveys for cross-reactive A(H1N1)pdm09 antibody, except in elderly persons who had substantial cross-reactive antibodies to A(H1N1) pdm09 virus (*14*,*15*). Unfortunately, because of the small sample size of our study and lack of serum from children <7 years of age, we could not assess whether cross-reactivity was an age-dependent phenomenon. However, all but 3 of the cross-reactive seroconverters (13/16 [81%]) were 7–30 years of age. To explore preferential responses to the original infecting virus (original antigenic sin), we assessed cross-reactivity for the older A/New Caledonia/99(H1N1) virus that was potentially the priming antigen for some of our younger participants or was closely related to the priming antigen in older participants. However, seroconversion rates for A/New Caledonia (10%) were comparable to those of the more recent A/Brisbane/59/2007(H1N1) strain (12%), and thus we could not distinguish original antigenic sin on that basis. These antigens may have been too closely related antigenically to demonstrate that in this young cohort.

Our work supports the notion that natural A(H1N1) pdm09 virus infection induces broad heterosubtypic (H1 and even H3) responses. It also highlights the need for further investigation of the mechanisms behind cross-protection because they could be keys to creating improved influenza vaccines with broader protection.

Acknowledgments

We are grateful to Kanta Subbarao for her critical review of the manuscript.

This work was supported by the Fonds de la Recherche en Santé du Québec.

Dr Baz is a postdoctoral visiting fellow in the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA. Her research interests include antiviral resistance to influenza virus and development of vaccines against seasonal and pandemic influenza viruses.

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Address for correspondence: Guy Boivin, CHUL, Rm RC-709, 2705 Blvd Laurier, Quebec City, Quebec G1V 4G2, Canada; email: guy.boivin@crchul.ulaval.ca

Influenza Virus Infection in Guinea Pigs Raised as Livestock, Ecuador

Victor H. Leyva-Grado, Samira Mubareka, Florian Krammer, Washington B. Cárdenas, and Peter Palese

To determine whether guinea pigs are infected with influenza virus in nature, we conducted a serologic study in domestic guinea pigs in Ecuador. Detection of antibodies against influenza A and B raises the question about the role of guinea pigs in the ecology and epidemiology of influenza virus in the region.

Influenza A virus infection causes disease in humans and domestic animals, including pigs, horses, and chickens, and seasonal epidemics among humans in the Northern and Southern Hemispheres result in hospitalizations and deaths worldwide. Influenza A virus transmission studies are often conducted in laboratory guinea pigs (*1*,*2*) because the virus can efficiently spread from infected animals to naive guinea pigs by direct and indirect (short-range infectious aerosols) contact (*3*,*4*). However, whether guinea pigs are naturally infected with influenza virus outside the laboratory setting is not known.

In some regions of South America, guinea pigs are part of the traditional cuisine and are produced as livestock and sold commercially for human consumption. Guinea pigs are customarily raised on small rural farms in proximity to other livestock. Circulation of influenza virus in these populations has not been studied. Given the effect of influenza virus on human health and the susceptibility of guinea pigs to influenza virus infection in the laboratory, it is worthwhile to determine whether influenza virus can spread among guinea pigs in agricultural settings. As an initial step in this endeavor, we obtained serum samples from domestic guinea pigs in Ecuador and tested them for the presence of influenza antibodies to determine whether the guinea pigs had been infected with influenza virus.

Author affiliations: Mount Sinai School of Medicine, New York, New York, USA (V.H. Leyva-Grado, F. Krammer, P. Palese); Sunnybrook Health Sciences Centre and Research Institute, Toronto, Ontario, Canada (S. Mubareka); and Escuela Superior Politécnica del Litoral (ESPOL), Guayaquil, Ecuador (W.B. Cárdenas)

DOI: http://dx.doi.org/10.3201/eid1807.111930

The Study

We obtained serum samples from 40 guinea pigs from 3 different regions of Ecuador (Figure 1), 20 from Cuenca and 10 each from Guayaquil and the Manabí region. Cuenca is located in the Andes region, 2,500 m above sea level; it is one of the main producers of guinea pig meat. Guayaquil, the most populated city in Ecuador, is located at the head of the Gulf of Guayaquil on the Pacific Ocean. The Manabí region is located in western Ecuador on the Pacific Ocean coast. Serum samples were collected from adult guinea pigs that we purchased from local farms (Cuenca), where they had been raised as livestock, or from live animal markets (Guayaquil and Manabí). The samples were collected by heart puncture under general anesthesia (combination of ketamine and xylazine). Animals were euthanized after samples were obtained.

For antigens in the serologic analyses, we used whole viruses and recombinant hemagglutinin, nucleoprotein, and neuraminidase (subtypes N1 and N2) proteins produced in our laboratory as described (*5*,*6*). The whole viruses were A/Brisbane/59/2007 (H1N1) (Brisbane07), A/New Caledonia/20/1999 (H1N1) (Newcal99), A/Wisconsin/67/2005

Figure 1. Three regions of Ecuador where guinea pig serum samples were obtained: Cuenca, Guayaquil, and Manabi. The country is bordered by Colombia to the north, Peru to the east and south, and the Pacific Ocean to the west. Cuenca is located in the Andes; the average annual mean temperature is 14.7°C, and the average annual relative humidity is 85%. Guayaquil is located at the head of the Gulf of Guayaquil; the mean temperature is 26.1°C, and relative humidity is 74%. The Manabí region is located on the Pacific Ocean coast; the mean temperature is 25.9°C, and relative humidity is 79%.

(H3N2) (Wisconsin05), A/Vietnam/1203/2004 (H5N1) (Vietnam04), and B/Yamagata/16/1988 (Yamagata88). The hemagglutinins were A/California/04/2009 (Cal09), NewCal99, Vietnam04, Wisconsin05, and Yamagata88. The nucleoproteins were Brisbane07, A/Puerto Rico/08/1934, and B/Florida/04/2006. The subtype N1 neuraminidases were Cal09 and Vietnam04; the N2 subtype was A/Hong Kong/1/1968.

ELISA was done as described (*7*), with slight modifications for the use of guinea pig serum. The cutoff for serum considered positive for influenza virus was the value of the negative control (naive guinea pig serum) +3 SD (from 3 repetitions). For Western blot (WB) analyses (*8*), serum samples were pooled into groups of 5 (groups 1–4, 5–6, and 7–8 were from Cuenca, Guayaquil, and Manabí region, respectively). Pooled samples were also used for the hemagglutination inhibition (HI) assay, as described (*9*), using influenza strains Brisbane07, Wisconsin05, and Vietnam04. As positive controls, we used serum from guinea pigs that we had infected with Cal09, Brisbane07, NewCal99, Wisconsin05, Vietnam04, or Yamagata88.

ELISA results for the 40 serum samples showed that 20, 18, and 14 were positive for influenza subtypes H1, H3, and H5, respectively (Table 1). The samples were also tested for the presence of antibodies against the influenza virus nucleoprotein: results for 29 were positive. Samples with positive results to ≥1 hemagglutinin antigens and to nucleoprotein were also analyzed for the presence of antibodies against proteins of the neuraminidase subtypes N1 (Cal09, Vietnam04) and N2 (influenza A/Hong Kong/1/1968 [H3N2]) (Table 1). Serum samples were also tested for the presence of influenza B virus by using whole virus, recombinant hemagglutinin, and recombinant nucleoprotein as described above. Samples from Cuenca showed the highest overall positivity to the 3 antigens (Table 2).

WB analysis of pooled samples from the 3 areas confirmed the ELISA results that showed antibodies against the hemagglutinin and nucleoprotein antigens; however, samples from Manabí region (*7*,*8*) showed immunoreactivity to the H3 antigen only (Figure 2). WB results for serum samples from Cuenca and Guayaquil were positive for influenza B virus, but results were not positive for samples from Manabí when whole virus was used as antigen (Figure 2). HI activity was observed in all pooled samples against all 3 viruses tested. Titers were highest in samples from Cuenca (320 for Brisbane07, 640 for Wisconsin05, and 80 for Vietnam04) and lowest for samples from Manabí (80 for Brisbane07 and 40 for Wisconsin05 and Vietnam04). Titers for samples from Guayaquil were 160 for Brisbane07 and 80 for Wisconsin05 and Vietnam04.

Conclusions

We showed the presence of influenza virus antibodies with HI activity to different virus subtypes in guinea pigs marketed through local farms and live animal markets in different regions of Ecuador. Seroprevalence was similar for influenza A virus subtypes H1 (50%) and H3 (45%). A study of influenza among humans in Ecuador also showed similar seroprevalences for subtypes H1 (5.1%) and H3 (5.5%) (*10*). This10-fold difference between seroprevalence rates among humans and the guinea pigs in our study may be explained by animal husbandry practices that facilitate influenza transmission (e.g., the crowding of animals in cages).

The presence of antibodies to influenza B virus in guinea pigs is notable because infection with this virus is thought to be restricted to humans (*11*). Recent studies in our laboratory demonstrated that guinea pigs can also be infected with influenza B viruses and that they readily transmit the virus to naive guinea pigs (*12*). These studies support our finding that influenza B virus has the potential to be transmitted from humans to other species. Further studies are needed to isolate and characterize the type B influenza virus present in the population of guinea pigs to determine if there has been an adaptation to the new host or if the guinea pig is only a transient reservoir for the human virus.

We found that several guinea pigs had antibodies against influenza A (H5). Ecuador is purported to be free of avian influenza (*13*); however, reports from other countries in South America demonstrate the presence of avian subtypes H3, H5N2, H7N3, and H13N9 in wild birds (*14*). In addition,

Table 1. ELISA results for the presence of influenza A virus antibodies in guinea pigs from different regions of Ecuador*

Region	No. (%) positive, by antigen†						
	H1	H3	H5	NP	N1 (Cal09)	N1 (Vietnam04)	N2
Cuenca, n = 20	11 (55)	12 (60)	6 (30)	16 (80)	8 (57)‡	8 (57)‡	10 (71)
Guayaquil, n = 10	8 (80)	5 (50)	8 (80)	10 (100)	8 (88)§	8 (88)§	8 (88)§
Manabí, n = 10	1 (10)	1 (10)	0	3 (30)	1 (50)¶	1 (50)¶	0¶
Total	20	18	14	29	17	17	18

*Samples were from adult guinea pigs raised as livestock on local farms (Cuenca) or from live animal markets (Guayaquil and Manabí). H1, recombinant hemagglutinin from A/California/04/2009 (Cal09); H3, recombinant hemagglutinin from A/Wisconsin/67/2005; H5, recombinant hemagglutinin from A/Vietnam/1203/2004 (Vietnam04); NP, recombinant nucleoprotein from A/Brisbane/10/2007; N1, recombinant neuraminidase from Cal09 or Vietnam04; N2, recombinant neuraminidase from A/Hong Kong/1/1968.
†Serum samples were considered positive if absorbance (read at 405 nm) was higher than the value of the negative control (naive guinea pig serum) +3 SD.
‡Only 14 samples were tested.
§Only 9 samples were tested.
¶Only 2 samples were tested.

Table 2. ELISA results for the presence of influenza B virus antibodies in guinea pigs from different regions of Ecuador*

Region	No. (%) positive, by antigen†		
	Whole virus (Yamagata88)	Recombinant hemagglutinin	Recombinant nucleoprotein
Cuenca, n = 20	17 (85)	18 (90)	18 (94)‡
Guayaquil, n = 10	8 (80)	9 (90)	9 (90)
Manabí, n =10	2 (20)	1 (1)	1 (10)
Total	27	28	28

*Samples were from adult guinea pigs raised as livestock on local farms (Cuenca) or from live animal markets (Guayaquil and Manabí). Whole virus, B/Yamagata/16/1988. Recombinant hemagglutinin was derived from B/Florida/04/2006; nucleoprotein was derived from B/Yamagata/16/1988.
†Serum samples were considered positive if absorbance (read at 405 nm) was higher than the value of the negative control (naive guinea pig serum) + 3 SD.
‡Only 19 samples were tested.

chickens, turkeys, and guinea pigs that are later sold in local farmers' markets are raised together by some families, thereby facilitating contact of guinea pigs with avian influenza viruses. We tested only for seroreactivity to the H5 hemagglutinin and the N1 neuraminidase; therefore, further studies are needed to determine whether different avian origin influenza viruses are present in the guinea pig population.

We did not determine whether guinea pigs are an incidental host for influenza virus infection or, if instead, the virus has been adapted to these animals or if guinea pigs are a natural reservoir for some influenza viruses. To this end, virus isolation and characterization would be necessary to determine the virus strains circulating in this population. In the laboratory, guinea pigs are infected and efficiently transmit influenza viruses to naive hosts without showing any overt clinical signs of disease (*1*). Therefore, further studies are needed to address the specific role of guinea pigs raised as livestock in the ecology and epidemiology of influenza viruses in the region.

This work was partially funded by the Center of Excellence for Influenza Research and Surveillance, National Institutes of Health (grant HHSN266200700010C to P.P.) and the Keck Foundation (P.P.). F.K. was supported by an Erwin Schrödinger fellowship (J 3232) from the Austrian Science Fund.

Dr. Leyva-Grado is a postdoctoral fellow at Mount Sinai School of Medicine in New York. His primary research interests include the study of the pathophysiology of virus-induced respiratory infections in humans and domestic animals and the development of therapeutics for virus infections.

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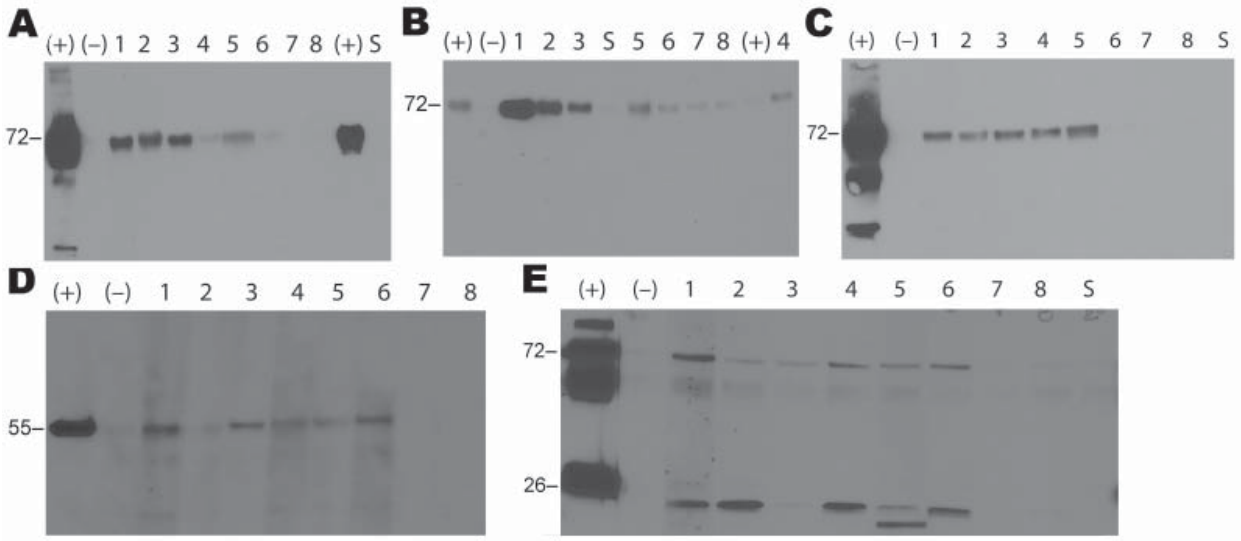

Figure 2. Results of Western blot analyses of pooled serum samples from adult guinea pigs in Ecuador. The guinea pigs were obtained from farms in Cuenca, where they had been raised as livestock, or from live animal markets in Guayaquil and Manabí. The results show different influenza antigens: recombinant hemagglutinin (rHA) A/New Caledonia/20/1999 (A); rHA A/Wisconsin/67/2005 (B); rHA A/Vietnam/1203/2004 (C); recombinant nucleoprotein A/Puerto Rico/08/1934 (D); and B virus (whole virus B/Yamagata/16/1988) (E). Molecular weights are shown at left. (+), serum from guinea pigs infected in the laboratory with influenza virus; (–), serum from naive guinea pig; lanes 1–4, pooled serum samples from Cuenca; lanes 5–6, pooled serum samples from Guayaquil; lanes 7–8, pooled serum samples from Manabí; S, secondary antibody only.

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Address for correspondence: Peter Palese, Mount Sinai School of Medicine,1 Gustave Levy Place, New York, NY 10029-6574, USA; email: peter.palese@mssm.edu

Multiple Introductions of Avian Influenza Viruses (H5N1), Laos, 2009–2010

Stephanie Sonnberg, Phouvong Phommachanh, Tri Satya Putri Naipospos, Joanna McKenzie, Chintana Chanthavisouk, Som Pathammavong, Daniel Darnell, Phetlamphone Meeduangchanh, Adam M. Rubrum, Mahanakhone Souriya, Bounkhouang Khambounheuang, Richard J. Webby, Bounlom Douangngeun, and Robert G. Webster

Avian influenza viruses (H5N1) of clades 2.3.4.1, 2.3.4.2, and 2.3.2.1 were introduced into Laos in 2009–2010. To investigate these viruses, we conducted active surveillance of poultry during March 2010. We detected viruses throughout Laos, including several interclade reassortants and 2 subgroups of clade 2.3.4, one of which caused an outbreak in May 2010.

Since 2003, highly pathogenic avian influenza virus (H5N1) has spread from southern China throughout Southeast Asia and to Europe and Africa (*1*,*2*). Since 2003, Laos has experienced outbreaks of clade 1 (2003), clade 2.3.4 (2006, 2007, 2008, 2009, 2010), and clade 2.3.2 viruses (twice in 2008) (*3*,*4*). Active surveillance of domestic ducks and chickens in Laos has been limited, but serum antibodies against subtypes H5 and H9 have been detected in ducks. In addition, subtype H5N1 virus was isolated from healthy ducks in 2006, and subtype H3N8 virus was detected in 2007 (*4*,*5*). To explore the diversity, extent, and endemicity of avian influenza viruses in Laos, we conducted a survey of healthy domestic poultry throughout the country in March 2010.

Author affiliations: St. Jude Children's Research Hospital, Memphis, Tennessee, USA (S. Sonnberg, D. Darnell, A.M. Rubrum, R.J. Webby, R.G. Webster); National Animal Health Centre, Vientiane, Laos (P. Phommachanh, P. Meeduangchanh, B. Douangngeun); Food and Agriculture Organization of the United Nations, Rome, Italy (T.S.P. Naipospos, J. McKenzie, C. Chanthavisouk, S. Pathammavong); and Ministry of Agriculture and Forestry, Vientiane (M. Souriya, B. Khambounheuang)

DOI: http://dx.doi.org/10.3201/eid1807.111642

The Study

Serum samples were collected in 9 of 17 provinces in Laos from healthy ducks and chickens in live-bird markets, village backyard flocks, and layer duck farms. Cloacal, tracheal, and environmental (fecal and water) swab specimens were also collected and placed immediately in transport medium (Figure). Swab specimens were screened in pools of 4 by using a real-time reverse transcription PCR for the matrix (M) gene segment (*6*). Positive pools were reextracted individually, retested, tested for hemagglutinin 5 (H5) by real-time reverse transcription PCR (*7*), and injected into 10–11-day-old embryonated chicken eggs.

Sequencing was conducted by using an Illumina (San Diego, CA, USA) platform (swabs and isolates) (*8*,*9*) and conventional Sanger sequencing (isolates). Illumina reads were first mapped to a database of publicly available human, avian, and swine influenza virus reference sequences from the Western Hemisphere Americas and Eurasia, and then remapped against references with the highest number of reads and best average coverage. Final average coverage varied between samples and segments with ranges of 123–24,163 (9 samples), 21–18,671 (12 samples) and 9–436 for sample A/duck/Lao/670/10. Isolate genotypes were verified by using Sanger

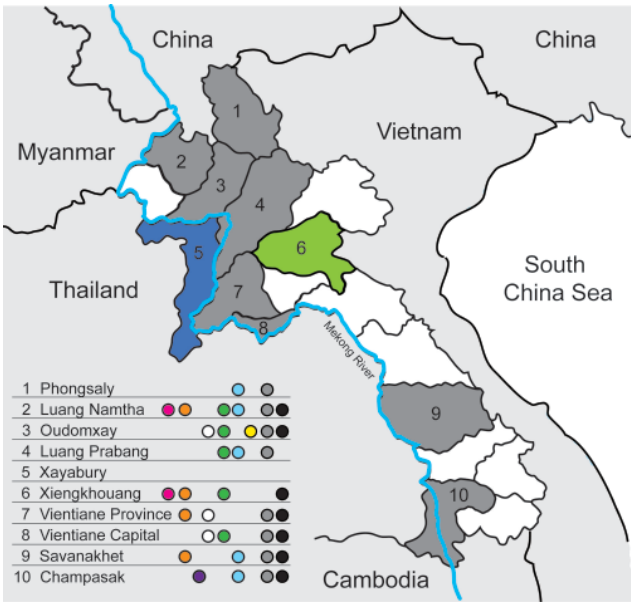

Figure. Areas sampled and location of subtyped avian influenza viruses (H5N1), Laos, 2009–2010. Provinces that had previous outbreaks of highly pathogenic avian influenza and were part of the survey are indicated in gray, the province that had a previous outbreak but was not part of the survey is indicated in blue, and the province that had not had an outbreak but was part of the survey is indicated in green. Colored dots indicate presence of viruses: light blue, anti-H5 (clade 2.3.4); gray, anti-H9 lineage G1; red, clade 2.3.4.1; orange, clade 2.3.4.2; green, anti-H5 (clade 2.3.2); black, anti-H9 lineage Y280; white, anti-H4; yellow, anti-H6; purple, clade 2.3.2.1 or virus-specific antibodies.

sequencing. Mixed infections could not be excluded for direct sequencing in the absence of an isolate.

Phylogenetic analysis (ClustalW [http://www.clustal.org/], neighbor-joining analysis, 1,000 bootstrap tests, maximum composite likelihood, pairwise deletions) was conducted by using MEGA version 5.02 (*10*). Sequences are available in GenBank (CY098294–CY098334, CY098336–CY098340, CY098342–CY098368, and CY098370–CY098464). Serum samples were screened by using an ELISA (FlockChek MultiS Screen; IDEXX Laboratories, Westbrook, ME, USA), and antibody-positive serum samples were tested by using a hemagglutinin inhibition assay for subtypes H3, H4, H5 (clades 2.3.2 and 2.3.4), H6, and H9 (lineages G1 and Y280) as described (*11*).

During March 2010, a total of 3,695 swab specimens were collected (1,928 duck and 279 chicken cloacal samples, 446 duck tracheal samples, 675 fecal samples, and 367 water samples). M gene prevalence was 4.0% (ducks), 1.8% (chickens), and 0.3% (environment samples). Five isolates were obtained (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/7/11-1642-FA1.htm). All M gene–positive swab specimens were collected in 13 locations (8 backyards, 2 markets, and 3 farms) (Table 1; Figure) Sample protection was suboptimal, and only 21 samples could be subtyped by real-time RT-PCR and sequencing (Table 1).

Phylogenetic analysis identified 3 groups of viruses: clades 2.3.2.1, 2.3.4.1, and 2.3.4.2 (online Appendix Figure). Two samples were closely related to A/chicken/Lao/LH1/2010–like virus (outbreak in Vientiane in April–May 2010) and to A/chicken/Laos/C100209–194-PTK/2009–like virus (outbreak in Phongsaly in February 2009) (clade 2.3.4.1) (online Appendix Figure; Table 1). These viruses were closely related to A/Guizhou/1/2009 and A/chicken/Vietnam/NCVD-404/2010 (online Appendix Figure).

Sixteen surveillance viruses of clade 2.3.4.2 were highly homogeneous (online Appendix Figure; Table 1) and closely related to A/environment/Guizhou/4/2009 and A/chicken/Vietnam/NCVD-394/2010–like viruses, although they contained the polymerase basic 2 (PB2) gene of a clade 2.3.2.1 donor virus and were therefore interclade reassortants (online Appendix Figure; Table 1). The genotype of the 4 isolates was verified by using Sanger sequencing. The PB2 gene was most closely related to A/grey_heron/Hong_Kong/1046/2008–like viruses. These reassortants were detected in 3 locations in northern (backyard), central (backyard), and southern (farm) Laos, suggesting multiple introductions of reassortants into Laos (online Appendix Figure; Table 1).

Three clade 2.3.2.1 viruses were detected in 2 ducks and 1 environmental sample (same trader). These viruses were interclade reassortants. Two (1 isolate and 1 direct sequence) contained 6 or 7 segments that were A/whooper swan/Mongolia/6/2009 like (clade 2.3.2.1), and the nucleoprotein gene was A/tree_sparrow/Jiangsu/1/08 like (clade 2.3.4). The environmental sample (direct

Table 1. Genotypes of 21 surveillance samples of avian influenza virus (H5N1) and outbreak virus A/chicken/Lao/LH1/2010, Laos, 2009–2010*

Sample ID	Sample type	Location†	Sampling site	Gene‡							
				PB2	PB1	PA	H5§	NP	N1	M	NS
A/ck/LH1	Chicken	Vientiane	Farm				2.3.4				
210317	Duck	Xiengkhouang-1	Market		NA	NA	2.3.4				
210265	Duck	Luang Namtha-1	Backyard		NA	NA	2.3.4				NA
210281	Env	Champasak-1	Market		NA	NA	2.3.2				NA
210287	Duck	Champasak-1	Market		NA		2.3.2				
210289	Duck	Champasak-1	Market				2.3.2				
210252	Duck	Luang Namtha-1	Backyard				2.3.4				
210253	Duck	Luang Namtha-1	Backyard				2.3.4				
210255	Duck	Luang Namtha-1	Backyard				2.3.4				
210358	Duck	Xiengkhouang-2	Backyard				2.3.4				
210360	Duck	Xiengkhouang-2	Backyard			NA	2.3.4				
210361	Duck	Xiengkhouang-2	Backyard				2.3.4				
210363	Env	Xiengkhouang-2	Backyard				2.3.4				
210367	Duck	Xiengkhouang-2	Backyard			NA	2.3.4				
210374	Duck	Xiengkhouang-2	Backyard				2.3.4				
210376	Env	Xiengkhouang-2	Backyard				2.3.4				
210378	Env	Xiengkhouang-2	Backyard				2.3.4				
210379	Duck	Xiengkhouang-2	Backyard				2.3.4				
210380	Duck	Xiengkhouang-2	Backyard				2.3.4				
210385	Duck	Xiengkhouang-2	Backyard				2.3.4				
210386	Duck	Xiengkhouang-2	Backyard				2.3.4				
210349	Duck	Savanakhet-1	Farm				2.3.4			NA	

*__Boldface__ indicates outbreak virus isolate and closely related isolates. ID, identification; PB, polymerase basic; PA, polymerase acidic; H, hemagglutinin; NP, nucleoprotein; N, neuraminidase; M, matrix; NS, nonstructural; NA, not available; env, environmental.
†Identical numbers indicate same sampling site.
‡Pink shading, A/Guizhou/1/2009-like clade 2.3.4.1; blue shading, A/whooper swan/Mongolia/6/2009-like clade 2.3.2.1; purple shading, A/tree_sparrow/Jiangsu/1/08-like clade 2.3.4; gray shading, A/environment/Guizhou/4/2009-like clade 2.3.4.2.
§Clade is indicated.

sequence) contained A/whooper swan/Mongolia/6/2009–like hemagglutinin and M genes and A/Guizhou/1/2009–like PB2, nucleoprotein, and neuraminidase genes (clade 2.3.4.1) (Table 1). The genotype of the isolate was verified by using Sanger sequencing. Hemagglutinin segments of the reassortants were identical (100% nt identity). This identity and the source of the 3 samples (1 trader) suggest that reassortment occurred recently, likely in Laos.

Table 2. Serum HI titers in ducks against avian influenza virus (H5N1) H3, H4, H5, H6, and H9 antigens, Laos, 2009–2010*

Serum			HI test antigen subtype‡ (clade or lineage)§					
sample ID	Location†	Sampling site	H4	H5 (2.3.2)	H5 (2.3.4)	H6	H9 (G1)	H9 (Y280)
790	Phongsaly-1	Backyard	<10	<10	<5	<10	10	<10
797	Phongsaly-1	Backyard	<10	<10	5	<10	<10	<10
798	Phongsaly-1	Backyard	<10	<10	<5	<10	10	<10
925	Phongsaly-2	Backyard	<10	<10	5	<10	<10	<10
11A	Luang Namtha-1	Backyard	<10	<10	<5	<10	10	10
11B	Luang Namtha-1	Backyard	<10	40	<5	<10	<10	10
12	Luang Namtha-1	Backyard	<10	160	<5	<10	<10	<10
14	Luang Namtha-1	Backyard	<10	160	40	<10	<10	<10
15	Luang Namtha-1	Backyard	<10	320	<5	<10	<10	<10
1686	Luang Namtha-2	Backyard	<10	<10	<5	<10	<10	640
1688	Luang Namtha-2	Backyard	<10	<10	<5	<10	10	<10
1696	Luang Namtha-2	Backyard	<10	<10	<5	<10	10	<10
1699	Luang Namtha-2	Backyard	<10	<10	<5	<10	<10	320
1736	Luang Namtha-3	Market	<10	<10	<5	<10	10	<10
757	Luang Namtha-3	Market	<10	<10	10	<10	<10	<10
1314	Oudomxay-1	Backyard	80	<10	<5	80	<10	<10
1319	Oudomxay-1	Backyard	40	10	<5	320	<10	<10
1322	Oudomxay-2	Market	<10	<10	<5	<10	<10	40
1324	Oudomxay-2	Market	<10	<10	<5	<10	<10	10
1325	Oudomxay-2	Market	<10	<10	<5	<10	<10	40
1350	Oudomxay-2	Market	<10	<10	<5	<10	<10	160
1362	Oudomxay-3	Market	80	<10	<5	<10	<10	<10
1372	Oudomxay-3	Market	<10	<10	<5	<10	<10	40
1459	Oudomxay-4	Backyard	<10	<10	<5	640	320	320
1470	Oudomxay-5	Farm	<10	<10	<5	<10	<10	10
24	Luang Prabang-1	Backyard	<10	10	<5	<10	<10	<10
36	Luang Prabang-1	Backyard	<10	<10	<5	<10	10	<10
37	Luang Prabang-1	Backyard	<10	<10	<5	<10	10	<10
38	Luang Prabang-1	Backyard	<10	<10	<5	<10	10	<10
951	Luang Prabang-2	Backyard	<10	<10	5	<10	<10	<10
958	Luang Prabang-2	Backyard	<10	<10	5	<10	10	<10
721	Xiengkhouang-3	Backyard	<10	10	<5	<10	<10	<10
1673	Xiengkhouang-4	Farm	<10	<10	<5	<10	<10	10
1378	Vientiane-1	Backyard	640	<10	<5	<10	20	40
1379	Vientiane-1	Backyard	<10	<10	<5	<10	10	<10
1381	Vientiane-1	Backyard	40	<10	<5	<10	20	<10
1382	Vientiane-1	Backyard	<10	<10	<5	<10	10	<10
1386	Vientiane-1	Backyard	40	<10	<5	<10	<10	40
1389	Vientiane-1	Backyard	<10	<10	<5	<10	10	<10
1393	Vientiane-1	Backyard	40	<10	<5	<10	<10	<10
1526	Vientiane-2	Farm	<10	<10	<5	<10	<10	10
463	Vientiane-1	Market	<10	<10	<5	<10	160	320
464	Vientiane-1	Market	<10	<10	<5	<10	>1,280	>1,280
467	Vientiane-1	Market	<10	<10	<5	<10	160	160
468	Vientiane-1	Market	<10	<10	<5	<10	<10	80
1245	Vientiane-2	Backyard	<10	<10	<5	<10	<10	10
1249	Vientiane-2	Backyard	<10	10	<5	<10	<10	<10
1277	Vientiane-2	Backyard	<10	10	<5	<10	<10	<10
1419	Vientiane-3	Market	20	<10	<5	<10	<10	<10
657	Savanakhet-2	Farm	<10	<10	<5	<10	10	10
990	Savanakhet-3	Farm	<10	<10	10	<10	<10	<10
589	Champasak-2	Backyard	<10	<10	<5	<10	40	<10
606	Champasak-3	Backyard	<10	<10	20	<10	10	10

*H, hemagglutinin; ID, identification; HI, hemagglutination inhibition.
†Identical numbers indicate same sampling site.
‡All titers for H3 (A/duck/Laos-Xaythany/A0573/2007) were <10. H4, A/duck/Czech/1956; H5 clade 2.3.2, A/common magpie/Hong Kong/5052/2007; H5 clade 2.3.4, A/duck/Laos/3295/2006; H6, A/turkey/Massachusetts/1965; H9 lineage G1, A/Hong Kong/33982/2009; H9 lineage Y280, A/duck/Hong Kong/Y280/1997.
§Values represent HI titers reciprocal to the highest dilution of serum that inhibited haemagglutination of 4 hemagglutination units of antigen by using 0.5% chicken erythrocytes.

Antibody titers to H5 and H9 and a subtype H3N8 virus isolate have been reported in Laos (*4*,*5*). Subtypes H4 and H6 also circulate in this region (*1*,*2*,*12*). For this study, 2,148 serum samples (1,899 from ducks, 200 from chickens, and 49 from unspecified species) were collected and 267 antibody-positive (seroprevalence 14%) duck and 15 (7.5%) chicken serum samples were detected. Hemagglutination inhibition testing for specific antibodies against H3, H4, H5, H6, and H9 detected all antibodies but to H3 (Table 2). Antibodies against H9 were detected at highest titers and most frequently (1.1% of ducks for each H9 lineage), although some cross-reactivity between G1 and Y280 likely occurred (Table 2). These ducks were most widely distributed (18 [19%] of 97 locations in all 9 provinces). Antibodies against H5 clade 2.3.4 were found at the detection limit in few serum samples (0.4% of ducks in 3 northern and 2 southern provinces). Antibodies against H5 clade 2.3.2 were detected in 0.4% of ducks in 4 northern provinces and the capital of Vientiane.

Among ducks, 34 (1.7%) were either shedding or had antibodies against avian influenza virus (H5N1), and there was ≥1 virus-positive or antibody-positive duck in each of the 9 provinces sampled. One village had 36% of sampled ducks exposed to this virus; 18% shed 2.3.4 virus and 18% had antibody to 2.3.2 virus (Table 2). One duck in this village had antibodies against 2.3.2 and 2.3.4 clade viruses. Exposure to 2.3.2 and 2.3.4 viruses was evident in ducks from locations in 3 other provinces (1 district each in Champasak and Xiengkhoung, and several districts in Luang Prabang).

Conclusions

This study showed that 3 groups of avian influenza viruses (H5N1) were likely introduced into Laos in 2009–2010, one of which resulted in 2 outbreaks (2009, 2010). In all 9 provinces where surveillance was conducted, ducks had been exposed to this virus. Evidence of clades 2.3.2 and 2.3.4 virus activity was detected in 4 provinces. Several interclade reassortants were identified, demonstrating the high genetic mobility of these viruses in the region. Since 2004, Laos has had repeated outbreaks of highly pathogenic avian influenza viruses, which have also been detected in China and Vietnam. There is no evidence that a particular virus lineage has established itself in Laos. The frequency of introduction, diversity, and extent of these viruses in Laos suggests considerable movement of viruses into the country from surrounding territories (China and Vietnam, but not Cambodia) and within the country.

Acknowledgments

We thank Jennifer DeBeauchamp, Scott Krauss, Mariette Ducatez, David Walker, Jerry Parker, Richard Elia, Betsy Williford, and David Galloway for technical assistance, data management, and help in preparing the figures and manuscript; and the Australian Animal Health Laboratory (Geelong, Victoria, Australia) for provision of Lao H5 sequences of avian influenza virus.

This study was supported by US Agency International Development project OSRO/RAS/604/USA Baby 3, National Institutes of Health contract HHSN266200700005C, and American Lebanese Syrian Associated Charities.

Dr Sonnberg is a postdoctoral research associate at St. Jude Children's Research Hospital, Memphis, Tennessee. Her research interests are surveillance, transmission propensity, and reassortment of influenza viruses.

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Address for correspondence: Stephanie Sonnberg, Division of Virology, St. Jude Children's Research Hospital, MS 330, Memphis, TN 38105, USA; email: stephanie.sonnberg@stjude.org

Human Infection from Avian-like Influenza A (H1N1) Viruses in Pigs, China

Huanliang Yang,[1] Chuanling Qiao,[1] Xu Tang, Yan Chen, Xiaoguang Xin, and Hualan Chen

In investigating influenza in an immunodeficient child in China, in December 2010, we found that the influenza virus showed high sequence identity to that of swine. Serologic evidence indicated that viral persistence in pigs was the source of infection. Continued surveillance of pigs and systemic analysis of swine influenza isolates are needed.

Humans have been infected with avian-like swine influenza A (H1N1) viruses (SIVs) several times since the first case was diagnosed in Switzerland in 1986 (*1*). These cases generally occur in persons who have direct exposure to pigs (*2*–*4*). On December 31, 2010, a 3-year-old boy in rural Jiangsu Province, People's Republic of China, who had chronic renal disease (for which he was given long-term steroid treatment), sought care with influenza-like symptoms. Laboratory tests at the Chinese Center for Disease Control and Prevention yielded a positive result for European avian-like A (H1N1) SIV, indicating that the European avian-like SIV also caused human infection in the Asia-Pacific region.

The Study

After notification of the boy's infection from the Ministry of Health, we performed active public health surveillance to locate the origin of the infection. A total of 60 nasal swab specimens were collected from pigs at the patient's family farm and a local slaughterhouse. Each swab was placed in 2 mL of minimal essential medium supplemented with penicillin (2,000 U/mL) and streptomycin (2,000 U/mL). Virus was isolated by using 10-day-old specific pathogen-free embryonated chicken eggs. Hemagglutinin (HA) and neuraminidase (NA) subtypes were determined as described (*5*). Three A (H1N1) SIVs were obtained, including 2 isolates from pigs in the slaughterhouse and 1 from a pig raised at the family's farm. Viral RNA was extracted and reverse transcribed under standard conditions by using the Uni12 (5′-AGCAAAAGCAGG-3′) primer. The viral genomes were amplified by PCR and sequenced by using segment-specific primers (sequences available on request). Genomic sequencing ultimately showed that the 3 isolates were virtually identical, and the sequence of the entire genome of the representative strain A/swine/Jiangsu/40/2011 (Sw/JS/40/11) is available in GenBank (accession nos. JQ319645–JQ319652). Unrooted phylogenetic trees were generated by using MEGA5 software (www.megasoftware.net). The A (H1N1) viruses isolated in this study fell into the European avian-like swine A (H1N1) lineage (Figure 1). The homology of the polymerase basic protein (PB) 2, PB1, polymerase acidic protein, HA, nucleocapsid protein, NA, matrix (M), and nonstructural protein genes between the Sw/JS/40/11 virus and the A/Jiangsu/1/2011 (JS/1/11) virus, which was isolated from the child, were 99.3%, 99.3%, 99.3%, 99.7%, 99.7%, 99.4%, 99.6%, and 99.1%, respectively, indicating that they might have been derived from the same ancestor.

The receptor-binding property of the HA protein is a major molecular determinant of host range. The amino acids at sites 190 and 225 of HA are major determinants of the receptor-binding specificity of the A (H1N1) virus, and the mutations E190D and D225E in HA switch the virus receptor-binding specificity from α-2,3–linked sialosides to α-2,6–linked sialosides (*6*). The Sw/JS/40/11 and JS/1/11 isolates have the amino acids D at site 190 and E at site 225 within the HA protein, which implies that these viruses might preferentially bind to α-2,6–linked sialosides. Potential glycosylation sites (PGSs) also have a major effect on the antigenic and receptor-binding properties of influenza A viruses. Molecular analysis showed that the 2 Jiangsu strains had 5 PGSs in their HA1 proteins, 4 of which were the same as those of the A/Netherlands/386/1986 virus (the cause of the first avian-like SIV infection in a human). Antigenic sites in the H1 HAs, i.e., Sa, Sb, Ca1, Ca2, and Cb, were compared between A/Netherlands/386/1986 and JS/1/11. Amino acid mutations H159N, K238R, and G239E were observed at the Ca2 site; R187G at the Ca1 site; and T202D, N203S, S207T, and A212N at the Sb site. Compared with JS/1/11, the unique mutation D204V, located at the Sb site, which is an antigenic site near the receptor-binding site in influenza virus (*7*), occurred in the HA1 of Sw/JS/40/11 (Figure 2). No oseltamivir resistance–conferring substitutions (H274Y and N294S) were observed in the NA proteins of the 2 viruses, which suggests that they are sensitive to NA inhibitors (*8*). The amino acid sequence of the M2 protein of the 2 isolates did not contain the I27T or S31N substitution, characteristic

Author affiliation: State Key Laboratory of Veterinary Biotechnology–Harbin Veterinary Research Institute, Harbin, People's Republic of China

DOI: http://dx.doi.org/10.3201/eid1807.120009

[1]These authors contributed equally to this article.

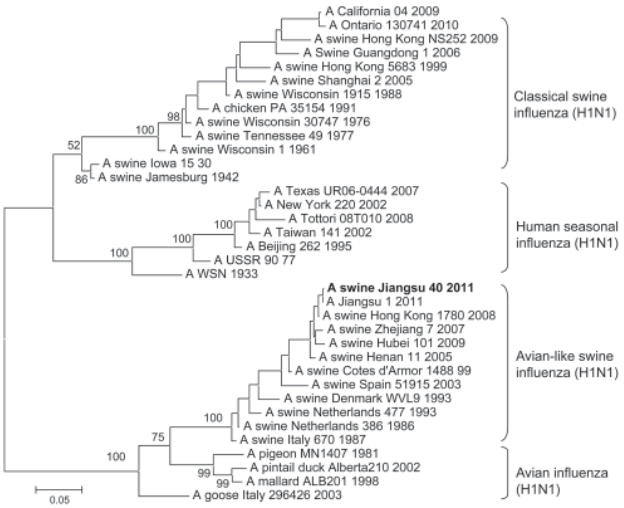

Figure 1. Phylogenetic tree of selected swine, human, and avian H1 hemagglutinin 1 sequences. An unrooted phylogenetic tree was generated by the distance-based maximum-likelihood method by using MEGA5 software (www.megasoftware.net). Bootstrap values were calculated on the basis of 1,000 replications; A/swine/Jiangsu/40/2011 is in **boldface**. Scale bar indicates nucleotide substitutions per site.

of amantadine resistance in influenza viruses (*9*,*10*). The 627K and 701N residues in the PB2 protein contribute to the replication and transmission of avian influenza viruses in mammalian hosts (*11*–*14*). Similar to most avian-like A (H1N1) SIVs, both isolates (JS/1/11 and Sw/JS/40/11) had 701N in their PB2 gene.

Chicken antiserum against different subtype H1N1 or H1N2 SIVs were used for antigenic analysis. The Sw/JS/40/11 virus reacted with the antiserum against the classical A (H1N1) SIV (A/swine/Guangdong/6/2010), the triple reassortant A (H1N2) SIV (A/swine/Tianjin/1/2007), influenza A(H1N1)pdm09 (A/swine/Heilongjiang/44/2009), and the avian-like SIV (A/swine/Henan/11/2005) (Table), but not with the antiserum against the human-like A (H1N1) SIV (A/swine/Hebei/15/2009). Antiserum against Sw/JS/40/11 reacted only with the avian-like A (H1N1) SIV and the human-like A (H1N1) SIV, but the HI titers against the human-like A (H1N1) SIV were 4-fold lower than those against the avian-like A (H1N1) SIVs. These results suggest that the H1 subtype SIVs circulating in China differ antigenically.

We investigated antibody responses in 20 serum samples from pigs at the patient's family farm and the local slaughterhouse. Serologic assays showed that the seroprevalence of antibodies to the avian-like A (H1N1) SIVs was 55% and to classical A (H1N1) SIVs and A(H1N1)pdm09 virus were 25% and 30%, respectively. Furthermore, antibodies against A(H3N2) SIVs were observed but at the low rate of 10%.

Conclusions

We showed that similar viruses were simultaneously prevalent in a local pig population when a child was infected with an avian-like A (H1N1) SIV. Specifically, isolation of avian-like SIV from a family farm provides direct evidence for the origin of the human infection. No further spread of the Sw/JS/40/2011-like swine strain occurred, according to the limited information available; however, the incident aroused interest in influenza in animals, especially in pigs. Antigenic analysis showed that this avian-like A (H1N1) SIV was antigenically divergent from classical A (H1N1) and human-like A (H1N1) SIVs currently circulating in China, which was further reinforced by the heterogeneity of their genetic relationships. Since early avian-like A (H1N1) SIV isolates in humans, amino acid mutations in the antigenic sites and PGS changes might have altered the antigenic properties in the avian-like A (H1N1) SIV cluster. Our data highlight the need to characterize circulating strains antigenically and genetically through regular influenza virus surveillance.

Pigs can serve as intermediate hosts for influenza viruses to evolve toward efficient replicability in humans. The classical A (H1N1) SIVs and European avian-like A (H1N1) SIVs have circulated worldwide in pigs since 1930 and 1979, respectively, and a classical A (H1N1) SIV emerged in humans as a triple reassortant, causing the 2009 influenza pandemics (*15*). Although the virulence and transmissibility of the avian-like A (H1N1) SIVs remain

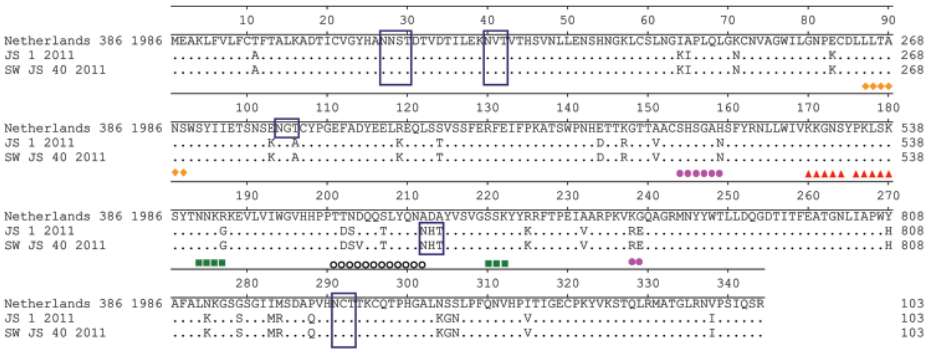

Figure 2. Multiple alignment of hemagglutinin protein sequences. Epitopes Sa, Sb, Ca1, Ca2, and Cb are indicated. Triangle, Sa; circle, Sb; square, Ca1; hexagon, Ca2; diamond, Cb. Putative glycosylation sites are indicated in blue-lined boxes.

Table. Antigenic analysis of H1 swine influenza viruses, People's Republic of China*

Virus	HI antibody titers of chicken antiserum against†					
	Classical swine A (H1N1)	Triple-reassortant A (H1N2)	A (H1N1) pdm09	Human seasonal A (H1N1)	Avian-like swine A (H1N1)‡	Avian-like swine A (H1N1)§
Classical swine A (H1N1)	**512**	512	512	¶	8	¶
Triple-reassortant A (H1N2)	1024	**512**	1,024	¶	8	¶
A(H1N1)pdm09	512	512	**1,024**	¶	16	¶
Human seasonal A (H1N1)	16	32	64	**32**	32	64
Avian-like swine A (H1N1)‡	128	64	128	¶	**512**	256
Avian-like swine A (H1N1)§	32	16	64	¶	256	**256**

*Classical swine A (H1N1), A/swine/Guangdong/6/2010; triple-reassortant A (H1N2), A/swine/Tianjin/1/2007; A(H1N1)pdm09, A/swine/Heilongjiang/44/2009; human seasonal A (H1N1), A/swine/Hebei/15/2009.
†Antiserum was generated by inoculating specific pathogen-free chickens with an oil-emulsified inactivated vaccine derived from the indicated viruses. Homologous titers are shown in **boldface**.
‡A/swine/Jiangsu/40/2011.
¶HI titer <2.
§A/swine/Henan/11/2005.

to be evaluated, recurrent human infections with avian-like A (H1N1) SIVs suggest that after long-term adaptation in pigs, the avian-like A (H1N1) SIVs already can replicate in humans. After further whole-genome adaptation to the human host or reassortment with other viruses, novel strains bearing the avian-like swine subtype H1N1 HA gene are highly likely to be generated with pandemic potential. Continued surveillance of swine and systemic analysis of swine influenza isolates are clearly needed.

Acknowledgments

We thank Susan Watson for editing the manuscript.

This study was supported by the 973 Program (2011CB505001, 2010CB534001), the Chinese National Science Fund for Distinguished Young Scholars (30825032), Harbin Municipal S&T Plan (2009AA6BN078), and the Scientific Research Program of the State Key Laboratory of Veterinary Biotechnology (NKLVSP201013).

Dr Yang is a veterinary microbiologist in the Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, China. His research interests are the surveillance and molecular epidemiology of SIVs.

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Address for correspondence: Hualan Chen, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, 427 Maduan St, Harbin 150001, People's Republic of China; email: hlchen1@yahoo.com

Electronic Event–based Surveillance for Monitoring Dengue, Latin America

Anne G. Hoen,[1] Mikaela Keller, Aman D. Verma, David L. Buckeridge, and John S. Brownstein

The current dengue epidemic in Latin America represents a major threat to health. However, surveillance of affected regions lacks timeliness and precision. We investigated real-time electronic sources for monitoring spread of dengue into new regions. This approach could provide timely estimates of changes in distribution of dengue, a critical component of prevention and control efforts.

Dengue, a potentially fatal viral disease, has been recognized for >200 years (*1*). Once sporadic and limited geographically, dengue viruses (DENVs) and their mosquito vectors have spread globally, putting an estimated 2.5 billion persons at risk throughout the tropical and subtropical regions of the world (*2*). Factors contributing to the dramatic expansion of DENV activity include demographic changes such as population growth, urbanization, and globalization, and reductions in vector control and other public health measures (*3*). Because of the nature of passive surveillance, conventional systems have limited ability in identifying new epidemics quickly (*1*), thus suggesting a role for alternative information sources.

Free or low-cost sources of unstructured information, such as Internet news outlets, health expert mailing lists, social media sites, and queries to online search engines, when computationally filtered and mined, can provide detailed local and near real-time data on potential or confirmed disease outbreaks (*4*). For dengue in particular, our group and others recently reported on a set of Google search terms that parallel temporal trends in official dengue case counts (*5*,*6*). These event-based data sources can provide insight into new and ongoing public health challenges in areas of the world with limited public health reporting infrastructure.

Few studies have investigated the value of unofficial sources for monitoring recent geographic expansion of infectious disease risk. Using dengue as a case study, we report on the utility of electronic outbreak surveillance for real-time monitoring of recent infectious disease spread.

The Study

We focused on the geographic range of DENVs in Latin America and the Caribbean, where dengue is widespread and expanding in range. We attempted to identify areas contiguous with previously known dengue-endemic zones where new DENV transmission is occurring by using reports of recent outbreaks.

Known dengue-endemic areas were defined as dengue risk areas identified by the US Centers for Disease Control and Prevention (Atlanta, GA, USA) Health Information for International Travel (commonly referred to as the Yellow Book), 2010 (*7*) and 2012 (*8*) editions. Each edition of this book reflects the known distribution of dengue risk in the prior 2 years. To characterize spread according to the Yellow Book, we identified areas that were classified as no known dengue risk in 2010 but were changed to risk areas in the 2012 edition (hereafter referred to as new dengue-endemic areas).

Outbreak data for December 1, 2009–March 18, 2011, were collected from HealthMap (http://www.healthmap.org/en/an), an open access online infectious disease outbreak monitoring system (*9*,*10*). HealthMap integrates outbreak-related data from >30,000 electronic sources, including the news media, ProMED-mail, and other electronic public health reporting sources, by using algorithms to classify the diseases and locations associated with each report. Because we wanted to identify spread into new dengue-endemic zones, we limited our analyses to areas that were identified as having no known dengue risk in the 2010 Yellow Book but that were contiguous with ≥1 risk areas in the 2010 Yellow Book. We identified 53 dengue outbreaks distributed in 60 of these areas.

We fitted a bivariate Gaussian mixture model to the extracted HealthMap alerts to model a continuous surface of outbreak density (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/12-0055-Techapp.pdf). This modeled outbreak probability density surface represents a risk map of recent DENV spread into areas of previously unknown dengue endemicity according to the 2010 Yellow Book (Figure 1). We compared our map with the geographic

Author affiliations: Children's Hospital Boston, Boston, Massachusetts, USA (A.G. Hoen, J.S. Brownstein); Harvard Medical School, Boston (A.G. Hoen, J.S. Brownstein); Institut de Recherche en Informatique et Automatique, Lille, France (M. Keller); Université de Lille, Lille (M. Keller); McGill University, Montreal, Quebec, Canada (A.G. Hoen, A.D. Verma, D.L. Buckeridge, J.S. Brownstein); and Agence de la Santé et des Services Sociaux de Montreal, Montreal (D.L. Buckeridge)

DOI: http://dx.doi.org/10.3201/eid1808.120055

[1]Current affiliation: The Geisel School of Medicine at Dartmouth College, Lebanon, New Hampshire, USA.

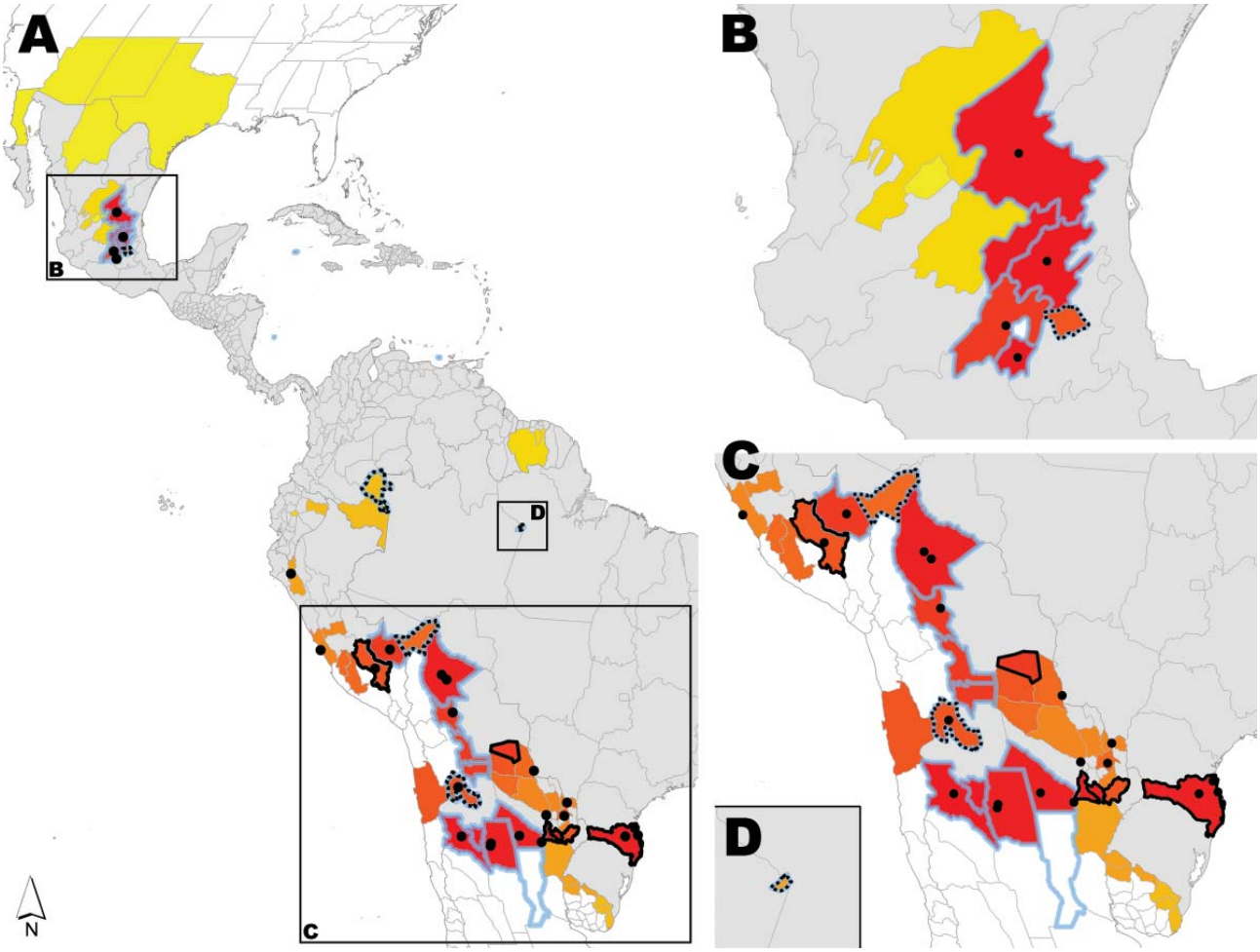

Figure 1. A) Regions in Latin America analyzed for dengue. B) Central Mexico; C) central South America; D) eastern Amazonas, Brazil. Thin gray lines indicate boundaries of first-level administrative units. Areas to which dengue was identified in the 2010 Yellow Book are shaded in gray. New dengue-endemic areas identified in the 2012 Yellow Book are outlined in blue. Dots indicate HealthMap dengue-related alerts. Modeled HealthMap alert probability density surface is shown in a gradient from yellow to red with yellow areas predicted as having lower alert densities and red areas predicted as having higher alert densities according to the model. Areas outlined with heavy black solid lines were classified as high HealthMap alert density but were not identified in either Yellow Book edition as dengue risk areas. Areas outlined with heavy black dashed lines were classified as low HealthMap alert density but were identified in the 2012 Yellow Book as areas at risk for dengue.

distribution of new dengue-endemic areas identified in the 2012 Yellow Book. Details of the datasets, models, and statistical methods are available in the online Technical Appendix.

Figure 1 shows that high dengue outbreak activity occurred adjacent to previously recognized dengue-endemic zones in 6 states in central Mexico and in parts of northern Argentina, southern Brazil, Bolivia, and Paraguay. We used receiver-operating characteristic analysis with cross-validation (Figure 2) to set a threshold dengue report density that best identifies new dengue-endemic areas (Figure 1; online Technical Appendix). Of the 19 new dengue-endemic areas reported in the 2012 Yellow Book, this threshold identified 14 (74%) as being at elevated risk of endemicity, according to the dengue outbreak probability density estimated by our model. Of the 41 areas that remained unidentified as dengue-endemic areas in the 2012 Yellow Book, our model classified 35 (85%) as having reduced risk of endemicity.

When compared with the Yellow Book, our model incorrectly classified 6 areas as at elevated risk (Figure 1). All alerts in these areas described outbreaks of cases acquired in a nearby known dengue-endemic region of the country. One alert also warned of the recent discovery of dengue vector mosquito larvae by the local surveillance program. The model also classified 5 Yellow Book dengue-endemic areas as at reduced risk (Figure 1). Four of these areas were rural or isolated. Although other explanations

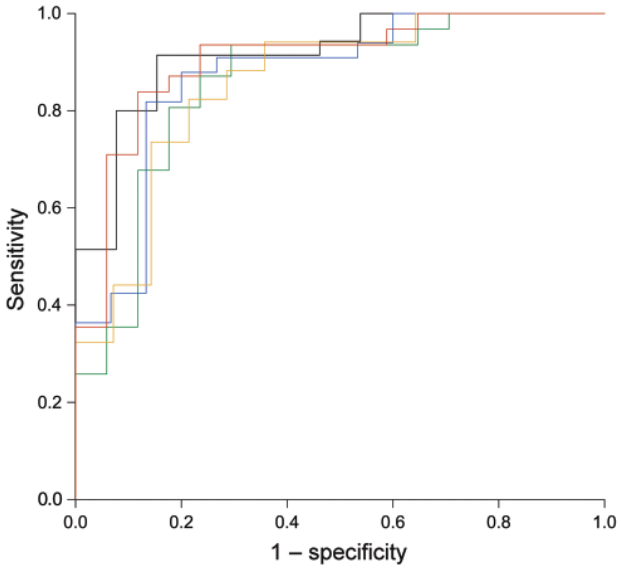

Figure 2. Receiver-operating characteristic plot of 5-fold cross-validated HealthMap alert density-based classification with new dengue-endemic areas identified by the 2012 Yellow Book as the standard.

likely exist, the low observed sensitivity in these areas illustrates certain limits of any system that relies on Internet-based information flow for monitoring disease spread.

Conclusions

Electronic event–based surveillance systems such as HealthMap and others are frequently used by public health authorities, travelers, physicians and patients, to gain a real-time understanding of global outbreak activity. The HealthMap dengue feed, DengueMap, is currently part of the online dengue information resource of the Centers for Disease Control and Prevention (http://www.cdc.gov/dengue/). Used in combination with traditional case reporting, HealthMap and other electronic surveillance systems have proven value for enhancing the timeliness of outbreak discovery and information dissemination (*11*). However, these information sources may also provide added value for monitoring ongoing spread.

Although the signal of DENV activity detected by HealthMap is relatively robust, it has certain limitations. First, the signal tends to be sparse in areas with limited reporting because of low population density or incomplete coverage by the news or social media. Second, the signal can be surrounded by background noise because separating reports caused by cases in travelers from true autochthonous transmission is difficult with automated methods. By limiting our analysis to areas contiguous with known dengue-endemic areas and smoothing outbreak alerts into an outbreak-density surface, we were able to identify a reliable signal of dengue spread. Although this analysis was performed retrospectively, the timeliness of this signal far outperforms any traditional surveillance data stream. Passive case report-based surveillance systems typically operate at a delay of weeks to months, which limits their value for providing a picture of geographic spread, especially on an international scale where surveillance delays may be even more prolonged.

We have demonstrated a novel approach to real-time monitoring of recent expansion of DENV activity in Latin America. Using outbreak reports captured by HealthMap, we identified a signal of geographic expansion of dengue activity that would precede official reports of the geographic distribution of dengue-endemic areas. Currently, no reliable surveillance system is in widespread use that reports the distribution of DENV activity on an ongoing basis and enables near real-time monitoring of trends in geographic expansion. Such a system should enhance the ability of regional and global public health authorities to dynamically allocate resources within a time frame that might effectively avert a full-blown epidemic. Like other large-scale surveillance data sources, our results must be interpreted cautiously. However, when used in conjunction with traditional surveillance methods, our approach has the potential to provide a timely estimate of changes in the geographic distribution of dengue, a critical component of targeted prevention and control efforts.

Acknowledgments

We thank Michael Johansson for critically revising the manuscript and providing Yellow Book data; and Katia Charland, Laura Pinheiro, Emily Chan, Susan Aman, Clark Freifeld, Katelynn O'Brien and Sumiko Mekaru for helpful discussions and database support.

This study was supported by National Institutes of Health grants R01 LM010812 and G08 LM009776.

Dr Hoen is an infectious disease epidemiologist in the Department of Community and Family Medicine at The Geisel School of Medicine at Dartmouth College. Her research interests include informatics-based approaches to understanding infectious disease risk, emergence and spread.

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Address for correspondence: Anne G. Hoen, Dartmouth-Hitchcock Medical Center, 1 Medical Center Dr, HB 7937, Lebanon, NH 03756, USA; email: anne.g.hoen@dartmouth.edu

Changing Socioeconomic Indicators of Human Plague, New Mexico, USA

Anna M. Schotthoefer,[1] Rebecca J. Eisen, Kiersten J. Kugeler, Paul Ettestad, Pamela J. Reynolds, Ted Brown, Russell E. Enscore, James Cheek, Rudy Bueno, Jr., Joseph Targhetta, John A. Montenieri, and Kenneth L. Gage

Socioeconomic indicators associated with temporal changes in the distribution of human plague cases in New Mexico were investigated for 1976–2007. In the 1980s, cases were more likely in census block groups with poor housing conditions, but by the 2000s, cases were associated with affluent areas concentrated in the Santa Fe–Albuquerque region.

Plague is a severe zoonotic disease caused by *Yersinia pestis*. An average of 11 cases per year have occurred in the United States (range 1–40 cases) since 1976 (*1*); most cases in recent decades have been found in New Mexico (*2*,*3*). The pathogen cycles between rodents and fleas, and humans most frequently become infected through flea bites (*4*). Living near habitats that support the sylvatic cycle is a major risk factor associated with human disease in New Mexico (*2*,*5*,*6*). However, even in areas defined as high risk on the basis of environmental characteristics (*6*), plague is rare, and the area defined as highly suitable for plague represents a large geographic region (≈52,626 km^2).

Poor socioeconomic status has been anecdotally associated with human plague cases, but this factor has rarely been investigated quantitatively in the United States, and such information has not been systematically collected for cases of *Y. pestis* infection. Identifying human socioeconomic or behavioral risk factors may enable a more refined definition of the highest risk populations for more targeted control efforts. To evaluate possible associations between socioeconomic factors and plague risk, we used US Census Bureau decennial data to compare census block groups (CBGs) in which human plague cases occurred and did not occur in New Mexico during 1976–2007.

The Study

We restricted our analyses to peridomestic cases that were reported in the geographic region previously determined to be at high risk on the basis of environmental factors (*6*). Thus, we included 123 (75.9%) of 162 cases reported in New Mexico during the study period. We also restricted our analyses to the CBGs that had population densities within the range of densities found in plague-positive CBGs (0.05 and 1,425.40 persons/km^2). This restriction avoided comparing rural to urban CBGs because plague tends to occur in rural to lightly suburbanized areas. The final area considered encompassed ≈17% of New Mexico (*6*) and included the entirety or portions of 483 CBGs (Figure; Table 1).

To relate plague occurrence to socioeconomic conditions of CBGs at times when cases occurred, we divided the study period into 3 time frames centered on the most recent census. Thus, 1976–1985 cases were associated with 1980 census data, 1986–1995 cases with 1990 census data, and 1996–2007 cases with 2000 census data. Variables that described economic status and housing conditions were extracted from each census, normalized to US Census 2000 CBG boundaries (Geolytics, Inc., East Brunswick, NJ, USA; www.geolytics.com), and compared between plague-positive and plague-negative CBGs by using Wilcoxon rank sum tests (Table 1). For each time frame, the risks of CBGs having at least 1 case of human plague on the basis of significant variables were then evaluated by using 2 × 2 tables. CBGs were divided into high and low categories by using median values of each variable as division points.

Plague risk was positively associated with CBGs that had an ecotone habitat identified by Eisen et al. (*6*) as especially suitable for human plague cases (e.g., convergence of the Rocky Mountain/Great Basin open and closed coniferous woodland habitats; odds ratio 4.18, 95% CI 2.66–6.57). Therefore, to ensure that we were measuring differences in socioeconomic conditions and not the presence or absence of the ecotone habitat in CBGs, we also calculated adjusted odds ratios for each variable and time frame by using Mantel-Haenszel tests.

Author affiliations: Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (A.M. Schotthoefer, R.J. Eisen, K.J. Kugeler, R.E. Enscore, J.A. Montenieri, K.L. Gage); New Mexico Department of Health, Santa Fe, New Mexico, USA (P. Ettestad, P.J. Reynolds [retired]); New Mexico Environment Department, Santa Fe (T. Brown [retired]); Indian Health Services, Albuquerque, New Mexico, USA (J. Cheek); Harris County Public Health and Environmental Services, Houston, Texas, USA (R. Bueno, Jr.); and City of Albuquerque Division of Environmental Health, Albuquerque (J. Targhetta)

DOI: http://dx.doi.org/10.3201/eid1807.120121

[1]Current affiliation: Marshfield Clinic Research Foundation, Marshfield, Wisconsin, USA.

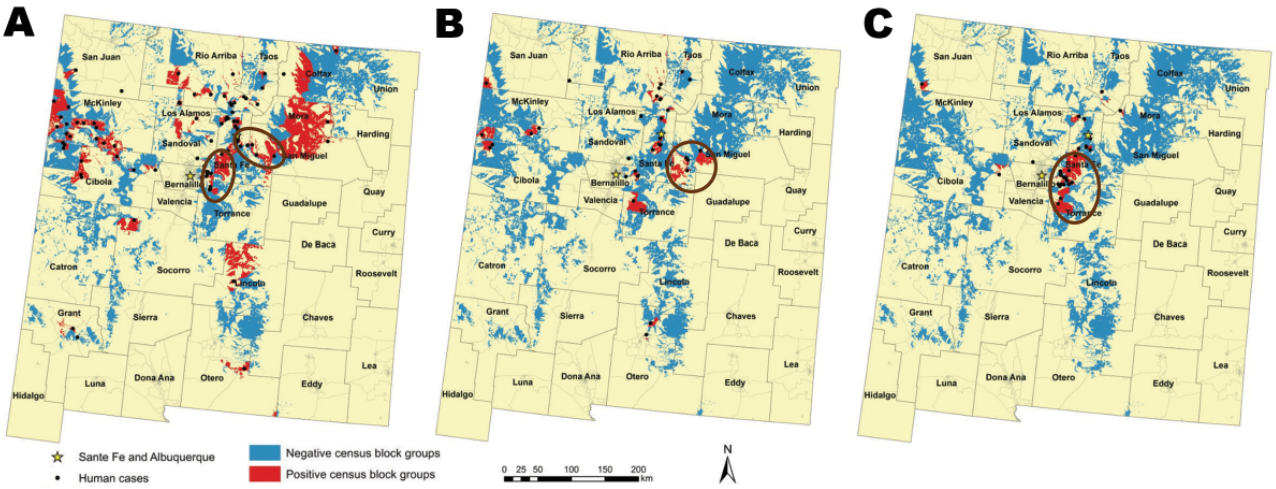

Figure. Areas of New Mexico, USA, considered in the current analysis on the basis of those defined as high risk for human plague by Eisen et al. (*6*) for each time frame examined. A) 1976–1985, B) 1986–1995, C) 1996–2007. Distributions of human cases are displayed and census block groups are color coded as negative or positive for plague cases. Census block group boundaries are indicated in light gray, and counties are outlined in dark gray. Ovals or circle indicate census block groups with significantly ($p<0.05$) high human plague incidence rates per 1,000 persons, identified by using the Kulldorff space scan statistic (*9*). Analyses were conducted by using the Poisson probability model and 999 Monte Carlo replications to test for significance.

Our results suggested temporal changes in socioeconomic factors associated with location of human plague cases. In the 1980s, plague tended to occur in CBGs with poor housing conditions (e.g., old homes with incomplete plumbing) and high proportions of the population living near or below the poverty line, but this second association was confounded by presence of ecotone habitat (Table 2; Figure). Beginning in the 1990s, plague cases began to be associated with CBGs with higher median incomes and home values, and by the 2000s, wealthier CBGs with higher proportions of newer homes were positively associated with plague cases (Tables 1, 2). High proportions of homes using wood fuel were consistently associated with positive CBGs for each time frame (Tables 1, 2), which supported suggestions from previous studies that availability of harborage for rodents (e.g., wood piles) in and around domestic environments may increase human plague risk (*2*,*5*–*8*).

A general change in the distribution of plague cases during the study period was also observed. In the 1980s, plague cases were more widely distributed across New Mexico and were particularly common in the northwestern region of McKinley and Cibola Counties (Figure). However, by the 1990s, plague cases became less common there and more focused in the north-central region of the state (Santa Fe–Albuquerque and surrounding counties; Figure). We implemented the Kulldorff spatial scan statistic (*9*) by using SaTScan (*10*) to identify clusters of CBGs with high incidence rates of plague cases per 1,000 persons for each of the time frames to quantify these changes. Significant clusters were detected only in the Santa Fe–Albuquerque region for each time frame (Figure). Changes consistent with the overall analysis in which plague occurrence shifted from poorer to wealthier CBGs and occurred in more new homes were observed when this region was considered alone.

Our analysis also suggested that migration of middle to upper–class families into suitable plague habitat throughout the high-risk areas of the state was associated with locations of plague cases. For example, in the 1990s, 28 (96.6%) of 29 plague-positive CBGs experienced population growth between the 1980 and 1990 censuses, in contrast to 337 (78.4%) of 430 nonplague CBGs that experienced growth. Likewise, 17 (85%) of 20 plague-positive CBGs in the 2000s occurred where there was growth between the 1990 and 2000 censuses versus growth in only 327 (75%) of 434 nonplague CBGs. Moreover, for the census 2000 period, population growth was more likely to have occurred in CBGs that had ecotone habitat than CBGs without ecotone habitat ($p = 0.004$, by Fisher exact test). Migration of persons into suitable plague habitat would potentially increase the likelihood of human exposure to infected rodents and their fleas (*7*).

Conclusions

Overall, our results confirmed the role of living in or near habitats that support maintenance of sylvatic plague as a risk factor for human *Y. pestis* infection, but also suggested migration of middle to upper–class families into such areas may be contributing to changes in the locations

Table 1. Characteristics of census block groups considered in analysis for human plague on the basis of 1980, 1990, and 2000 US Census data, New Mexico, USA*

Variable	1976–1985		1986–1995		1996–2007	
	Positive, n = 64	Negative, n = 405	Positive, n = 29	Negative, n = 430	Positive, n = 20	Negative, n = 434
Population density/km^2	**4.34 (11.54)**	**22.68 (264.48)†**	8.31 (34.66)	23.46 (238.31)	13.70 (31.82)	25.96 (267.56)
Housing density/km^2	**1.46 (4.18)**	**8.38 (107.56)†**	3.33 (12.57)	9.33 (95.91)	5.87 (12.13)	11.30 (107.09)
Poverty rate‡	**0.34 (0.23)**	**0.22 (0.23)§**	0.32 (0.42)	0.28 (0.23)	**0.16 (0.18)**	**0.24 (0.24)¶**
% Housing units						
Vacant	12.0 (8.84)	10.8 (7.09)	14.0 (15.54)	14.5 (16.34)	8.4 (10.77)	10.7 (18.46)
Rural farms	**2.1 (5.19)**	**1.3 (6.03)§**	0 (1.18)	0 (0.66)	0 (1.02)	0 (1.24)
Occupied, incomplete plumbing#	**8.6 (15.93)**	**2.5 (6.22)†**	**7.5 (30.57)**	**2.3 (9.21)§**	1.5 (5.77)	1.1 (4.47)
Occupied mobile homes	16.2 (5.89)	15.5 (17.88)	17.4 (23.31)	23.7 (20.25)	17.1 (13.19)	18.4 (24.21)
Built before 1940	**16.0 (14.98)**	**11.4 (16.92)§**	6.7 (16.81)	5.0 (15.67)	3.7 (10.38)	3.8 (11.72)
≥40 y old	**16.0 (14.98)**	**11.4 (16.92)§**	14.3 (20.38)	11.1 (22.71)	8.3 (22.42)	15.8 (25.83)
≤5 y old	20.6 (11.06)	21.7 (19.65)	17.1 (21.42)	13.6 (13.97)	21.5 (15.43)	14.5 (14.86)§
Heated with wood fuel	**18.5 (20.80)**	**6.8 (16.73)†**	**31.56 (34.65)**	**13.0 (31.99)†**	**16.9 (21.79)**	**6.8 (20.91)¶**
Occupied by ≥6 persons	**9.5 (6.28)**	**7.1 (4.89)§**	5.4 (11.97)	5.0 (6.90)	3.3 (4.48)	3.5 (5.94)
Household income**	**$28,477 ($11,544)**	**$30,190 ($16,367)¶**	$29,644 ($30,784)	$28,822 ($16,775)	**$44,098 ($30,563)**	**$31,323 ($18,541)§**
Value of homes**	$85,280 ($67,672)	$95,791 ($78,706)	$83,614 ($104,492)	$80,237 ($75,854)	**$132,350 ($90,050)**	**$84,300 ($90,400)¶**
Year housing unit built	NA	NA	1972 (9)	1974 (11)	**1987 (13)**	**1979 (11)§**
% Census block group area						
Ecotone habitat††	**2.8 (11.36)**	**0 (3.34)†**	**5.5 (13.44)**	**0 (4.33)†**	**10.7 (23.28)**	**0 (4.63)†**
Water	0.60 (0.45)	0.56 (0.83)	0.60 (0.31)	0.56 (0.71)	0.43 (0.33)	0.58 (0.67)

*Values are medians (interquartile ranges) for plague-positive and negative census block groups in the respective time frames. **Boldface** indicates variables significantly different between positive and negative groups. NA, not available.
†p≤0.001, by Wilcoxon rank sum test.
‡Defined by the US Census Bureau as the proportion of the population living near or below the federal poverty line, which is based on household income adjusted for number of household members. The definition of the federal poverty line changed between the 1980 and 1990 censuses. Therefore, rates are not directly comparable across decennials.
§p≤0.01, by Wilcoxon rank sum test.
¶p≤0.05 by Wilcoxon rank sum test.
#Defined by the US Census Bureau as lacking ≥1 of the following: hot and cold piped water, a flush toilet, and a bathtub or shower. Values are not directly comparable among censuses. In 1980, a housing unit was considered to have complete plumbing only if plumbing fixtures were for exclusive for the residents of that unit. In 1990, the requirement of exclusive use was dropped.
**In US year 2000–adjusted dollars, rounded to the nearest dollar.
††Identified as the convergence of the Rocky Mountain/Great Basin open and closed coniferous woodland habitats by Eisen et al. (*6*).

Table 2. Socioeconomic indicators and human plague cases among CBGs, New Mexico, USA*

Indicator	1976–1985		1986–1995		1996–2007	
	% Positive	OR (95% CI)	% Positive	OR (95% CI)	% Positive	OR (95% CI)
Population in poverty†						
High	64.1	**1.81 (1.05–3.12)**	55.2	1.24 (0.58–2.65)	35.0	0.52 (0.21–1.34)
Low	35.9		44.8		65.0	
Value of homes						
High	40.6	0.64 (0.38–1.10)	51.7	1.07 (0.50–2.27)	75.0	**3.11 (1.11–8.71)‡**
Low	59.4		48.3		25.0	
Housing 0–5 y old						
High	48.4	0.93 (0.55–1.58)	65.5	1.97 (0.90–4.34)	75.0	**3.14 (1.12–8.79)‡**
Low	51.6		34.5		25.0	
Incomplete plumbing§						
High	84.4	**6.68 (3.31–13.49)‡**	65.5	1.97 (0.90–4.34)	55.0	1.23 (0.50–3.04)
Low	15.6		34.5		45.0	
Use of wood fuel						
High	84.4	**6.68 (3.31–13.49)‡**	79.3	**4.17 (1.66–10.44)‡**	75.0	3.14 (1.12–8.79)
Low	15.6		20.7		25.0	

*__Boldface__ indicates significant associations (p<0.05). CBGs, census block groups; OR, odds ratio.
†Defined by the US Census Bureau as the proportion of the population living near or below the federal poverty line, which is based on household income adjusted for number of household members. The definition of the federal poverty line changed between the 1980 and 1990 censuses, Therefore, rates are not directly comparable across decennials.
‡Variables that continued to be significant after controlling for presence of ecotone habitat.
§Defined by the US Census Bureau as lacking ≥1 of the following: hot and cold piped water, a flush toilet, and a bathtub or shower. Values are not directly comparable among censuses. In 1980, a housing unit was considered to have complete plumbing only if plumbing fixtures were for exclusive for the residents of that unit. In 1990, the requirement of exclusive use was dropped.

of plague cases. The north-central region of New Mexico surrounding Santa Fe and Albuquerque was identified as a persistent focus of human plague cases, and it appears to be the predominate region for current cases. It is unclear why cases have become rare in the northwestern region of New Mexico because socioeconomic conditions have not generally improved there. However, the high numbers of cases observed there in the 1980s were associated with favorable climatic conditions for plague (*11*).

Although we detected changes in the socioeconomic indictors associated with the locations of plague-positive CBGs, what shifting individual behavioral factors may have accompanied these trends are unknown. In particular, we were unable to determine whether the socioeconomic status of individual plague case-patients has changed from poor to middle or upper–income classes. Future investigations are needed to characterize the characteristics and behaviors of persons to verify and fully understand the changing factors associated with plague cases in New Mexico.

Acknowledgments

We thank Brad Biggerstaff and Heidi Brown for statistical advice and for providing helpful comments that improved the manuscript.

Dr Schotthoefer completed this work while a research fellow at the Division of Vector-Borne Diseases, Bacterial Diseases Branch, Centers for Disease Control and Prevention, Fort Collins, Colorado. She is currently a project scientist at the Marshfield Clinic Research Foundation, Marshfield, Wisconsin. Her research interests are in the epidemiology and ecology of vector-borne and zoonotic diseases.

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Address for correspondence: Anna M. Schotthoefer, Marshfield Clinic Research Foundation, 1000 N. Oak Ave, Marshfield, WI 54449, USA; email: schotthoefer.anna@mcrf.mfldclin.edu

Disseminated Microsporidiosis in an Immunosuppressed Patient

Eric G. Meissner, John E. Bennett, Yvonne Qvarnstrom, Alexandre da Silva, Emily Y. Chu, Maria Tsokos, and Juan Gea-Banacloche

We report a case of disseminated microsporidiosis in a patient with multiple myeloma who had received an allogeneic stem cell transplant requiring substantial immunosuppression. The causative organism was identified as *Tubulinosema acridophagus,* confirming this genus of microsporidia as a novel human pathogen.

Microsporidia fungi are human pathogens known for causing diarrheal illness in persons infected with HIV; however, there is growing awareness of their involvement in other cases of host immunosuppression. A case of *Tubulinosema* sp. microsporidian myositis was recently reported in a patient with chronic lymphocytic leukemia (*1*). We describe a second case of disseminated microsporidiosis caused by a *Tubulinosema* sp. in an immunosuppressed patient who received an allogeneic stem cell transplant for multiple myeloma.

Case Report

The patient was a 33-year-old woman with multiple myeloma. After receiving an autologous stem cell transplant (SCT), she experienced a relapse of disease and was enrolled in an experimental protocol of immunoablative chemotherapy, followed by hematopoietic SCT at the National Institutes of Health Clinical Center in Bethesda, Maryland (www.ClinicalTrials.gov identifier NCT00520130). Patients in this study are given a conditioning regimen of chemotherapy drugs (including fludarabine and cyclophosphamide), followed by prophylaxis against graft-versus-host disease (alemtuzumab and cyclosporine).

Author affiliations: National Institutes of Health, Bethesda, Maryland, USA (E.G. Meissner, J.E. Bennett, E.Y. Chu, M. Tsokos, J. Gea-Banacloche); and Centers for Disease Control and Prevention, Atlanta, Georgia, USA (Y. Qvarnstrom, A. da Silva)

DOI: http://dx.doi.org/10.3201/eid1807.120047

The patient received a 7/8 HLA-matched allogeneic peripheral blood SCT (with a single mismatch at the DRB1 locus) from an unrelated donor. Her clinical course was complicated by vancomycin-resistant *Enterococcus faecium* bacteremia, meningitis, and concomitant noncommunicating hydrocephalus and retinal hemorrhages. The bone marrow did not reconstitute, and 35 days after the initial transplant, the patient received a second SCT from the same donor after a conditioning regimen with antithymocyte globulin. Engraftment took place on day 49, 14 days after the second transplant. Progressive respiratory failure and pulmonary infiltrates had developed over the preceding week despite administration of broad-spectrum antimicrobial drugs. Results of a bronchoscopy on day 49 showed diffuse alveolar hemorrhage and did not identify a pathogen. Treatment with activated factor 7 and corticosteroids was given with some clinical improvement as well as improvement shown on chest radiograph.

A second bronchoalveolar lavage (BAL), performed on day 64, again showed diffuse alveolar hemorrhage and absence of pathogens. The patient received a second course of corticosteroids and activated factor 7. On day 77, an ophthalmologic examination was performed during a routine follow-up, and new retinal lesions suggestive of candida chorioretinitis were seen. Liposomal amphotericin B was substituted for prophylactic anidulafungin, and an intravitreal injection of amphotericin B was given for a subfoveal lesion.

At this time, the patient also had increasing hyperbilirubinemia and elevation of liver aminotransferases, together with diarrhea, abdominal distension, and new ascites. Graft-versus-host disease of the gut and liver was suspected. A colonoscopy on day 79 showed that, with the exception of 1 ulcer, the colonic mucosa appeared normal; biopsy samples showed nonspecific inflammation and a few apoptotic bodies. A liver biopsy and paracentesis were performed on day 85. Samples were stained with calcofluor white, which revealed yeast-like organisms 2–3 μm in diameter (Figure 1, panel A; online Appendix Figure, panels A and B, wwwnc.cdc.gov/EID/article/18/6/12-0047-FA1.htm). The samples were also cultured for the presence of fungi, but results were negative.

Over the next 10 days, despite treatment with broad-spectrum antimicrobial drugs and corticosteroids, the patient had intermittent fever, continued elevation of liver enzymes, and progressive respiratory insufficiency with episodes of diffuse alveolar hemorrhage. Examination of BAL samples on days 90, 92, 96, and 97 was unrevealing. On day 96, multiple discrete, nonblanchable red macules and papules developed on the patient's face, arms, legs, and trunk; the macules and papules were initially suggestive of disseminated candidiasis. Skin biopsy showed cysts mostly within the epidermis and follicular epithelium, similar to

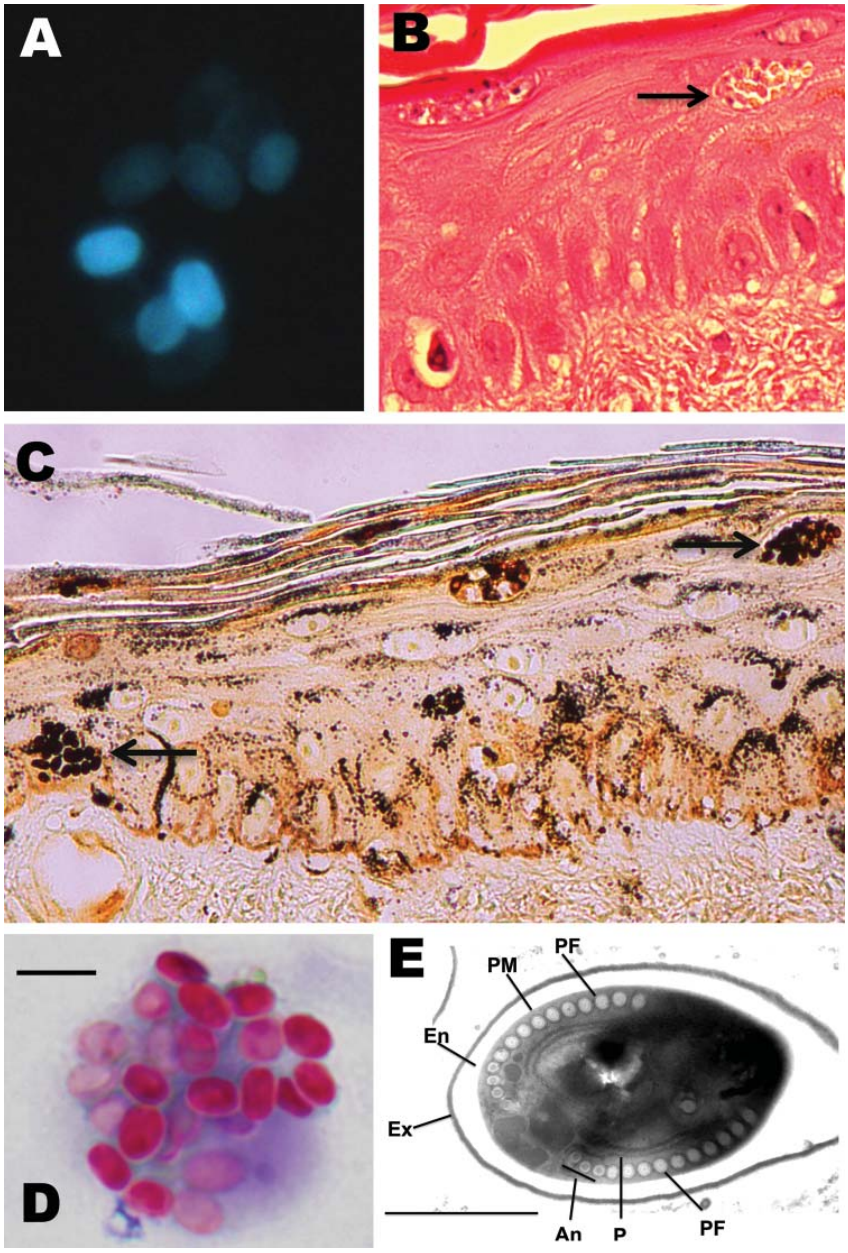

Figure 1. Microsporidium detected in clinical specimens from a stem cell transplant patient who had undergone substantial immunosuppression. A) Calcofluor white–stained ascitic fluid (original magnification ×500). B) Hematoxylin and eosin–stained skin biopsy sample (original magnification ×400). The arrow indicates clusters of spores. C) Warthin-Starry–stained skin biopsy sample (original magnification ×400). The arrows indicate clusters of spores. D) Modified trichrome–stained material from bronchoalveolar lavage. Scare bar = 5.0 µm. E) Transmission electron micrograph depicting 1 of the microsporidian spores identified in a skin biopsy sample. The image shows the polar filament (PF), containing 13 to 14 coils, in a single layer with anisofilar arrangement (An); the plasma membrane (PM); the exospore (Ex); the endospore (En); and polyribosomes (P). Scale bar = 1 µm.

those seen previously in the liver biopsy sample and in ascitic fluid; these cysts featured closely packed, uniform, oval basophilic structures (Figure 1, panels B and C).

On day 96, diffuse alveolar hemorrhage recurred. BAL was performed, and staining with Diff-Quik (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA) and modified trichrome showed that the fluid samples were now positive for intracellular yeast-like forms. In retrospect, we determined that the BAL samples from day 90 also contained yeast-like forms, but they could not be identified at that time (Figure 1, panel D). Refractory hypoxemia developed, and the patient died on day 97 after the initial transplant. No postmortem examination was performed.

The remaining clinical specimens were subsequently examined to determine if the yeast-like forms were microsporidia. Warthin-Starry staining showed that all organisms in the skin and ascitic fluid were consistent with microsporidia (Figure 1, panel C; online Appendix Figure, panel C). Similar organisms were identified in the BAL samples from days 90 and 96; spore size averaged 3.5 µm (range 3.3–3.9 µm) (Figure 1, panel D). Results of calcofluor white and modified acid-fast staining of the organisms were also positive, consistent with microsporidia.

Electron micrographs of skin and peritoneal fluid revealed microsporidian spores with features that were compatible with the genus *Tubulinosema*. Spores had a

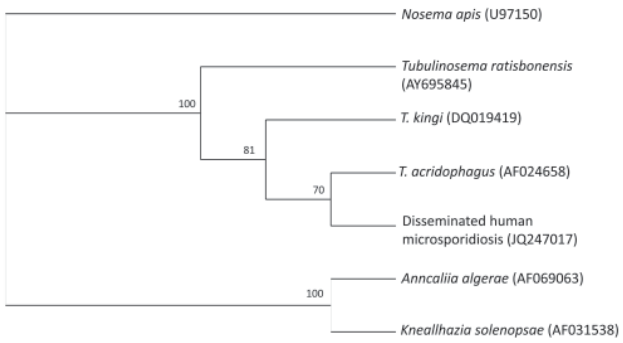

Figure 2. Cladogram of *Tubulinosematidae* spp. based on small subunit ribosomal RNA gene sequences. *Nosema apis* was added as an outgroup. The phylogenetic tree was created by using the quartet puzzling maximum likelihood program TREE-PUZZLE (www.tree-puzzle.de). The numbers at the nodes are quartet puzzling estimations of support to each internal branch.

single layer of polar tubule or filament with 11–14 coils; the last 3 coils were clearly smaller in some spores (i.e., they had an anisofilar polar tubular arrangement) (Figure 1, panel E). The quality of the electron micrograph was limiting and did not allow visualization of the diplokaryon nucleus.

BAL samples were sent to the reference diagnostic laboratory for parasitology at the Centers for Disease Control and Prevention for molecular analysis. A nested PCR approach was applied, using the outer primers microFN18 (5′-CACCAGGTTGATTCTGCC-3′) and microR9 (5′-GTATGATCCTGCCACAGATTCTTCTAT-3′) and the inner primers Ta50f (5′-GGTTGATTCTGCCTGTT ATATGT-3′) and Ta1300r (5′-GGACACATTCATC GTAACTTAGT-3′). Phylogenetic analysis of the resulting 1,246-bp sequence (GenBank accession no. JQ247017) revealed a high similarity to the small subunit ribosomal RNA gene from *T. acridophagus* (Figure 2), with only 1 nucleotide difference between the 2 sequences. Our final clinical diagnosis was disseminated *T. acridophagus* microsporidiosis with skin, liver, peritoneal, lung, and possibly chorioretinal involvement. Histopathology was notable for a paucity of inflammation around the organisms.

Conclusions

Along with another case of *Tubulinosema* sp. microsporidiosis (*1*), this case of disseminated microsporidiosis in an immunosuppressed SCT patient solidifies *Tubulinosema* sp. as a novel pathogen in immunosuppressed persons. By using molecular analysis, we characterized the microsporidian from the patient in our study to the species level (*T. acridophagus*).

Of 3 other reported cases of pulmonary microsporidiosis after SCT, 2 were in T cell–depleted patients (*2*,*3*), and the third was in a patient receiving high-dose methylprednisolone (1 g/m^2) to treat graft-versus-host disease (*4*). The patient in our study was substantially immunosuppressed. She received alemtuzumab with her first allogeneic transplant and conditioning with antithymocyte globulin before her second transplant, resulting in significant in vivo T-cell depletion; she was also receiving adrenal corticosteroids. Because the patient had retinal changes suggestive of candidemia, we initially suspected that she had a disseminated infection. Although we did not identify the cause of these retinal changes, a case of endopthalmitis caused by microsporidia has been reported (*5*).

Microsporidia have only rarely been reported in the skin (*6*–*8*). The organisms we found in the skin and liver of the patient in our study were similar, providing the clue to the eventual postmortem diagnosis of disseminated microsporidiosis. This report reinforces the need to consider microsporidiosis as a life-threatening condition and a potential cause of diffuse alveolar hemorrhage after SCT (*2*,*3*), particularly in patients with extreme T-cell dysfunction.

Acknowledgments

We thank Daniel Fedorko for help with microbiological analysis and Govinda Visvesvara for help with interpretation of the microsporidia electron micrographs.

This research was supported in part by the Intramural Research Program of the National Institutes of Health (National Institute of Allergy and Infectious Diseases and National Cancer Institute).

Dr. Meissner is an infectious diseases fellow at National Institutes of Health. His research interests include HIV and hepatitis C co-infection.

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Address for correspondence: Eric Meissner, NIH/NIAID, 10 Center Dr, Room 11N204, Bethesda, MD 20892, USA; email: eric.meissner@nih.gov

Salmonellosis Outbreak Traced to Playground Sand, Australia, 2007–2009

Michael Staff, Jennie Musto, Geoff Hogg, Monika Janssen, and Karrie Rose

A community outbreak of gastroenteritis in Australia during 2007–2009 was caused by ingestion of playground sand contaminated with *Salmonella enterica* Paratyphi B, variant Java. The bacterium was also isolated from local wildlife. Findings support consideration of nonfood sources during salmonellosis outbreak investigations and indicate transmission through the animal–human interface.

Variants of *Salmonella enterica* serovar Paratyphi B that use D-tartrate as a carbon source (known as *S. enterica* var. Java) primarily cause gastroenteritis. In contrast to experience in other countries (*1*–*4*), in Australia, *S. enterica* var. Java outbreaks have not been linked to food sources; the only outbreaks reported before 2007 were associated with imported ornamental fish (*5*). Sand in recreational sandboxes has been identified as a risk factor for infection of children with *S. enterica* serovar Typhimurium (*6*), and given the popular nature of this activity, there is a potential for sandboxes to pose a substantial public health hazard unless managed appropriately. We describe a protracted localized community outbreak associated with playground sand during 2007–2009, and highlight the need to consider nonfood sources when investigating salmonellosis outbreaks.

The Study

In mid-2007, a routine review of serotypes among cases of *Salmonella* spp. infection reported to the New South Wales Health Department identified a probable *S. enterica* var. Java outbreak in a single local government area (population 57,000), and an investigation was initiated. We defined a case-patient as a person reported to health authorities during 2007–2009 who had had diarrhea for at least 24 hours, either lived in or had visited the local government area during the 7 days before the onset of diarrhea, and had provided a fecal sample from which *S. enterica* var. Java was isolated. Seventy-five case-patients were identified: ages ranged from 1 month through 60 years (median age 2 years, 10 months.); 34 were female. Three children were admitted to a hospital for 1–2 days; none died.

All isolates were sensitive to ampicillin, streptomycin, tetracycline, chloramphenicol, sulfathiazole, and spectinomycin. Results of phage typing by using standard techniques (*7*) were available for 74 isolates; 54 were classified as phage type Dundee, 19 as a uniform "reactions do not conform," which closely resembled Dundee, and 1 as untypeable. Seventy-two isolates underwent multilocus variable number tandem repeat analysis (MLVA) typing (*8*); 69 were classified as 1-(12-17)-0-0-493 and 3 as distinctly different types. This MLVA type was unique to case-patients from this outbreak; it was not seen in isolates from other parts of New South Wales.

After an extensive investigation involving case-patient interviews and food and environmental sampling during 2007 and early 2008, exposure to playground sand at public parks and childcare centers was identified as the likely immediate source of the outbreak. *S. enterica* var. Java was isolated from 50 of 207 sand samples taken from 39 locations; no other *Salmonella* serotypes were detected. All 19 playgrounds with sandboxes from within the local government area were sampled, regardless of whether they were linked to case-patients. All 35 playground isolates came from 6 playgrounds that had received sand from a central depot within the past 12 months. *Salmonella* spp. were isolated from surface and deep (as far as 50 cm below the surface) sand samples. Despite multiple samples being taken, no isolates were obtained from the depot. Antimicrobial drug sensitivity testing, phage typing, and MLVA typing of isolates produced results indistinguishable from those of the outbreak strain. One contaminated playground was closed to human access, left undisturbed, and sequentially sampled every 3 months. Nine months passed before all samples taken from this playground were clear of the bacterium.

To confirm the hypothesis that sand from playgrounds contaminated with *S. enterica* var. Java was the immediate source of the outbreak, we performed an age-matched case–control study of case-patients 1 month to 4 years of age involving 16 case-patients and 32 controls during May 2008. Controls were selected from the registers of 2 local community child-health clinics. Exposure to playgrounds with contaminated sand within 7 days of symptom onset was associated with illness (matched odds ratio 3.7, 95% CI 1.1–12.1). In May 2008, the local authority began closing sandboxes, replacing the sand, and reopening. A substantial reduction in the number of case-patients reported occurred (Figure 1), although new case-patients continued to be reported throughout the study period.

Author affiliations: New South Wales Health, Sydney, New South Wales, Australia (M. Staff, J. Musto); Public Health Laboratory, Melbourne, Victoria, Australia (G. Hogg); and Taronga Conservation Society Australia, Sydney (M. Janssen, K. Rose)

DOI: http://dx.doi.org/10.3201/eid1807.111443

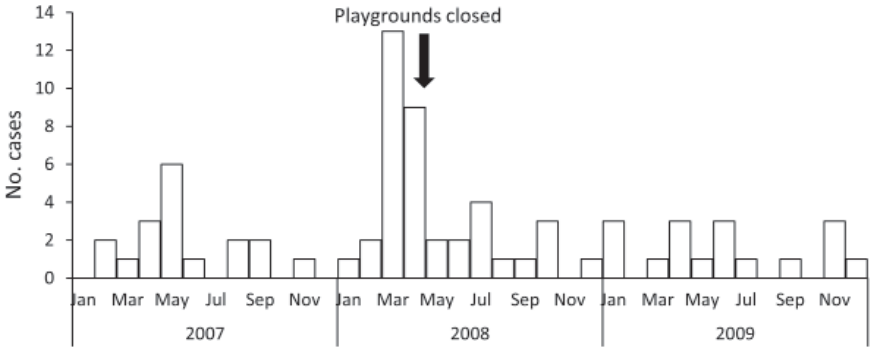

Figure 1. Number of cases of *Salmonella enterica* variant Java infection and month of onset in children playing in sandboxes, Australia, 2007–2009.

To better characterize the distribution of the bacterium in the local ecosystems, we collected fecal and cloacal samples from 261 free-ranging animals of various species (Table). Thirty-four isolates of *S. enterica* var. Java were identified: most were from a marsupial species native to the local area, the long-nosed bandicoot, *Perameles nasuta* (Figure 2). Phage and MLVA types were indistinguishable from human and environmental isolates. The Australian Registry of Wildlife Health had no recorded evidence of disease associated with the isolation of *S. enterica* var. Java from long-nosed bandicoots (K. Rose, Taronga Conservation Society Australia, pers. comm.).

The isolation of *S. enterica* var. Java from playground sand with the same phage type, MLVA pattern, and antibiogram as that of human isolates supported the hypothesis that ingestion of sand from playgrounds was the human exposure pathway for this outbreak. The case–control study found an association between infection in humans and exposure to at least 1 playground where an *S. enterica* var. Java isolate was found in the sand; these findings confirmed sand as the immediate source of infection. Although sand from the central depot was a common factor in all contaminated playgrounds where case-patients contracted the illness, the infection source for this facility remains unknown. It was located in a wild bushland setting, and it is feasible that transmission of the bacterium from local wildlife occurred.

Conclusions

Native animals and wildlife have been implicated in previous community-wide outbreaks of salmonellosis (*9*,*10*). The isolation of *S. enterica* var. Java of common phage and MLVA types from human, animal, and environmental samples implies that the organism can survive within multiple ecosystems. The organism probably has multiple transmission pathways, involving interactions among humans, animals, and the environment. The persistence of the bacterium in playground sand for up to 9 months demonstrates that the organism can survive for a relatively long period in this environment and might

Table. *Salmonella enterica* var. Java isolates from animals in or near local government area, Australia, 1998–2009*

Species	Year	Reason for collection	Specimen type	No. animals tested	No. positive	MLVA (no.)
Bandicoot†	1998	Opportunistic screening	Feces	1	1	ND
Dog‡	2001	Unclear	Rectal swab	1	1	ND
Black duck	2008	Near contaminated playground	Feces from ground	5	2	1-16-0-0-493
Black rat	2008	Trapped in contaminated playground	Rectal swab	1	1	1-16-0-0-493
Bandicoot	2008	Trapped in contaminated playground	Cloacal swab	1	1	1-16-0-0-493
Brushtail possum	2009	Trapped in state park	Cloacal swab	11	1	1-14-0-0-493
Bandicoot	2009	Trapped in state parks	Cloacal swab and feces	67	18	1-13-0-0-493 (6), 1-14-0-0-493 (3), 1-15-0-0-493 (3), 1-16-0-0-493 (3), 1-17-0-0-493 (3)
Bandicoot	2008–2009	Wildlife rescue	Cloacal swab	12	2	1-13-0-0-493 (1), 1-15-0-0-490 (1)
Bandicoot	2009	Trapped in yard of a case-patient	Cloacal swab	3	2	1-16-0-0-493 (2)
Bandicoot§	2009	Wildlife rescue	Cloacal swab	3	3	01-13-0-0-493 (3)

*With the exceptions of samples from 1 bandicoot that reacted but did not conform (PT RDNC/A001), and 1 dog that was not tested, all phage types were Dundee. Var., variant; MLVA, multilocus variable number tandem repeat analysis; ND, not done.
†Adult, sampled 10 km south of local government area.
‡Sampled10 km south of local government area.
§Juvenile siblings.

Figure 2. Long-nosed bandicoot (*Perameles nasuta*). Photograph courtesy of Taronga Zoo, Sydney, New South Wales, Australia.

provide information about the natural history of the bacterium and how it can infect humans.

We detected *S. enterica* var. Java with the outbreak phage type and MLVA typing in cloacal swab specimens from 31% of native long-nosed bandicoots sampled (Table); illness in the animals was not apparent. It is unclear whether the bacterium has a predilection for this species or whether the bandicoot exhibits a particular behavior that predisposes it to being colonized. Further research is merited.

The study identifies accidental sand ingestion as a previously unrecognized pathway for humans acquiring illness caused by *S. enterica* var. Java and provides further evidence for the need to manage this medium in playgrounds to protect public health. The emergence of human disease caused by *S. enterica* var. Java from this environmental source and the local colonization of wildlife with the same bacterium highlights the consequences of the interface between human and wildlife health. Further research and a more extensive systematic sampling program that includes the local government area that was the focus of this study are required to better understand the ecology of *S. enterica* var. Java and the potential role of wildlife in sustaining human disease outbreaks. Ultimate control of the outbreak might require a strategy that involves altering human behavior, the environment, and wildlife habitat.

Acknowledgements

We thank the staff of the Microbiological Diagnostic Unit, University of Melbourne, for phage and MLVA typing of sand samples and phage typing human isolates; the staff of the Institute for Clinical Pathology and Medical Research, Westmead for MLVA human isolate typing; the Public Health Unit and Public Health Officer training staff for performing case-patient follow-up and interviews of controls; Taronga Wildlife Hospital for conducting preliminary microbial culture of many of the wildlife samples, and Jane Hall for her attention to detail in collating animal records; Ben Hope, Rachel Miller, Geoff Ross, and Ron Haering from New South Wales Office of Environment and Heritage for organizing the trapping of bandicoots in state parks; and the Australian Biosecurity Intelligence Network, for wildlife health information management and communication systems that contributed to this research.

Dr Staff is a physician for the public health system of New South Wales, known as NSW Health, Sydney, and a clinical senior lecturer at the University of Sydney. His research interests include the public health aspects of communicable disease control and environmental health.

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Address for correspondence: Michael Staff, Public Health Unit, Hornsby Hospital, Palmerston Rd, Hornsby, New South Wales 2077, Australia; email: mstaff@nsccahs.health.nsw.gov.au

Probable Transmission of Coxsackie B3 Virus from Human to Chimpanzee, Denmark

Sandra C. Abel Nielsen, Tobias Mourier, Ulrik Baandrup, Tine Mangart Søland, Mads Frost Bertelsen, M. Thomas P. Gilbert, and Lars Peter Nielsen

In 2010, a chimpanzee died at Copenhagen Zoo following an outbreak of respiratory disease among chimpanzees in the zoo. Identification of coxsackie B3 virus, a common human pathogen, as the causative agent, and its severe manifestation, raise questions about pathogenicity and transmissibility among humans and other primates.

Six serotypes of coxsackie B viruses (CBVs) (family *Picornaviridae*, genus *Enterovirus*) are recognized: CB1–6. CBV infections are common in humans and usually cause minor symptoms. However, CBVs are also linked to several serious acute manifestations in infants, children, and adults. CBVs are one of the most common causes of meningitis and can also cause encephalitis (*1*). In addition, enteroviruses are well documented as a cause of acute myocarditis, with viruses in the species *Human enterovirus B*, particularly CBVs, being the most common etiologic agents (*2*).

The common chimpanzee (*Pan troglodytes*) and bonobo (*P. paniscus*) are the closest living relatives to humans (*3*). Research has shown that wild chimpanzees from Cameroon excrete different types of enteroviruses in their feces and that some of the enteroviruses are closely related to those known to infect humans (*4*). It has also been shown that fatal myocarditis caused by CBV can occur in other primates, including the orangutan and the snub-nosed monkey (*5*,*6*). We report an incident of likely human-to-chimpanzee enterovirus transmission, which raises the question of whether enteroviruses are regularly transmitted between humans, chimpanzees, and other primates.

Author affiliations: University of Copenhagen, Copenhagen, Denmark (S.C.A. Nielsen, T. Mourier, M.T.P. Gilbert, L.P. Nielsen); Aalborg University, Aalborg, Denmark (U. Baandrup, L.P. Nielsen); Copenhagen Zoo, Copenhagen (T.M. Søland, M.F. Bertelsen); and Statens Serum Institut, Copenhagen (L.P. Nielsen)

DOI: http://dx.doi.org/10.3201/eid1807.111689

The Case

Chimpanzees at the Copenhagen Zoo (Copenhagen, Denmark) zoo are housed together and allowed contact with each other at all times. In October 2010, respiratory symptoms (coughing, sneezing, and serous nasal discharge) developed in all chimpanzees at the zoo; however, a 7-year-old female chimpanzee was particularly affected. She was a West African chimpanzee (*P. troglodytes verus*) that had been born at the zoo; typical lifespan for such chimpanzees is 50-60 years. Following 36 hours with respiratory symptoms, lethargy, and decreased appetite, the chimpanzee died on October 25.

Postmortem examination revealed marked hepatic congestion and mild congestion of the small intestine. The heart was flaccid, and pale streaks were observed in the myocardium. On histographic examination, the lungs showed mild mononuclear interstitial infiltration, and there was marked infiltration in the myocardium consisting mainly of CD3 positive T lymphocytes mixed with a few granulocytes. In addition, multifocal severe myonecrosis was observed; cardiac myocytes were completely engulfed by inflammatory cells (Figure 1).

One-step real-time PCR was performed on RNA extracted from blood, myocardial tissue, and feces to assay for the presence of enterovirus sequences. All results were strongly positive; cycle threshold values of samples were unusually low, in comparison with general values observed for enterovirus-infected samples from humans (*7*,*8*). On the basis of histopathologic and microbiologic findings, we concluded that the chimpanzee died from generalized infection by an enterovirus, which caused an overwhelming inflammation of the heart muscle.

We further characterized the virus by using RNA extracted from the heart. Virus was reverse-transcribed by using SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA), and then subjected to PCR amplification by using an enterovirus-specific, long-range PCR approach. A near complete viral genome (≈7,200 nucleotides; 96%) was amplified with primer sequences 5′-GGTGCGAAGAGTCTATTGAGC-3′ and 5′-CACCGAAYGCGGAKAATTTACCCC-3′. PCR amplification was performed by using the Platinum *Taq* DNA Polymerase High Fidelity Kit (Invitrogen). Reactions were performed in 25μL volumes comprised of 1× HiFi buffer, 2 mmol/L $MgSO_4$, 0.2 mmol/L dNTPs, 0.4 μmol/L each primer, 1 μL template cDNA, and 1 U enzyme. Cycling conditions were 94°C for 2 min; 35 cycles of 94°C for 30 s, 55°C for 30 s, and 68°C for 7 min 45 s; followed by a final extension at 72°C for 7 min.

After PCR amplification, the amplicons were fragmented, converted into a sequencing library, and sequenced by using the Genome Sequencer FLX System (Roche, Copenhagen, Denmark). A local

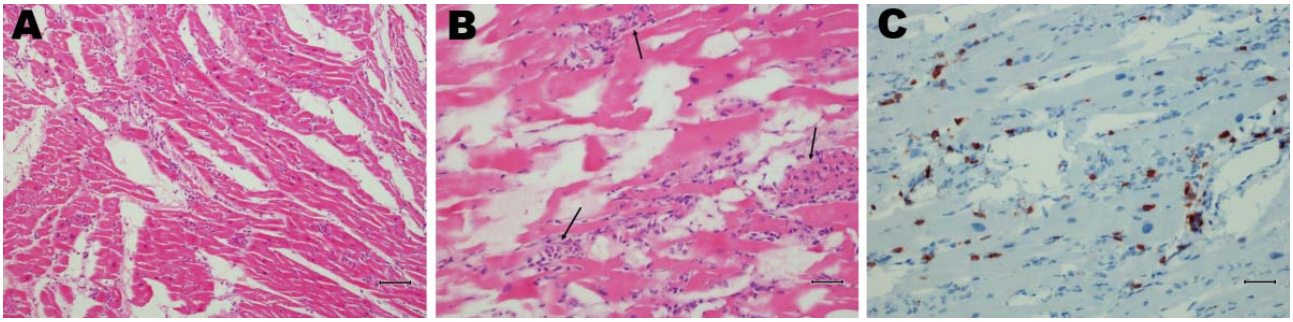

Figure 1. Postmortem tissue sections from chimpanzee with coxsackie virus B infection, Denmark. A) Myocardial section showing artifacts of freezing and a diffuse lymphocytic infiltration. Scale bar = 80 μm. B) Myocytic degeneration (arrows) is evident. Scale bar = 40 μm. (Hematoxylin and eosin stain.) C) CD3 marker reaction showing T lymphocytes. Scale bar = 40 μm.

database containing all virus sequences, except those for HIV, retrieved from the Viral Genomes database in GenBank (downloaded June 7, 2011; www.ncbi.nlm.nih.gov/genomes/GenomesHome.cgi?taxid=10239), was constructed, against which all sequence reads were compared by using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). This comparison revealed 89,121 sequence reads with similarity to CB3 virus sequences (expectation value $<10^{-6}$). Using a complete genome of a CB3 virus (strain PD, GenBank accession no. AF231765) as reference, we mapped all sequence reads with similarity to enteroviruses by using SMALT software (www.sanger.ac.uk/resources/software/smalt). A consensus sequence constituting the reported CB3 virus was constructed from the mapped reads (GenBank accession no. JN979570). To assess the likelihood of the reported CB3 virus being of human origin, we performed phylogenetic analysis by using a neighbor-joining method. The phylogeny was generated by using published full-genome CBV sequences; all CBV serotypes and the chimpanzee CB3 virus were represented (Figure 2). The phylogeny shows a topology in which the new CB3 virus is clustered within a clade containing human CB3 viruses. Thus, it is most likely that the CB3 virus that infected the female chimpanzee was of human origin rather than a novel type.

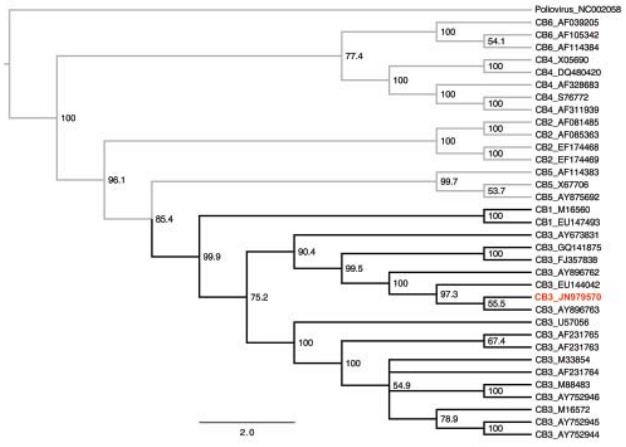

Figure 2. Phylogenetic tree of coxsackie B viruses inferred by using neighbor-joining analysis. The tree was generated by using the Tamura-Nei distance model and 1,000 bootstrap replicates. Scale bar represents estimated phylogenetic divergence. Specific coxsackie B virus serotypes (CB1–6) and corresponding GenBank accession number are shown on the right. Poliovirus was included as an outgroup. Coxsackie virus B clade shown in **boldface**; the reported coxsackie B virus sequence is listed in red. CB, coxsackie B virus.

In addition, a similarity plot with the chimpanzee CB3 virus protein sequence and the CB3 reference strain protein sequence (GenBank accession no. AAA74400) was generated (data not shown). Overall, the plot showed >95% similarity between the 2 sequences; however, 83% similarity was shown in the 2A region of the sequences. Such a change in the 2A protein could theoretically contribute to altered pathogenicity of the CB3 virus, although whether this is the case would require additional analyses.

Conclusions

A CB3 virus was detected at extremely high concentration in the heart muscle of a chimpanzee that died from myocarditis. Given the close similarity of the near-complete viral genome sequence to that of human CB3 viruses, the source of the virus was likely a human. Infection of the chimpanzee colony most likely occurred through close contact with an animal keeper or other zoo employee. Alternate explanations, such as transmission by contact between the chimpanzee colony and other zoo animals or zoo visitors, are unlikely because the chimpanzees had no contact with other animals, and they are separated from the public by a 4-m-high glass wall.

Within the *Enterovirus* genus, some enteroviruses that infect humans also infect animals (e.g., polioviruses can infect nonhuman primates and humans), and some primates can be infected with several other enterovirus types. CB5 virus was isolated from an infant chimpanzee with a fatal illness (*9*), and mice can be infected by several coxsackie viruses. Moreover, swine vesicular disease virus, which

infects swine, is serotypically identical and antigenically closely related to human coxsackie B5 virus (*10*–*12*).

This report shows that transmission of viruses from humans to chimpanzees is possible and can be fatal. Whether enteroviruses are regularly transmitted between humans, chimpanzees, and other primates, and whether some primates serve as reservoirs for mutation of enteroviruses that can then infect other primates remains to be investigated.

Acknowledgments

We thank Helle Flaga and the Danish National High-throughput DNA Sequencing Centre for support and technical assistance.

This study was supported by The Lundbeck Foundation (grant R52-A5062), the Danish National Research Foundation (geogenetics grant), and the Kornerup Foundation.

Ms Nielsen is a PhD student at the Centre for GeoGenetics, University of Copenhagen, Denmark. Her research interests include infectious disease, high-throughput sequencing, and virus discovery.

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Address for correspondence: Sandra C. Abel Nielsen, University of Copenhagen, Natural History Museum of Denmark, Oester Voldgade 5-7, Copenhagen DK-1350, Denmark; email: s.abelnielsen@gmail.com

Transmission of *Bordetella holmesii* during Pertussis Outbreak, Japan

Hajime Kamiya, Nao Otsuka, Yuka Ando, Fumito Odaira, Shuji Yoshino, Kimiko Kawano, Hirokazu Takahashi, Toshihide Nishida, Yoshio Hidaka, Hiromi Toyoizumi-Ajisaka, Keigo Shibayama, Kazunari Kamachi, Tomimasa Sunagawa, Kiyosu Taniguchi, and Nobuhiko Okabe

We describe the epidemiology of a pertussis outbreak in Japan in 2010–2011 and *Bordetella holmesii* transmission. Six patients were infected; 4 patients were students and a teacher at the same junior high school. Epidemiologic links were found between 5 patients. *B. holmesii* may have been transmitted from person to person.

Bordetella holmesii, a small gram-negative coccoid bacillus that was first reported in 1995 (*1*), was originally identified as a member of the Centers for Disease Control and Prevention nonoxidizer group 2. The organism is associated with bacteremia, endocarditis, and pneumonia, usually in patients with underlying disorders such as asplenia or sickle cell anemia, and has been isolated from blood and sputum samples (*1–5*). *B. holmesii* may also be responsible for causing symptoms similar to those of pertussis (whooping cough) in otherwise healthy patients (*6*). Large surveillance studies in the United States and Canada have shown that the organism was detected in nasopharyngeal swab (NPS) specimens of patients with pertussis-like symptoms (*7*,*8*). Although humans may be infected with *B. holmesii*, transmission of *B. holmesii* between humans has not yet been fully elucidated.

Pertussis is a highly contagious disease caused by the bacterium *B. pertussis*. The organism is known to be transmitted from person to person by airborne droplets (*9*). During a recent pertussis outbreak, we conducted a laboratory-based active surveillance study and detected 76 suspected cases of pertussis. Among these cases, we identified not only *B. pertussis* infection but also *B. holmesii* infection. The purpose of this study was to describe the epidemiology of the pertussis outbreak and to determine the epidemiologic relatedness of *B. holmesii* transmission.

The Study

During 2010–2011, a pertussis outbreak occurred in a suburban town (town A) in Nobeoka City in Miyazaki Prefecture, Japan. Town A has a population of 4,227 persons and an elementary school and junior high school. The first pertussis case (in a 17-year-old boy) was reported in September 2010. From that time, we conducted a laboratory-based active surveillance study in the area until April 2011, in cooperation with clinics, hospitals, and local health departments. Pertussis-suspected cases were defined as an illness with cough lasting for ≥2 weeks, and pertussis-confirmed cases were defined as the presence of 1 of the following laboratory findings: a culture-positive result for *Bordetella* species from NPS specimens, or a positive result for molecular testing for *Bordetella* species.

For molecular testing, we conducted conventional PCR specific for insertion sequence IS*481*, which is known to detect *B. pertussis* and *B. holmesii*, and *B. pertussis*–specific loop-mediated isothermal amplification assays (*10–12*). To further confirm *B. holmesii* infection, *B. holmesii*–specific real-time PCR specific for the *recA* gene was also performed as described (*8*). For confirmed cases of *B. holmesii* infection, we collected the general information for patients (clinical symptoms, treatment, contact information, and outcome) by face-to-face interview or questionnaire.

During the pertussis outbreak, we identified 76 suspected pertussis cases. Among these suspected cases, 35 cases were confirmed by laboratory testing and involved persons 2–63 years of age (median age 13 years); 1 case occurred in a 2-year-old child, 14 in 6- to 12-year-old children, 14 in 13–15-year-old children, and 6 in persons >16 years of age. Despite pertussis vaccination rates in childhood of 82.3%–92.6%, most (80%) patients were students 6–12 and 13–15 years of age. Among the 35 confirmed case-patients, 29 and 6 patients showed *B. pertussis* and *B. holmesii* infection, respectively. Confirmed cases of *B. holmesii* infection were observed within the last half of the epidemic curve, i.e., weeks 1–12 of 2011 (Figure 1). There were no cases of co-infection with *B. pertussis* and *B. holmesii*.

All NPS specimens from patients with *B. holmesii* infection showed a negative result for the *B. pertussis* loop-mediated isothermal amplification, but showed positive results in the IS*481* PCR and *B. holmesii recA* real-time PCR (Table 1). *B. holmesii*-like organisms were obtained

Author affiliations: National Institute of Infectious Diseases, Tokyo, Japan (H. Kamiya, N. Otsuka, Y. Ando, F. Odaira, H. Toyoizumi-Ajisaka, K. Shibayama, K. Kamachi, T. Sunagawa, K. Taniguchi, N. Okabe); Miyazaki Prefectural Institute for Public Health and Environment, Miyazaki, Japan (S. Yoshino, K. Kawano); Takahashi Clinic, Miyazaki (H. Takahashi); and Nobeoka Public Health Center, Miyazaki (T. Nishida, Y. Hidaka)

DOI: http://dx.doi.org/10.3201/eid1807.120130

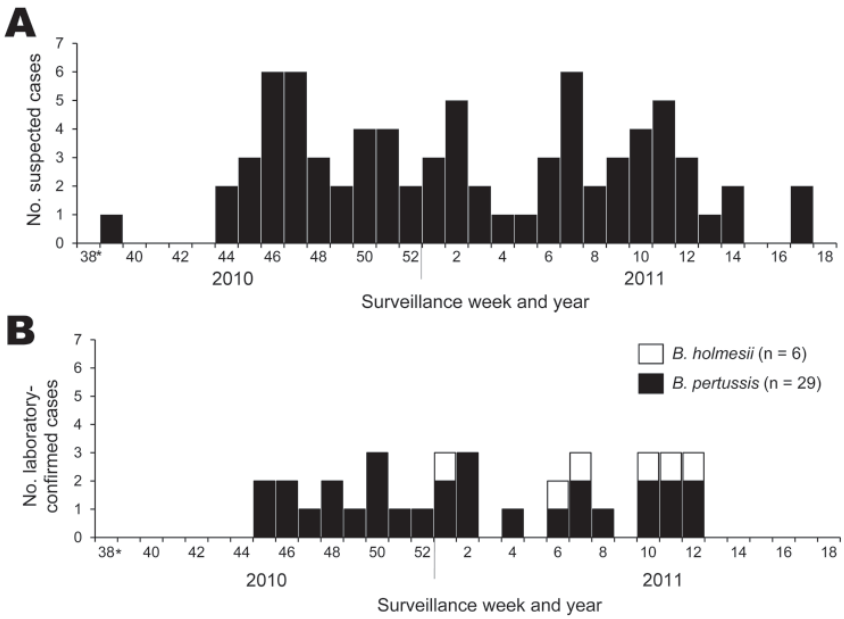

Figure 1. Epidemic curve of a pertussis outbreak, September 2010–April 2011, Japan. A) Suspected cases of pertussis. B) Laboratory-confirmed cases of *Bordetella pertussis* and *B. holmesii* infection. *As of September 20–26, 2010.

from 5 patients, and these were identified as *B. holmesii* by *recA* gene sequencing. Patient 6 had been treated with antimicrobial drugs before the culture test, which probably resulted in a culture-negative test result in this patient. Real-time PCR confirmed that patient 6 had a low *B. holmesii* DNA load (threshold cycle 36.6) in her NPS specimen. All patients experienced paroxysms of coughing without posttussive vomiting, especially at night, and 3 also experienced a whoop (Table 2). Five of 6 patients had a persistent cough lasting >2 weeks. No patients experienced any complications, and they were treated mainly with azithromycin, resulting in complete recovery. None of the patients were immunocompromised.

Our surveillance study showed epidemiologic linkage between 5 patients (patients 2–6), but not for patient 1 (Figure 2). Patient 1, a student in high school A, had the first case of *B. holmesii* infection (probable index case). He lived in a dormitory outside Nobeoka City. However, his family home was in town A in Nobeoka City, and he returned to his home at the end of 2010 and remained there in 2011. Patients 2, 3, and 4 were students at the same junior high school (B) in town A and were close friends. Patient 5 was a teacher at junior high school B and was in charge of patient 4. Patient 6 was a medical practitioner at clinic C, which is 1 of 2 clinics in town A. Patients 1–5 visited clinic C in early January, late February, late February, early March, and late March, 2011, respectively. The duration of illness in patient 1 did not overlap with that of the other patients, whereas that of patients 2–6 clearly overlapped.

Conclusions

In the past 16 years, ≈70 *B. holmesii* clinical strains have been isolated from human patients in several countries, mainly the United States. All reported cases of *B. holmesii* infection have been sporadic occurrences. Thus, the reservoir of *B. holmesii* is currently unknown. Moreover, whether *B. holmesii* is transmitted between humans is not known. In this report, we have demonstrated that 5 patients infected with *B. holmesii* showed epidemiologic linkage. In particular, the fact that 4 of these patients attended the same junior high school suggests that *B. holmesii* may be transmitted from person to person.

Table 1. Characteristics of *Bordetella holmesii* infection in 6 patients during pertussis outbreak, Japan, September 2010–April 2011*

Patient no.	Age, y/sex	Duration of cough, d†	*B. holmesii* test results	
			recA real-time PCR (C_t)‡	Culture§
1	17/M	5	+ (28.7)	+
2	15/F	4	+ (23.4)	+
3	15/F	>14	+ (21.6)	+
4	14/F	8	+ (25.1)	+
5	40/M	8	+ (27.0)	+
6	45/F	15	+ (36.6)	–

*All patients had negative results for *B. pertussis* loop-mediated isothermal amplification and positive results for IS*481* PCR. C_t, cycle threshold; –, negative; +, positive.
†At time of specimen collection.
‡Detection limit was a C_t value of 38.7, corresponding to 100 fg of DNA of *B. holmesii* ATCC51541.
§All strains were isolated from nasopharyngeal swab specimens.

Table 2. Clinical and epidemiologic characteristics for 6 *Bordetella holmesii*–infected patients during pertussis outbreak, Japan, September 2010–April 2011*

Patient no.	Whoop	Duration of cough	Treatment	DTP vaccine status, no. doses	Medical history	Epidemiologic findings
1	+	10 d	AZM	4	Asthma	Student at high school A. His 14-year-old sister, who was given a diagnosis of pertussis, was a student at junior high school B.
2	+	28 d	AZM	4	˜–	Student at junior high school B. Her 18-year-old brother had similar symptoms, but laboratory test results were negative.
3	–	>4 wk	AZM	4	–	Student at junior high school B. Her close friends began coughing after her disease onset.
4	+	15 d	AZM	4	Chlamydial pneumonia	Student at junior high school B. Her 11-year-old sister was given a diagnosis of pertussis before her disease onset.
5	˜–	28 d	AZM	UNK	Allergic rhinitis	Teacher at junior high school B in charge of patient 4.
6	–	23 d	AZM, CFPN-PI, GRNX	UNK	Rheumatoid arthritis	Medical staff at clinic C, which was visited by patients 1–5.

*All patients had a paroxysmal cough and coughed at night; none had posttussive vomiting. DTP, diphtheria-tetanus-pertussis; +, positive; AZM, azithromycin; –, negative; UNK, unknown; CFPN-PI, cefcapene pivoxil; GRNX, garenoxacin.

A previous report suggested that *B. holmesii* and *B. pertussis* may co-circulate in young adults (*7*). However, the relationship between pertussis epidemics and *B. holmesii* infection is not fully understood. Our active surveillance study showed that *B. holmesii* infection spread concurrently with the *B. pertussis* epidemic, but that there was no co-infection of *B. holmesii* and *B. pertussis*. Our observations demonstrate that accurate diagnosis is needed to discriminate between *B. holmesii* and *B. pertussis* infections during a pertussis outbreak because symptoms associated with these 2 diseases are similar.

In 2012, a patient with *B. holmesii* infectious pericarditis was reported in Japan (*13*). This is probably the first case report of *B. holmesii* infection in Asia. Previous surveillance studies conducted in the United States and Canada have shown low rates (0.1%–0.3%) of *B. holmesii* infection in patients with cough (*7*,*8*). However, in a recent study, *B. holmesii* DNA was detected in 20% of NPS specimens collected from patients in France who had been given a diagnosis of *B. pertussis* infection (*14*). These surveillance data indicate that *B. holmesii* infection is present in adolescents and adults, and that the organism is associated with pertussis-like symptoms. However, other causes of viral or bacterial respiratory infection cannot be excluded. Because of lack of specific diagnostic tools to detect bordetellae, *B. holmesii* infection may have been underestimated. Accurate diagnosis and further studies are required to fully elucidate the nature of *B. holmesii* infection.

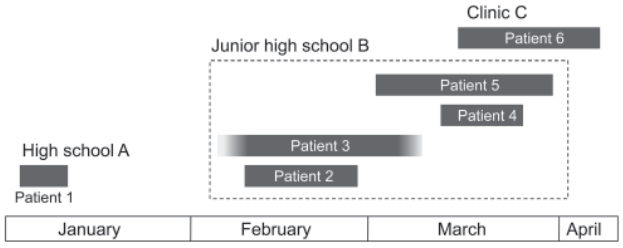

Figure 2. Epidemiologic linkage in 6 patients infected with *Bordetella holmesii* during pertussis outbreak, Japan, 2011. Duration of illness for each patient is shown as a gray box. Patient 3 provided unreliable information about the date of onset and recovery, but the patient's cough lasted for ≥1 month. Epidemiologic linkage was observed between 5 patients (patients 2–6), but not for patient 1.

Acknowledgments

We thank all medical staff for cooperating during the active surveillance study.

This study was supported by a grant for Research on Emerging and Re-emerging Infectious Diseases from the Ministry of Health, Labor, and Welfare of Japan.

Dr Kamiya is a pediatrician and medical officer at the National Institute of Infectious Diseases in Tokyo, Japan. His research interests focus on surveillance and control of vaccine-preventable diseases.

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Address for correspondence: Kazunari Kamachi, Department of Bacteriology II, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan; email: kamachi@nih.go.jp

etymologia

Rabies

[ra′bēz]

From the Latin *rabere* (to rage), which may have roots in the Sanskrit *rabhas* (to do violence). Acute progressive fatal encephalomyelitis caused by neurotropic viruses in the genus Lyssavirus—from the Greek *lyssa* (frenzy or madness). In Greek mythology, Lyssa was the goddess of rage, fury, and rabies, known for driving mad the dogs of the hunter Acteon and causing them to kill their master.

Democritus (460–370) described rabies, and Hippocrates is believed to refer to the disease when he said that "persons in a frenzy drink very little, are disturbed and frightened, tremble at the least noise, or are seized with convulsions." According to Aristotle, "Dogs suffer from the madness. This causes them to become irritable and all animals they bite to become diseased." The disease in humans was characterized by hydrophobia, in which the sick person was simultaneously tormented with thirst and fear of water. The Roman writer Cardanus described the saliva from a rabid dog as a *virus*, the Latin word for poison.

Canine rabies has been eliminated in the continental United States. However, dog bites remain a concern for travelers to areas where the disease is enzootic.

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Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30333, USA; email: boq3@cdc

Trap-Vaccinate-Release Program to Control Raccoon Rabies, New York, USA

Sally Slavinski, Lee Humberg, Martin Lowney, Richard Simon, Neil Calvanese, Brooke Bregman, Daniel Kass, and William Oleszko

In 2009, an outbreak of raccoon rabies in Central Park in New York City, New York, USA, infected 133 raccoons. Five persons and 2 dogs were exposed but did not become infected. A trap-vaccinate-release program vaccinated ≈500 raccoons and contributed to the end of the epizootic.

Central Park, described as an oasis in the midst of an urban jungle, spans 843 acres. Raccoons thrive in Central Park, an ideal habitat with an abundance of human refuse as food. Although not actually counted, the estimated raccoon population in the park is ≈500. Each year, Central Park receives >25 million visitors, offering ample opportunity for humans and off-leash dogs to be exposed to raccoons.

On August 27, 2009, a sick raccoon collected from Central Park in Manhattan tested positive for rabies virus, marking the emergence of an enzootic of raccoon rabies in Central Park. From December 2009 through December 2011, rabies test results for 133 raccoons collected in or near Central Park were also positive (Figure 1). The New York City Department of Health and Mental Hygiene (DOHMH) quickly assembled a task force with the objective of developing a response plan. The task force comprised members of the New York City DOHMH, the US Department of Agriculture Wildlife Services, the Central Park Conservancy, the New York City Department of Parks and Recreation, the New York State Department of Health, New York City Animal Care and Control, and the New York State Department of Environmental Conservation. A trap-vaccinate-release (TVR) plan was developed and implemented.

Author affiliations: New York City Department of Health and Mental Hygiene, New York, New York, USA (S. Slavinski, B. Bregman, D. Kass, W. Oleszko); US Department of Agriculture, Rockville, Maryland, USA (L. Humberg, M. Lowney); New York City Department of Parks and Recreation, New York (R. Simon); and Central Park Conservancy, New York (N. Calvanese)

DOI: http://dx.doi.org/10.3201/eid1807.111485

The Program

The trap-vaccinate-release program goals were to reduce transmission of rabies among raccoons and prevent human and pet exposure to rabid raccoons. The few examples of raccoon rabies epizootics in similar settings often used a point infection control approach: oral rabies vaccine, depopulation of up to 80% of the raccoons, and TVR (*1*–*6*).

For the Central Park outbreak, oral rabies vaccine was ruled out because of the small but potential risk for vaccinia infections of humans (*7*,*8*), given the large volume of park visitors and poor raccoon seroconversion rates (9%–61%) (*9*–*14*). Depopulation was also eliminated because it would have overwhelmed the animal shelter system with demand for humane euthanasia and decapitations and because a national animal welfare organization and the public voiced opposition. Thus, the task force chose TVR.

The makeup of Central Park and the surrounding Manhattan area creates a fishbowl-style habitat; inside the park are acres of ideal living habitat, surrounded by a mass of concrete, roadways, vehicles, and pedestrians, to contain the raccoons. Central Park and 2 small parks in

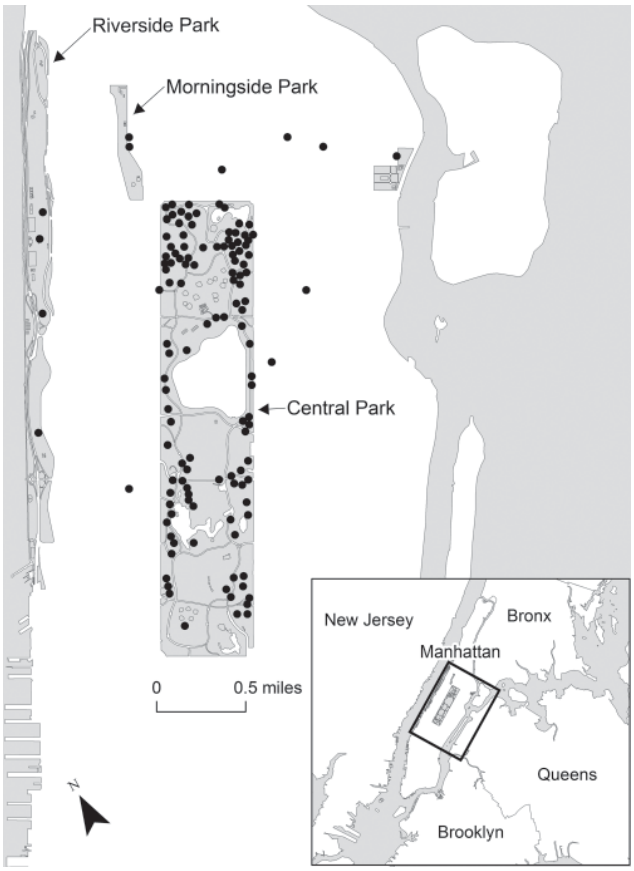

Figure 1. Location of rabid raccoons in and around Central Park, New York City, New York, USA, December 1, 2009–December 1, 2011. Each dot represents a rabid raccoon.

Table. Results of raccoon trap-vaccinate-release program, New York, New York, USA, 2010

Round	Trap nights	No. trapped	No. vaccinated and released	No. recaptured	No. sick, injured, or dead
1	1,697	460	237	165	58
2	1,409	459*	148	307	1
3	716	210	99	114	1
Total	3,822	1,129	484	586	60

*Three raccoons were submitted for rabies testing after human exposures.

close proximity were targeted for 3 rounds of TVR. The public was notified through press releases, posters, flyers, electronic messaging, and the New York City 311 telephone information service. Community boards, political leaders, and human and animal health communities were notified directly. The DOHMH website was kept updated.

The 3 rounds of TVR were conducted: February 16–April 7, 2010, September 20–November 5, 2010, and November 28–December 16, 2011. Trapping efforts were focused in Central Park, followed by Morningside and Riverside Parks. Humane cage traps baited with marshmallows and anise oil as a scent attractant were placed in sites that were off limits or of limited access to the public and their dogs. Each trap had a rabies warning sign with emergency contact information.

Trapped raccoons were visually assessed for evidence of injury, illness, or death. Raccoons that were ill or injured were humanely euthanized and, along with those found dead, were submitted for rabies testing at the DOHMH Public Health Laboratory. Healthy raccoons were immobilized in the trap by a squeeze comb, given 1 mL of rabies vaccine in a thigh muscle, identified by placement of an ear tag, and then released at the capture site. Healthy, tagged raccoons that were later recaptured during the same round of TVR were released, but those recaptured during a subsequent TVR round were revaccinated.

During each round of TVR, 26–73 traps were set per night, resulting in 3,822 trap-nights (Table). A total of 1,129 raccoons were trapped (range 0–34/night), of which 484 raccoons were vaccinated and 112 were revaccinated. Among 232 raccoons, there were 586 instances of recapture (median 1, range 1–9).

During round 1, of 460 raccoons trapped, 237 were deemed healthy, processed, and released (Table). Of 58 raccoons deemed unhealthy or found dead, 11 were rabies positive; of these, 0/6 were dead, 5/8 were sick, and 6/44 were injured. During round 2, of 459 raccoons trapped, 148 were newly vaccinated, tagged, and released, and 68 were revaccinated. One injured raccoon was euthanized; rabies test result was negative. None were found dead or sick. During round 3, of 210 raccoons trapped, 99 were newly vaccinated, tagged, and released, and 44 were revaccinated. One sick raccoon was euthanized; rabies test result was positive.

Among the vaccinated raccoons, rabies later developed in 14. The time between vaccination and recapture was 1–26 days (mean 10 days), suggesting that they were probably incubating the virus at the time of vaccination.

Exposures (confirmed or possible raccoon bites, contact with raccoon saliva) were identified for 5 persons and 2 dogs, all during January–June, 2010. Each person received rabies postexposure prophylaxis. Each dog was currently vaccinated against rabies, received a booster dose, and was monitored. Rabies did not develop in these persons or dogs.

At the peak of the epizootic, 11 rabid raccoons were reported per week. By April, the epizootic started to decline (Figure 2), probably attributable to the natural

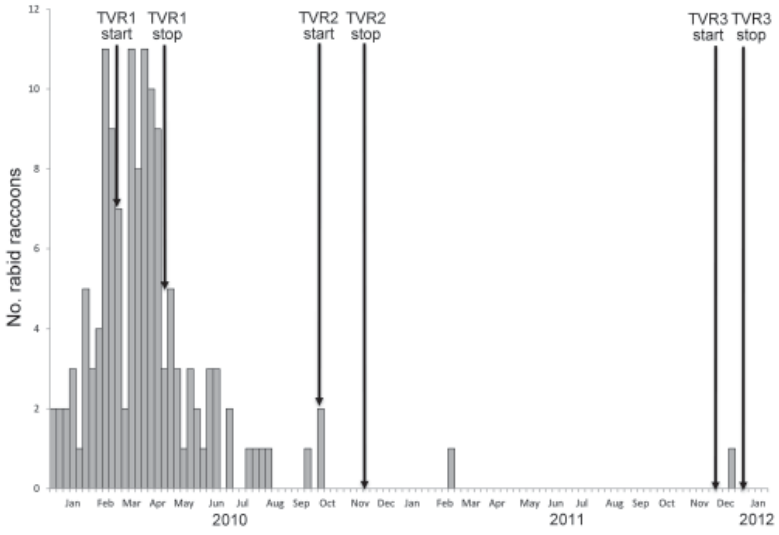

Figure 2. Number of rabid raccoons, Manhattan, New York, USA, by week, during and after the epizootic in Central Park and the corresponding dates of the 3 rounds of the trap-vaccinate-release program (TVR),

depopulation resulting from rapid spread of the virus and to the population immunity resulting from TVR. The last cases of rabid raccoons were reported on February 2 and December 9, 2011.

Conclusions

TVR seems to have effectively stemmed this epizootic of rabies in an established raccoon population. Critical to its success was the collaboration among federal, state, and local agencies and the private organizations responsible for park stewardship and animal control. This example suggests that a TVR program tailored to the geography, scope, and specifics of an epizootic in an urban area can successfully immunize a large population of raccoons and limit the potential for human and pet exposure to rabies virus.

Ongoing surveillance suggests that raccoon rabies has been successfully controlled in Manhattan. Had TVR not been implemented, the epizootic would probably have reached a state of continuous low-level enzootic activity. Given the natural border around Manhattan, it is unknown how rabies was initially introduced, but theories include illegal release of a raccoon or raccoon entry by bridge, tunnel, or even vehicle.

Annual use of TVR is not likely. However, after the immunized raccoon population declines and subsequent generations of susceptible animals predominate, another large epizootic could occur. Given the favorable park environment in which raccoon numbers can grow almost unchecked, population control should be explored as another way to prevent a recurrent epizootic with a similar explosive pattern. Public health and wildlife officials, along with academicians, should continue to explore efforts to develop safe, effective, and acceptable population control measures to help manage the unchecked growth of wildlife supported by urban centers.

Acknowledgments

We thank the members of the Rabies Task Force, Thomas Farley, the New York City Department of Parks and Recreation and the Urban Park Rangers, the Central Park Conservancy, the US Department of Agriculture, the DOHMH Public Health Laboratory Rabies Unit, and New York City Animal Care and Control for their assistance. We also thank the US Department of Agriculture Wildlife Services for their field service, which was instrumental to the program.

Dr Slavinski is assistant director of the Zoonotic, Influenza and Vector-Borne Disease Unit of the New York City DOHMH. Her research interests are surveillance and investigation of diseases such as rabies, Lyme disease, West Nile virus infection, and several reportable animal diseases.

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Address for correspondence: Sally Slavinski, New York City Department of Health and Mental Hygiene, 2 Gotham Center, CN# 22A, 42-09 28th St, Queens, NY 11101-4132, USA; email: sslavins@health.nyc.gov

Potential International Spread of Multidrug-Resistant Invasive *Salmonella enterica* Serovar Enteritidis

Irene Rodríguez, M. Rosario Rodicio, Beatriz Guerra, and Katie L. Hopkins

In developing countries, *Salmonella enterica* serovar Enteritidis causes substantial illness and death, and drug resistance is increasing. Isolates from the United Kingdom containing virulence-resistance plasmids were characterized. They mainly caused invasive infections in adults linked to Africa. The common features in isolates from these continents indicate the role of human travel in their spread.

Worldwide, nontyphoidal *Salmonella enterica* is a major cause of foodborne illness, and Enteritidis is one of the most commonly reported serovars (www.who.int/salmsurv/links/GSSProgressReport2005.pdf). In industrialized countries, *S. enterica* serovar Enteritidis commonly causes self-limiting gastroenteritis, for which treatment with antimicrobial drugs is usually not needed. However, in developing countries, this serovar, together with serovar Typhimurium, frequently causes invasive infections and substantial illness and death among young children with underlying diseases and among adults with HIV infection (*1*). Although antimicrobial drug resistance is not as high in *S. enterica* serovar Enteritidis as in other zoonotic disease serovars, multidrug-resistance (resistance to ≥4 antimicrobial drugs) has been increasingly reported (*2*), threatening treatment success for patients with severe infections. In recent years, in association with multidrug resistance, another trend has arisen: the emergence of virulence-resistance (VR) plasmids; these are hybrid plasmids that harbor resistance (R) and virulence (V) determinants. The appearance of these plasmids is of concern because they could lead to the co-selection of virulence (in addition to resistance) through the use of antimicrobial drugs (*3*,*4*). One such plasmid, pUO-SeVR1, has been recently reported in a multidrug-resistant (MDR) clinical isolate of *S. enterica* serovar Enteritidis (CNM4839/03) from Spain (*5*). This mobilizable plasmid of ≈100 kb derives from pSEV, the serovar-specific V plasmid of *S. enterica* serovar Enteritidis, and carries most of its V determinants, including the *spvRABCD* locus (*Salmonella* plasmid virulence). This plasmid greatly increases the ability of salmonellae to proliferate intracellularly and has been associated with severe infections in humans (*6*). The plasmid also harbors several R genes—$bla_{\text{TEM-1}}$, *catA2, strA-strB, sul1, sul2, tet*(A)—and a class-1 integron with the 700-bp/*dfrA7* variable region, which confer resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline, and trimethoprim (R-type ACSSuTTm). To investigate their international spread, we studied the presence of *S. enterica* serovar Enteritidis isolates carrying pUO-SeVR1–like plasmids in the United Kingdom.

Author affiliations: Federal Institute for Risk Assessment, Berlin, Germany (I. Rodríguez, B. Guerra); Universidad de Oviedo, Oviedo, Spain (M.R. Rodicio); and Health Protection Agency, London, UK (K.L. Hopkins)

DOI: http://dx.doi.org/10.3201/eid1807.120063

The Study

We screened 31,615 *S. enterica* serovar Enteritidis isolates that had been collected from clinical specimens during 2005–2010 and deposited in the culture collection of the Health Protection Agency *Salmonella* Reference Unit. We screened the isolates for R-type ACSSuTTm. A total of 14 serovar Enteritidis isolates showing this resistance phenotype were detected and subsequently examined for the presence of integron-located *dfrA7*. Of the 14 isolates, 11 were positive and their plasmid content was analyzed by S1 pulsed-field gel electrophoresis (PFGE) (*2*) and by the Kado and Liu methods (*7*); we used serovar Enteritidis strains NRL-Salm-PT4 and CNM4839/03 as controls for pSEV– and pUO-SeVR1–carrying isolates, respectively. The 11 isolates harbored 1 plasmid of variable size (60–95 kb); among these, 9 isolates hybridized with *dfrA7*-specific and *spvC*-specific probes (with plasmids of 85–95 kb) (Figure). These 9 isolates contained a VR-hybrid plasmid similar to pUO-SeVR1 and were selected for further analyses (Tables 1, 2). The remaining 2 isolates carried the normal pSEV plasmid (60 kb), in which *spvC* hybridized; *dfrA7* was chromosomally located.

In the 9 isolates carrying VR-hybrid plasmids, the R-type ACSSuTTm was encoded by the R-genes $bla_{\text{TEM-1}}$, *catA2*, *strA-strB*, *sul1*, *sul2*, *tet*(A), and *dfrA7*, which were located on the pUO-SeVR1–like plasmids as determined by Southern blot hybridization. By PCR amplification, using previously described primers and conditions (*5*,*8*) (Table 2), and by Southern blot hybridization (*5*), we tested for the presence of IncFII and IncFIB replicons, *parAB* (partition), *spvRABCD*, *rck* (resistance to complement killing), *mig-5* (macrophage-induced gene), *pefABCDI* (Pef fimbriae operon), and *srgA* (SdiA-regulated gene; next

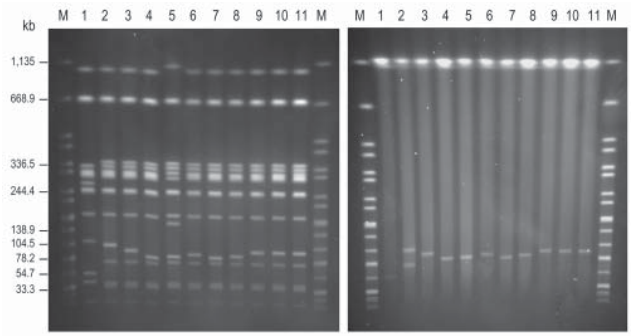

Figure. Genomic macrorestriction of *Salmonella enterica* serovar Enteritidis isolates: pulsed-field gel electrophoresis profiles for *Xba*I (left panel) and S1 (right panel). Lane M, *Xba*I-digested DNA of *S. enterica* serovar Braenderup H9812, used as size standard; lane 1, NRL-Salm-PT4; lane 2, CNM4839/03; lane 3, H051860415; lane 4, H070360201; lane 5, H070420137; lane 6, H073180204; lane 7, H091340084; lane 8, H091800482; lane 9, H095100307; lane 10, H100240198; lane 11, H101700366. The strain NRL-Salm-PT4 was used as control for the most commonly found *Xba*I-profile in *S. enterica* serovar Enteritidis.

to *orf7*), all carried by pSEV. The 9 plasmids were positive for the 2 replicons and for all genes screened except *pefC* and *pefD* (absent in H070360201 and H070420137), *pefI-orf7* (absent in all), and *srgA* (either absent [H051860415, H073180204, and H091800482] or truncated [in the remaining isolates]) (Table 2).

Isolate subtyping was conducted by phage typing, multilocus variable number tandem repeat analysis (MLVA), multilocus sequence typing (MLST), and *Xba*I-PFGE (*9*,*10*) (http://mlst.ucc.ie/mlst/dbs/Senterica; www.pulsenetinternational.org). The 9 *S. enterica* serovar Enteritidis isolates belong to phage type (PT) 42, in contrast with CNM4839/03, which belongs to PT14b (Table 2). We identified 4 MLVA profiles, which were all single-locus variants of the highly variable locus SENTR5, indicating that the isolates are closely related (Table 2). The isolate from Spain and 3 of the 9 isolates from the United Kingdom, selected as representative of each MLVA profile, were also analyzed by MLST (Table 2). CNM4839/03 and H091340084 were assigned to sequence type (ST) 11, the most commonly found ST in serovar Enteritidis (http://mlst.ucc.ie/mlst/). The remaining 2 isolates could be ascribed to ST1479, the first examples of this single-locus variant of ST11 in the MLST database (Table 2). According to *Xba*I-PFGE, the control strain NRL-Salm-PT4 showed a clearly distinct profile in comparison with CNM4839/03 and the 9 isolates containing pUO-SeVR1–like plasmids, which generated 6 closely related patterns (Figure). Most isolates differed by 1 band of variable size (85–95 kb), which corresponded to the *Xba*I-linearized VR-hybrid plasmids. As an exception, isolate H070420137 showed additional differences in chromosomal bands of ≈150 and ≈300 kb. Because isolates H070420137 and H070360201 came from the same patient (Table 1) and shared the same V and R genotypes and other typing markers, 1 isolate could have evolved from the other; however, co-infection of the patient with 2 closely related strains cannot be ruled out. In addition, considering the typing results of H095100307, H100240198, and H101700366, the identical size of their plasmids, and the fact that they were isolated from the same patient (Table 1), these 3 isolates can be considered the same strain.

All except 2 of the UK isolates carrying pUO-SeVR1–like plasmids were recovered from the blood of patients who had recently returned from an African country or who had an African name (Table 1). Supporting the possible African origin, similar *Xba*I-PFGE profiles and MDR phenotypes (ampicillin, trimethoprim, sulfonamides, and tetracycline) have been identified in clinical *S. enterica* serovar Enteritidis isolates involved in outbreaks and community infections in different African countries. These isolates caused bacteremia, meningitis, diarrhea (*11*–*13*), and high case-fatality rates; they affected mainly children, whereas most clinical isolates analyzed in our study were obtained from adults with bacteremia (Table 1). The detection of the 700-bp/*dfrA7* integron in *S. enterica* serovar Enteritidis isolates from Africa (*14*) also supports an African origin of the MDR serovar Enteritidis isolates harboring pUO-

Table 1. Epidemiologic information for multidrug-resistant *Salmonella enterica* serovar Enteritidis isolates, 2005–2010, UK*

Isolate no.	Date of isolation	Source	Recent travel history	African patient name	Patient age, y
CNM4839/03†	2003	Feces	Unknown	No	3
H051860415	2005 Apr 19	Blood	Nigeria	No	38
H070360201‡	2007 Jan 14	Blood	Unknown	Yes	35
H070420137‡	2007 Jan 15	Feces	Unknown	Yes	35
H073180204	2007 Jul 31	Blood	Unknown	Yes	34
H091340084	2009 Mar 15	Feces	Uganda	No	59
H091800482	2009 Apr 17	Blood	Unknown	Yes	30
H095100307§	2009 Dec 7	Blood	Unknown	Yes	68
H100240198§	2010 Jan 9	Blood	Unknown	Yes	68
H101700366§	2010 Apr 22	Blood	Unknown	Yes	68

*All isolates contained a virulence-resistance hybrid plasmid similar to pUO-SeVR1.
†Control isolate from Spain.
‡Recovered from the same patient.
§Recovered from the same patient.

Table 2. Characteristics of *Salmonella enterica* serovar Enteritidis isolates harboring pUO-SeVR1-like plasmids, 2005–2010, UK*

Isolate no.	Phage type	Resistance phenotype/ genotype	Class 1 integron†	pSEV genes‡	MLVA	MLST	VR plasmid, kb
CNM4839/03	PT 14b	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, srgB, srgC, pefA, pefB, pefC, pefD*	2-12-9-4-4-3-NA-8-8	ST11	100
H051860415	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, srgB, srgC, pefA, pefB, pefC, pefD*	2-13-9-4-4-3-NA-8-8	ST1479	95
H070360201	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, ΔsrgA, srgB, srgC, pefA, pefB*	2-15-9-4-4-3-NA-8-8	ST1479	88
H070420137	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, ΔsrgA, srgB, srgC, pefA, pefB*	2-15-9-4-4-3-NA-8-8	ND	88
H073180204	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, srgB, srgC, pefA, pefB, pefC, pefD*	2-13-9-4-4-3-NA-8-8	ND	92
H091340084	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, ΔsrgA, srgB, srgC, pefA, pefB, pefC, pefD*	2-9-9-4-4-3-NA-8-8	ST11	90
H091800482	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, srgB, srgC, pefA, pefB, pefC, pefD*	2-13-9-4-4-3-NA-8-8	ND	92
H095100307	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, ΔsrgA, srgB, srgC, pefA, pefB, pefC, pefD*	2-13-9-4-4-3-NA-8-8	ND	95
H100240198	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/d*frA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, ΔsrgA, srgB, srgC, pefA, pefB, pefC, pefD*	2-13-9-4-4-3-NA-8-8	ND	95
H101700366	PT 42	AMP, CHL, STR, SUL, TET, TMP/*bla*$_{TEM-1,}$ *catA2, strA-strB, sul1, sul2, tet*(A), *dfrA7*	700 bp/*dfrA7*	*spvA, spvB, spvC, spvR, rsk, rck, mig-5, ΔsrgA, srgB, srgC, pefA, pefB, pefC, pefD*	2-13-9-4-4-3-NA-8-8	ND	95

*pSEV, serovar-specific V plasmid of *S.enterica* serovar Enteritidis; MLVA, multilocus variable number tandem repeat analysis; MLST, multilocus sequence typing; VR, virulence-resistance; PT, phage type; NA, no amplification from this locus; AMP, ampicillin; CHL, chloramphenicol; STR streptomycin; SUL, sulfonamides; TET, tetracycline; TMP, trimethoprim; ST, sequence type; ND, not done.
†Size of the variable region amplified with the 5′CS and 3′CS primers (*2*).
‡All plasmids were positive for IncFIIA, IncFIB and the *par* locus. Two new primer pairs were devised for detection of *srgA*: srgAB-Fw1/Rv1 (5′-CGCCTTCCGTGTATGTCC/GCGAGTCACTCACCGACAG-3′) and srgAB-Fw2/Rv2 (5′-GTTGCACAGGAGTGGGAGTC/GTCCGGGTTCCATGTCAG-3′). The forward primers anneal at different positions within *srgA*; the reverse primers anneal at different positions within *srgB*.

SeVR1–like plasmids. Of note, resistance derivatives of pSLT, the V-plasmid specific to *S.* Typhimurium, have been found in the epidemic ST313 clone of this serovar, which has been considered a major cause of invasive disease in sub-Saharan Africa (*15*).

Conclusions

Closely related MDR *S. enterica* serovar Enteritidis isolates carrying pUO-SeVR1–like plasmids were recovered in the United Kingdom. Most were isolated from the blood of patients linked to Africa, and they showed common features with serovar Enteritidis isolates involved in outbreaks on that continent. The possibility that this potentially invasive clone of *S. enterica* serovar Enteritidis can be spread through human travel, together with the detection of VR-plasmids in the serovar most frequently associated with human infections, is of public health concern and requires surveillance.

Acknowledgments

We thank B. Malorny and E. Junker for help with MLVA typing; A. Schroeter, J. Ledwolorz, and staff of the Health

Protection Agency *Salmonella* Reference Unit for phage typing; and I. Montero for help with MLST. We are grateful to R. Helmuth for his advice and critical reading of the manuscript.

This study was supported by the Health Protection Agency, the Federal Institute for Risk Assessment (BfR-46-001; 45-005), and the "Fondo de Investigación Sanitaria" of the "Instituto de Salud Carlos III" of Spain (project FIS PI11/00808, cofunded by the European Regional Development Fund of the European Union: a Way of Making Europe). I.R. received a postdoctoral grant from Fundación Ramón Areces, Madrid, Spain.

For strain requests, contact the Health Protection Agency Colindale, email: katie.hopkins@hpa.org.uk.

Dr Rodríguez is a postdoctoral fellow at the Federal Institute for Risk Assessment in Berlin, Germany, in the Unit of Antimicrobial Resistance and Resistance Determinants of the Department of Biological Safety. Her research focuses on the genotypic characterization of antimicrobial drug resistance and the molecular epidemiology of gram-negative drug-resistant bacteria.

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Address for correspondence: Irene Rodríguez, Federal Institute for Risk Assessment, Diedersdorfer Weg 1, D-12277 Berlin, Germany; email: rodriguezfirene@uniovi.es

Outbreak-associated *Vibrio cholerae* Genotypes with Identical Pulsotypes, Malaysia, 2009

Cindy Shuan Ju Teh, Zarizal Suhaili, King Ting Lim, Muhamad Afif Khamaruddin, Fariha Yahya, Mohd Hailmi Sajili, Chew Chieng Yeo, and Kwai Lin Thong

A cholera outbreak in Terengganu, Malaysia, in November 2009 was caused by 2 El Tor *Vibrio cholerae* variants resistant to typical antimicrobial drugs. Evidence of replacement of treatable *V. cholerae* infection in the region with antimicrobial-resistant strains calls for increased surveillance and prevention measures.

Vibrio cholerae, the causative agent of cholera, is endemic in many parts of the world, especially in countries that lack clean water supplies and adequate public health facilities (*1*). In Malaysia, cholera outbreaks caused by the El Tor O1 *V. cholerae* serogroup occur periodically, cases from the 0139 serogroup occur sporadically, and the non–O1/non–O139 *V. cholerae* serogroup has not been implicated in any major outbreak (*2*–*4*). Contaminated drinking water, cooked food, and raw or undercooked seafood served as vehicles of transmission in Malaysia (*5*).

The Study

In November 2009, a cholera outbreak occurred in Terengganu, Peninsular Malaysia. The outbreak began in the capital, Kuala Terengganu, and spread to several districts within a week. Approximately 400 persons were hospitalized for treatment of acute diarrhea and its complications during the outbreak period. One death occurred before the local health authorities declared an outbreak. Five ice factories, 2 fish cracker factories, and several restaurants and street cart food vendors were ordered closed because they were suspected of being possible sources of the outbreak (Ministry of Health, Malaysia, unpub. data).

For this study, 75 rectal swab samples, collected from patients admitted to Hospital Sultanah Nur Zahirah in Kuala Terengganu who had acute diarrhea during the outbreak period, were available for analysis. In addition, 60 environmental samples (6 water samples, 54 environmental swab samples) were collected from 2 of the ice factories (factories A and B) in Kuala Terengganu by the Terengganu State Department of Health during the outbreak period and were provided to us for analysis. Environmental swab samples were obtained from several areas within the ice-making factories. The rectal swab and environmental samples were enriched overnight in alkaline-buffered peptone water, pH 8.6 (Oxoid, Basingstoke, UK) and cultured on thiosulfate citrate-bile salts-sucrose agar (Oxoid). The presumptive colonies were subjected to conventional biochemical tests, such as string, salt tolerance, Voges-Proskauer, lysine iron agar, Kliger iron agar, and arginine dihydrolase testing. PCRs targeting *ompW*, *hlyA*, *rfb*, *ctxA*, *toxR*, *tcpI*, *rtxC*, *rstR*, and *tcpA* genes as described (*6*,*7*) were run in parallel to confirm and characterize *V. cholerae* isolates. Template DNA was also prepared directly from the water samples as described (*6*) for detection of viable but nonculturable *V. cholerae* and its virulence genes.

Antimicrobial drug susceptibility of the confirmed *V. cholerae* isolates was determined by the disk diffusion method according to Clinical and Laboratory Standards Institute guidelines (*8*). Six antimicrobial agents (Oxoid) were used: ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), trimethoprim/sulfamethoxazole (25 μg), erythromycin (15 μg), and tetracycline (30 μg). To determine the genetic relatedness of the isolates, pulsed-field gel electrophoresis (PFGE) was performed according to the established PulseNet protocol (*9*) and analyzed with BioNumerics 6.0 (Applied Maths, Kortrijk, Belgium); *ctxB* genotyping was also performed as described (*10*).

On the basis of conventional biochemical tests and PCR, 37 isolates from the rectal swab samples and 1 isolate from the washroom swab sample of ice factory B were confirmed as *V. cholerae*, showing an isolation rate of 48.0% for the clinical samples and 1.9% for the environmental samples. In addition, the 37 clinical isolates were identified as El Tor O1 on the basis of Voges-Proskauer tests and were positive for *hlyA*^{El}, *tcpA*^{El}, *rstR*^{El}, *rtxC*, and *rfb*O1 genes. The *ctxA*, *toxR*, and *tcpI* genes were present in all of the clinical isolates. The isolate from the restroom specimen of factory B was identified as a non–O1/non–O139 *V. cholerae* strain that had *hlyA*^{El}, *rstR*^{El}, and *toxR* genes. This finding indicated that this isolate was likely not related to the outbreak in question. No amplification of *V. cholerae*–specific genes was observed for the DNA extracted directly from the water samples.

Author affiliations: University of Malaya, Kuala Lumpur, Malaysia (C.S.J. Teh, K.T. Lim, K.L. Thong); and Universiti Sultan Zainal Abidin, Kuala Terengganu, Malaysia (Z. Suhaili, M.A. Khamaruddin, F. Yahya, M.H. Sajili, C.C. Yeo).

DOI: http://dx.doi.org/10.3201/eid1807.111656

Although the environmental non-O1/non-O139 *V. cholerae* isolate was sensitive to all the antimicrobial agents tested, the 37 clinical O1 *V. cholerae* isolates were resistant to ampicillin, trimethoprim/sulfamethoxazole, erythromycin, and tetracycline. In Malaysia, tetracycline generally has been considered the drug of choice for cholera treatment; however, it has been replaced by erythromycin because the number of tetracycline-resistant strains has increased since a 1992 outbreak in the state of Kelantan (*11*). The emergence of erythromycin-resistant isolates in this outbreak will likely contribute to decreased efficacy of erythromycin.

PFGE of *Not*I-digested chromosomal DNA from all of the human isolates resulted in 1 pulsotype with 24 fragments (≈30 kb to ≈370 kb); the environmental isolate showed a distinct pulsotype (F = 0.83) (Figure 1). PFGE was repeated 2× with identical results. In addition, the pulsotype of the clinical isolates in this study was identical to the pulsotype of an O1 isolate (123/08) from a cholera patient in Kuala Lumpur in 2008 (Figure 1) (*12*). Isolate 123/08 also showed identical antibiograms with the Terengganu O1 outbreak isolates. This finding suggests that isolate 123/08 and the Terengganu 2009 O1 outbreak isolates were possibly linked.

Several *ctxB* alleles have been identified among O1 *V. cholerae* strains on the basis of a few point mutations: 1) classical and El Tor, US Gulf Coast (39His, 46Phe, 68Tyr); 2) El Tor, Australia (39His, 46Leu, 68Tyr); and 3) El Tor, seventh pandemic, and El Tor, Latin American epidemic (39Tyr, 46Phe, 68Ile) (*10*). Of the 37 isolates, 33 were classified as genotype 3 on the basis of multiple sequence alignments of *ctxB* (*10*). Similarly, our previous multilocus sequence typing study (*2*) subtyped isolate 123/08 as an El Tor biotype characterized by the *ctxB3* gene (Figure 2). We postulate that this particular clone has reemerged since its isolation in Kuala Lumpur in 2008 and was likely linked to the 2009 Terengganu outbreak.

Four of the 37 clinical isolates showed amino acid substitutions Tyr39His and Ile68Thr (Figure 2) and were classified as genotype 1. Genotype 1 strains that carry both classical and El Tor *rstR* allele have been detected in Asian and African countries since the 1990s (*13*). In 2001, El Tor variant strains that harbored *rstR*^{El}/*ctxB1* superseded the typical El Tor strains in Bangladesh and other Asian countries. Since 2007, outbreaks in Vietnam and Thailand have been mainly caused by El Tor variant strains (*7*,*14*). Ang et al. (*3*) reported that El Tor variant strains could have emerged earlier in Malaysia because 1 such strain was responsible for the 2000 outbreak in Kelantan. In contrast to neighboring countries, our current study indicates that the El Tor strains have not been replaced by El Tor variant strains in Malaysia; typical El Tor strains are still found in this country. In our current study, the isolates could not be differentiated by PFGE, although they belonged to 2 *ctxB* genotypes. This finding might be attributed to the genetic events resulting in the transfer of the different *ctxB* alleles among the *V. cholerae* populations in Malaysia or with the strains from neighboring countries.

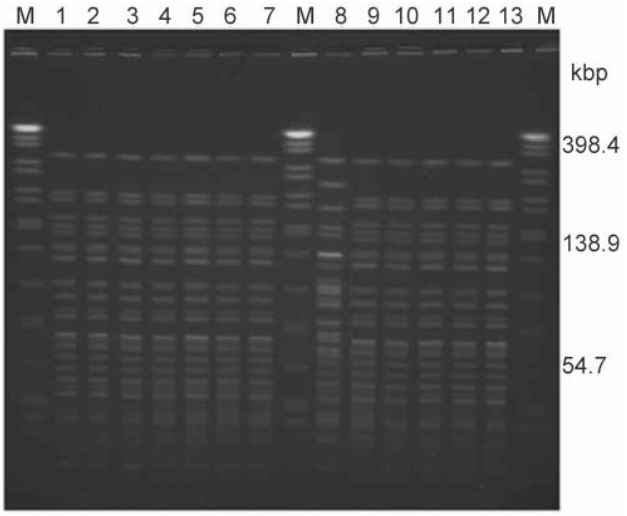

Figure 1. *Not*I-pulsed-field gel electrophoresis profiles of *Vibrio cholerae* isolated during the outbreak, Terengganu, Malaysia, 2009. Lane M: *Xba*I-digested *Salmonella enterica* serovar Braenderup H9812 as DNA standard; lanes 1–7 and 9–12: isolates of El Tor O1 serogroup (rectal swab); lane 8: isolate of non–O1/non–O139 serogroup (swab from ice factory); lane 13: El Tor O1 *V. cholerae* isolated in 2008 (Kuala Lumpur).

Conclusions

The 2009 cholera outbreak in Terengganu was controlled in late November; 187 cases and 1 death were confirmed (www.myhealth.gov.my/myhealth). We found no evidence of an association between 2 ice factories considered as possible sources of infection and the

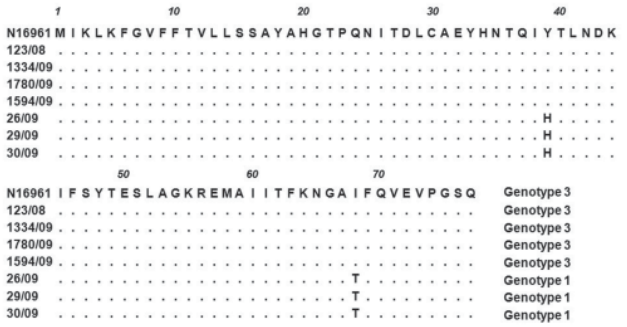

Figure 2. Amino acid sequence alignment of the *ctxB* subunit of representative *Vibrio cholerae* isolates from the cholera outbreak, Terengganu, Malaysia, 2009. El Tor O1 N16961 (*ctxB3*) was used as the reference strain in the alignment. Identical amino acid residues are indicated by dots. Two genotypes (*1*,*3*) were observed in the outbreak strains.

outbreak. The health authorities later ruled out the other ice factories, fish cracker factories, and eateries suspected of being sources and were unable to trace the source of the outbreak.

Two genotypes (ctxB1 and ctxB3) of the El Tor O1 *V. cholerae* serogroup with identical pulsotypes were likely responsible for the cholera outbreak in Terengganu in late 2009. Our findings support the need for increased surveillance in the region to document the prevalence of such strains. Preventive activities such as water sanitation, public education on proper food handling, and personal cleanliness are crucial to reduce the risk of spread of cholera.

Acknowledgments

We thank Ahmad Rushdi, Tan Abdullah, and Afandi B Ahmad for technical support; and Hospital Sultanah Nur Zahirah and the State Department of Health, Kuala Terengganu, for the samples.

The study was partially supported by the Higher Impact Research grant (UM.C/625/1/HIR/MOHE/02) from University of Malaya.

Dr Teh is a postdoctoral research fellow at University of Malaya. Her research interests are surveillance of enteric pathogens, detection of outbreaks, genomic diversity of enteric pathogens with reference to strain evolution, and molecular pathogenesis.

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Address for correspondence: Kwai Lin Thong, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia; email: thongkl@um.edu.my

Dobrava Hantavirus Infection Complicated by Panhypopituitarism, Istanbul, Turkey, 2010

Nevin Sarıgüzel,[1] Jörg Hofmann,[1] Alper Tunga Canpolat, Ali Türk, Jakob Ettinger, Deniz Atmaca, Işın Akyar, Serap Yücel, Ender Arıkan, Yavuz Uyar, Dilek Y. Çağlayık, Ayşe Sesin Kocagöz, Ayşin Kaya, and Detlev H. Kruger

We identified Dobrava-Belgrade virus infection in Turkey (from a strain related to hantavirus strains from nearby countries) in a patient who had severe symptoms leading to panhypopituitarism, but no known risk for hantavirus. Our findings emphasize the need for increased awareness of hantaviruses in the region and assessment of symptomatic persons without known risk factors for infection.

Hemorrhagic fever with renal syndrome (HFRS) is caused by infection with hantaviruses. Most patients with HFRS recover completely, but acute and chronic complications may develop. HFRS patients with severe lung involvement resembling hantavirus cardiopulmonary syndrome have been described (*1*). In addition, pituitary hemorrhage, followed by hypopituitarism, is a possible complication of HFRS. Involvement of the pituitary gland has been observed in some patients infected with Puumala virus, a hantavirus commonly found in western and central Europe (*2*–*4*).

We report on a patient who experienced shock, pulmonary failure, and panhypopituitarism as complications of HFRS. By testing for neutralizing antibodies and by amplification and molecular characterization of virus samples, we identified the causative pathogen as a strain of Dobrava-Belgrade virus (DOBV) that is closely related to a hantavirus strain typically carried by the yellow-necked field mouse (*Apodemus flavicollis*), strain DOBV-Af.

Author affiliations: Acıbadem Hospital, Istanbul, Turkey (N. Sarıgüzel, A.T. Canpolat, D. Atmaca, Serap Yücel, Ender Arıkan); Charité University Medicine, Berlin, Germany (J. Hofmann, J. Ettinger, D.H. Kruger); Labor Berlin Charité-Vivantes GmbH, Berlin (J. Hofmann, J. Ettinger, D. H. Kruger); Acıbadem University, Istanbul (A.Türk, I. Akyar, A. S. Kocagöz); Refik Saydam National Public Health Agency, Ankara, Turkey (Y. Uyar, D. Y. Çağlayık); and University of Geneva, Geneva, Switzerland (A. Kaya)

DOI: http://dx.doi.org/10.3201/eid1807.111746

The Case-Patient

A 34-year-old man with fever (38°C), tender cervical lymph nodes, and symptoms of pharyngeal infection was admitted to a hospital in Istanbul, Turkey on February 3, 2010. His blood pressure was 120/70 mm Hg and heart rate was 96 beats/min. Laboratory findings included thrombocytopenia (63,000 platelets/mm^3 [reference 130–450×10^3 platelets/mm^3]) and mild elevation of transaminase levels (alanine transaminase 95 IU/L [reference 10–40 IU/L]; Aspartate aminotransferase 99 IU/L [reference 15–40 IU/L]) (Table 1). Results of urinalysis, chest radiograph, and ultrasound of the abdomen were normal. Diarrhea developed during the first day of hospitalization, and conjunctival suffusion was observed on ocular examination. Ciprofloxacin was given to treat suspected salmonellosis at a dosage of 400 mg every 12 hours.

On day 2 of hospitalization, the patient became oliguric and hypotensive. He had persisting fever. His platelet count decreased to 20,000/mm^3, and his serum creatinine level increased to 1.8 mg/dL (reference 0.7–1.2 mg/dL). Urinalysis results showed microscopic hematuria and proteinuria. Results of a transthoracic echocardiogram showed heart chambers of normal size, a left ventricular ejection fraction of 60%, and moderate pericardial effusion. The patient was transferred to the intensive care unit. Broad-spectrum antimicrobial drugs were initiated as treatment for suspected sepsis, and platelet and albumin transfusions were given as supportive therapy.

On day 3 of hospitalization, the patient experienced tachycardia, petechial lesions appeared on his extremities, and his platelet count dropped to 13,000/mm^3. Results of a bone marrow aspiration showed proliferation of histiocytes and prominent hemophagocytosis. Intravenous methylprednisolone therapy (100 mg/day) was started. Septic shock developed in the patient, and inotropic therapy was initiated. On the same day, acute respiratory distress syndrome developed, and assisted ventilation was started. Results of a thoracic computerized tomographic scan showed focal infiltration on the upper zone of the left lung and minimal pleural effusion on the right lung. Results of abdominal computerized tomographic scan revealed ascites and multiple mesenteric lymphadenomegalies. On the basis of these clinical and laboratory findings, hantavirus infection was suspected. Blood samples were obtained for hantavirus testing, and oral ribavirin was added at an initial dose of 30 mg/kg, followed by 15 mg/kg every 6 hours for 4 days, then 7.5 mg/kg for 6 days. On the same day,

[1]These authors contributed equally to this article.

Table 1. Hematologic and biochemical parameters of DOBV hantavirus patient, Istanbul, Turkey, 2010*

Laboratory test (reference range)	Day of hospitalization								
	0	1	2	3	4	5	15	20	71
Leukocytes, $\times 10^3$ cells/mm^3 (4.5–11)	5.8	9.9	15.2	27.6	24	25	11.3	4.7	7.7
Hemoglobin, g/dL (11.7–15.5)	16	16.5	18	16	13.3	10.7	8.2	8.9	12.1
Hematocrit, % (36–46)	46.7	48	51.8	45.2	38.3	31.2	24.6	27	36.6
Platelets, $\times 10^3$/mm^3 (130–450)	63	19	20	13	37	55	180	171	254
ALT, U/L (10–40)	95	92	58	67	157	539	79	60	20
AST, U/L (15–40)	99	117	–†	–	497	2234	106	84	25
BUN, mg/dL (4.67–23.3)	–	18	23	30	41	41	129	74	19
Creatinine, mg/dL (0.7–1.2)	–	0.8	1.8	2.4	3.3	3.1	7.3	6.4	1.92
aPTT, s (4–40)	–	39	43	–	32.5	37.1	30.8	–	–
PT, s (10–14 s)	–	13.3	11.6	–	17.5	20	14.1	–	–
Ferritin, ng/mL (15–200)	–	5,304	–	–	14,036	–	–	–	–
TSH, U/mL (0.4–4.2)	–	1.71	–	–	–	–	–	2.83	0.92
Free thyroxin, pmol/L (10.3–23.2)	–	–	–	–	–	–	–	0.18	14.3
Cortisol, µg/dL (6.2–19.4)	–	–	–	–	–	45.0	–	0.98	4.7
ACTH, pg/mL (0–46)	–	–	–	–	–	3.02	–	6.80	–
ADH, pg/mL (0.8–4.5)	–	–	–	–	–	–	–	2.2	–
Total testosterone, ng/mL (2.8–8.0)	–	–	–	–	–	–	–	<0.025	1.9
LH, mIU/mL (1.7–8.6)	–	–	–	–	–	–	–	<0.1	–
FSH, mIU/mL (1.5–12.4)	–	–	–	–	–	–	–	0.66	–
Prolactin, mIU/mL (4.6–21.4)	–	–	–	–	–	–	–	10.05	–
Somatomedin-C/IGF-1, ng/mL (115–307)	–	–	–	–	–	–	–	<25	–

*DOBV, Dobrava-Belgrade virus; ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; aPTT, activated partial thromboplastin time; PT, prothrombin time; TSH, thyroid stimulating hormone; ACTH, adrenocorticotropic hormone; ADH, antidiuretic hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; IGF, insulin-like growth factor.
†Test not performed.

hemofiltration was started and continued for 6 consecutive days. The patient's urinary output gradually increased, pulmonary symptoms regressed, and his ventilation tube was removed on day 12 of hospitalization. The patient stayed in the intensive care unit for 18 days and was then transferred to a standard care unit.

No microbial growth was observed in the cultures of samples taken during the first days of hospitalization. Serum samples were tested for antibodies against a panel of pathogens, including *Salmonella typhi, S. paratyphi* A and B, *Brucella* spp., HIV, Crimean-Congo hemorrhagic fever virus, Epstein-Barr virus, cytomegalovirus, parvovirus B19, adenovirus, dengue virus, *Rickettsia* spp., *Leptospira* spp., *Treponema pallidum*, and hepatitis viruses A, B, C, and E. Results of these serologic tests were negative or inconspicuous. Results of PCR analysis conducted for leptospirosis, respiratory viruses, and cytomegalovirus were below the detection limit of the assays.

Serologic testing for hantavirus was performed by using the *recom*Line Bunyavirus IgG/IgM immunoassay (Mikrogen, Neuried, Munich, Germany); results showed strong reactivity for IgG and IgM antibodies, indicating an acute hantavirus infection. The blot data provided evidence of an infection with DOBV; to confirm the results, we performed serotyping by using focus reduction neutralization tests (Table 2). The results confirmed a DOBV infection most likely caused by a strain of DOBV-Af. Reverse transcription PCR results for hantaviral RNA were positive for the first acute-phase blood sample. Subsequent nucleotide sequence determination of parts of the 3 genomic segments and molecular phylogenetic analysis of these small, medium (Figure 1), and large gene sequences (data not shown) showed that the isolate was most closely related to DOBV-Af.

On day 19 of hospitalization, the patient displayed generalized weakness. The results of the basal levels of hormonal studies are shown in Table 1. On day 20 of hospitalization, panhypopituitarism was diagnosed. Hormone replacement therapy using hydrocortisone, thyroxin, and testosterone was immediately initiated. On day 22 of hospitalization, magnetic resonance imaging showed pituitary hemorrhage and pituitary atrophy (Figure 2). After the administration of hormone replacement therapy, symptoms improved markedly. The patient was discharged on day 33 of hospitalization. At the final outpatient followup visit, the patient had normal urine output and his serum creatinine level was 1.5 mg/dL. His pulmonary examination results and chest radiograph findings were normal, but he continued to require hormonal replacement therapy at the 16th month of followup care.

Table 2. Characterization of DOBV hantavirus in a patient's serum by focus reduction neutralization tests, Istanbul, Turkey, 2010*

Day of hospitalization	Focus reduction neutralization test endpoint titer			
	Puumala hantavirus	Hantaan virus	DOBV-Aa (strain SK)	DOBV-Af (strain Slo)
5	<1:40	<1:40	1:640	1:1,280
26	<1:40	<1:40	1:160	1:320

*DOBV, Dobrava-Belgrade virus; Aa, *Apodemus agrarius*; Af, *Apodemus flavicollis;* SK, strain Slovakia; Slo, strain Slovenia.

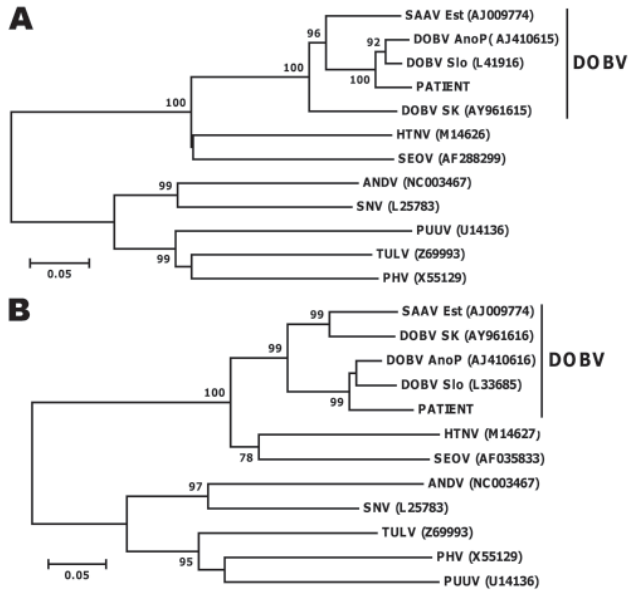

Figure 1. Molecular phylogenetic analysis of small (S) and medium (M) gene segments. Consensus neighbor-joining phylogenetic tree (Tamura-Nei 93 evolutionary model) of hantavirus strains was constructed as described (*9*) based on partial sequences of the S (panel A) and M segment (panel B). Bootstrap values >70%, calculated from 10,000 replicates, are shown at the tree branches. Sequences taken from GenBank are indicated by their accession numbers. SAAV Est, Saaremaa virus from Estonia; DOBV AnoP, Dobrava-Belgrade virus (lineage DOBV-Af) from Greece; DOBV Slo, Dobrava-Belgrade virus (lineage DOBV-Af) from Slovenia; DOB SK, Dobrava-Belgrade virus (lineage DOBV-Aa) from Slovakia; HTNV, Hantaan virus; SEOV, Seoul virus; ANDV, Andes virus; SNV, Sin nombre virus; PUUV, Puumala virus; TULV, Tula virus; PHV, Prospect Hill virus. Scale bars indicate an evolutionary distance of 0.1 substitutions per position.

Conclusions

The general symptoms and results of the serologic tests and molecular analyses for this patient were consistent with a hantavirus infection. After the acute phase of the illness, characterized by renal and pulmonary failure, panhypopituitarism developed in the patient. Pituitary hemorrhage and atrophy were observed on the magnetic resonance image. Case reports have documented that pituitary hemorrhage followed by panhypopituitarism may complicate HFRS (*2*–*4*), and Puumala virus was reported as the causative agent in most of these cases. In addition, hormonal deficiencies have been shown to be common features of Puumala virus infections, and chronic hormonal deficits develop in some patients (*5*). Our findings demonstrate that development of hypopituitarism can also be associated with infection by DOBV. Since the patient does not belong to any groups that have high exposure to hantaviruses (such as farmers, forest workers, and military recruits) and did not report any travel in hantavirus-endemic areas, the source of infection remains unclear.

In contrast to what is known about hantavirus presence in other Balkan states, little is known about the distribution, diversity, or host range of hantaviruses in Turkey (*6*). In 2009, a hantavirus outbreak occurred in Turkey; 3 of the 5 infected persons died (*7*). Serologic assays have been used to detect DOBV infection in patients in Turkey, but focus reduction neutralization tests have not been used for serotyping. DOBV RNA was found only in 1 urine sample from a hantavirus-infected patient from Turkey; however, sequence data were not provided (*8*). Prevalence studies of rodents collected in Turkey revealed 6% seropositivity to Puumala virus in *Microtus* voles and no appearance of hantavirus antibodies in the *Apodemus* species of rodents (*9*). Focus reduction neutralization testing and virus sequencing confirmed that the patient in our study was infected with DOBV-Af. This strain is associated with *A. flavicollis* field mice and yet has not been reported in animals in Turkey (see *10*,*11* for the molecular classification of *Apodemus* spp.–associated DOBV virus lineages).

This case should alert physicians that hantavirus infection should be considered in the differential diagnosis of patients who have high fever and thrombocytopenia, including those without known risk factors for hantavirus

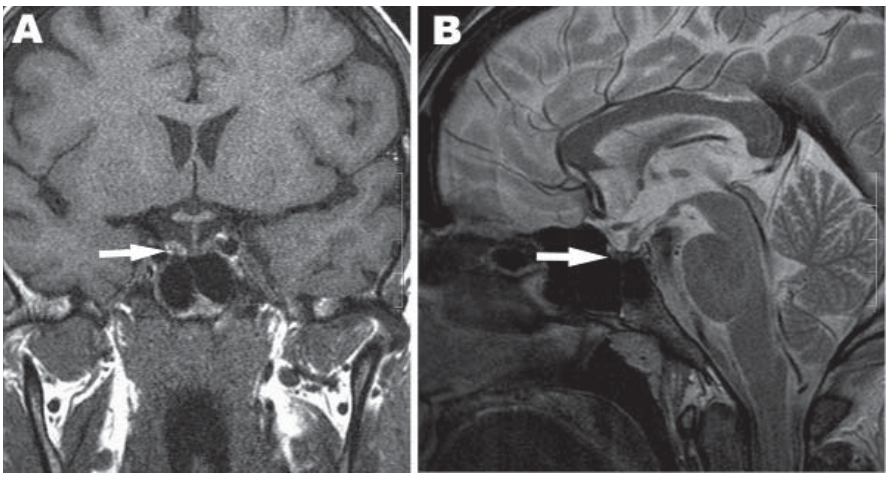

Figure 2. Magnetic resonance images showing hemorrhage of the pituitary gland and pituitary atrophy as indicated by arrows. A) T1-weighted coronal image shows high signal intensity on the right side of the adenohypophysis consistent with hemorrhage. B) T2-weighted sagittal image shows decreased pituitary gland height and heterogenous low signal intensity of the central adenohypophysis due to hemorrhagic infarction.

exposure. Since severe hormonal deficiencies are life-threatening, neuroendocrinologic complications should be taken into account and the endocrine status should be investigated to prevent panhypopituitarism even after recovery from HFRS.

Work in the laboratory in Berlin was supported by the Robert Koch Institute with funds from the German Ministry of Public Health (grant no. 1369-382 and 1369-435).

Dr Sarıgüzel is a specialist in clinical microbiology and infectious diseases at Acıbadem Health Group in Istanbul. Her research interests are infectious diseases, tuberculosis, and nosocomial infections.

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Address for correspondence: N. Sarıgüzel, Acıbadem Hospital, Tekin St, Acıbadem, Kadıköy-Istanbul, Turkey; email: sariguzel@hotmail.com

Timeliness of Nongovernmental versus Governmental Global Outbreak Communications

Luke Mondor, John S. Brownstein, Emily Chan, Lawrence C. Madoff, Marjorie P. Pollack, David L. Buckeridge, and Timothy F. Brewer

To compare the timeliness of nongovernmental and governmental communications of infectious disease outbreaks and evaluate trends for each over time, we investigated the time elapsed from the beginning of an outbreak to public reporting of the event. We found that governmental sources improved the timeliness of public reporting of infectious disease outbreaks during the study period.

Rapid public communication of incipient disease threats, even with incomplete information, might enable quicker response measures, including enhanced disease surveillance and initiation of protective measures, for those at risk (*1*). Traditionally, public notifications are communicated through a hierarchical infrastructure from which local, provincial or state, and national health authorities obtain information by interacting with health care providers and diagnostic laboratories (*2*). However, many health authorities now rely on informal outbreak-reporting systems, such as ProMED-mail, for timely signals of infectious threats (*3*,*4*), as encouraged by the revised WHO International Health Regulations (*5*). Research has suggested that globally, informal sources provide outbreak warnings faster than traditional governmental reporting mechanisms (*6*). However, existing research of this assertion has been limited to disease-specific evaluations (*7*) or descriptive summaries (*8*). We compared the timeliness of initial outbreak communications cited by nongovernmental sources to those of governmental sources and examined temporal trends in the time from outbreak onset to public communication for outbreaks communicated by each source, independently.

Author Affiliations: McGill University, Montreal, Quebec, Canada (L. Mondor, D.L. Buckeridge, T.F. Brewer); Harvard–Massachusetts Institute of Technology, Boston, Massachusetts, USA (J.S. Brownstein, E. Chan); Children's Hospital Boston, Boston (J.S. Brownstein, E. Chan); International Society for Infectious Diseases, Brookline, Massachusetts, USA (L.C. Madoff, M.P. Pollack, T.F. Brewer); University of Massachusetts, Worcester, Massachusetts, USA (L. Madoff); and Harvard Medical School, Boston (J.S. Brownstein).

DOI: http://dx.doi.org/10.3201/eid1807.120249

The Study

The study database consisted of 398 unique human infectious disease outbreak events collected from Disease Outbreak News, published online by the World Health Organization during 1996–2009 (*9*). For each outbreak, we defined the initial source or sources of the first public communication as the individual, organization, or website that first publicly communicated information regarding the disease threat (locally or internationally, orally or in writing). The corresponding date of communication was identified by using outbreak reports disseminated by ProMED-mail (*10*). All outbreaks were categorized as having been first communicated by ≥1 nongovernmental or governmental source, or simultaneously by both types of sources. When an outbreak was simultaneously first communicated by nongovernmental and governmental sources (n = 5), the outbreak was repeated in the dataset and each source was given credit. This adjustment increased the number of outbreak events to 403.

To characterize the timeliness of outbreak communications, for each reporting source of an event, we calculated the median time in days, and bootstrapped 95% CI, from outbreak start to public communication (Table 1). Median reporting times were calculated for the entire study period (1996–2009), before and after public recognition of severe acute respiratory syndrome (SARS) (March 12, 2003), and for each WHO-defined geographic region. The effect of the initial reporting source on the timeliness of outbreak communication was quantified by using negative binomial regression after adjusting for geographic region and whether the outbreak occurred before or after SARS. These variables were included in the model on the basis of a priori assumptions that public health infrastructure can vary by geographic and political region and that new pandemic preparedness strategies, including use of informal information to initiate public health responses, were developed in response to the SARS epidemic (*11*). Interaction terms between each variable were examined but were not included in the final model because none reached statistical significance ($p>0.05$). Temporal trends were assessed by using univariate negative binomial regression models, stratified by source category. These models included 1 covariate for the year of outbreak start.

Of all initial outbreak reports identified, 137 were excluded from analysis for ≈1 of the following reasons (Figure 1): 117 (85%) of the excluded reports were missing information on the estimated outbreak start date;

Table 1. Time from the estimated start of an outbreak to its earliest communication by source*

Variable	Governmental sources		Nongovernmental sources		p value
	No. outbreaks	Median no. days (95% CI)	No. outbreaks	Median no. days (95% CI)	
Period					
1996–2009	163	33.0 (30–44)	103	23.0 (20–32)	0.200
Pre-SARS	90	39.5 (31–51)	61	29.0 (20–50)	0.161
Post-SARS	73	29.0 (25–37)	42	21.5 (17–32)	0.613
Geographic location					
Africa	85	37.0 (29–51)	41	31.0 (23–57)	0.733
Americas	13	30.0 (21–63)	12	25.0 (20–34)	0.568
Eastern Mediterranean	24	41.0 (23–51)	9	31.0 (16–82)	0.903
Europe	11	31.0 (23–79)	9	20.0 (13–184)	0.909
Southeast Asia	8	28.0 (10–62)	11	14.0 (11–51)	0.431
Western Pacific	22	26.0 (12–52)	21	18.0 (13–33)	0.789

*Bootstrapping with 10,000 replicates was used to calculate 95% CIs for median values. SARS, severe acute respiratory syndrome.

20 (15%) were not found in the ProMED-mail archives; and 1 (1%) outbreak estimated start date occurred after the date of public communication of the outbreak. Of the 266 (66%) outbreaks included in analysis, 163 (61%) were first publicly communicated by governmental sources, and 103 (39%) were first communicated by nongovernmental sources. Chi-square tests showed no significant differences in the proportions of governmental and nongovernmental sources included in the analysis versus those excluded (p = 0.315).

The median time from estimated outbreak start to initial public communication was 10 days shorter for nongovernmental sources (23 days, 95% CI 20–32) than for governmental sources (33 days, 95% CI 30–45), although this difference was not significant according to the Wilcoxon rank-sum test (p = 0.200) (Table 1). Additionally, multivariate modeling showed no significant difference after covariates were adjusted for (incidence rate ratio [IRR] 0.95, 95% CI 0.77–1.18) (Table 2). The effect of missing data was assessed in sensitivity analyses for all outbreaks for which we had an estimated outbreak start date (17 of 20). When we used the WHO Disease Outbreak News communication date, our results did not change when crediting either governmental sources (IRR = 0.88, 95% CI 0.71–1.09) or nongovernmental sources (IRR = 1.086, 95% CI 0.882–1.336).

Examination of temporal trends over the study period (Figure 2) showed that nongovernmental sources generally communicated outbreak signals to the public faster after 1996, although the trend did not reach statistical significance (IRR = 0.96, 95% CI 0.91–1.01). Governmental sources, in contrast, made significant improvements in lessening the time in which they publicly communicated initial outbreak signals (IRR = 0.94, 95% CI 0.91–0.97).

Conclusions

Our data suggest that, from 1996 through 2006, outbreaks reported initially by nongovernmental sources were communicated publicly an average of 10 days earlier than those reported initially by governmental sources. Though the differences varied, nongovernmental sources tended to report outbreaks faster than governmental sources when we compared outbreaks before and after SARS, or by WHO-defined region. The lack of statistically significant differences in initial communication timeliness by source is probably attributable to a lack of statistical power rather than a lack of effect.

Our results also provide support for the International Health Regulations 2005 revisions that allow WHO to use unofficial information to request verification from member states. Slightly more than one-third of all unique infectious disease outbreaks in the WHO Disease Outbreak

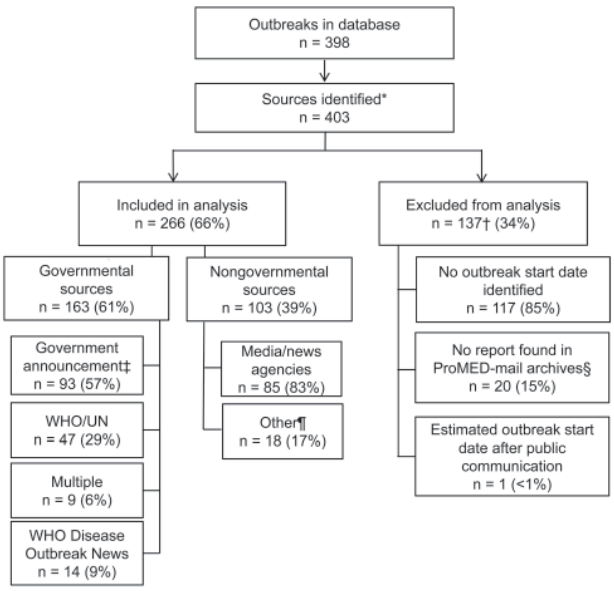

Figure 1. Exclusion criteria applied to database of 398 outbreak events publicly reported through the World Health Organization (WHO) Disease Outbreak News during 1996–2009 and breakdown of nongovernmental and governmental sources used to compare the timeliness of outbreak communications. UN, United Nations. *More than one source may be identified for a given outbreak; †categories for exclusion are not mutually exclusive; ‡health officials, ministries of health, laboratories, hospitals, etc.; §included in sensitivity analysis; ¶includes nongovernmental organizations, individual accounts, ProMED requests for information, and multiple sources.

Table 2. Comparison of the timeliness of outbreak communications by nongovernmental and governmental sources*

Variable	IRR (95% CI)	p value
Source		
Governmental	Ref	
Nongovernmental	0.950 (0.765–1.180)	0.645
Chronological order		
Pre-SARS	Ref	
Post-SARS	0.713 (0.576–0.884)	0.002
Geographic location		
Africa	Ref	
Americas	0.773 (0.531–1.126)	0.180
Eastern Mediterranean	0.912 (0.654–1.272)	0.587
Europe	1.100 (0.731–1.669)	0.637
Southeast Asia	0.602 (0.394–0.918)	0.019
Western Pacific	0.780 (0.577–1.054)	0.106

*Methods: The multivariate negative binomial regression model compared the timeliness of outbreaks first communicated by nongovernmental sources to those by governmental sources, while adjusting for geographic region and whether the outbreak occurred before or after public recognition of SARS. IRR, incidence rate ratio; SARS, severe acute respiratory syndrome; ref, reference value = 1. Reference categories: (1) source: governmental, (2) SARS: pre-SARS, (3) geographic region: Africa.

News during this 14-year period were initially reported by informal information sources.

Traditional governmental public health reporting mechanisms remain an integral source for outbreak information, accounting for almost two-thirds of all initial reports over this period. Our results also show that these sources made statistically significant improvements in reporting early warnings of outbreak threats more rapidly to the public, which might result in part from a shift toward automated, electronic methods that improve the timeliness of communication (*12*,*13*). It is possible that enhancements in nongovernmental outbreak reporting systems also contributed to improvements in governmental outbreak reporting timeliness over the study period, but we were unable to test this assumption with the current data.

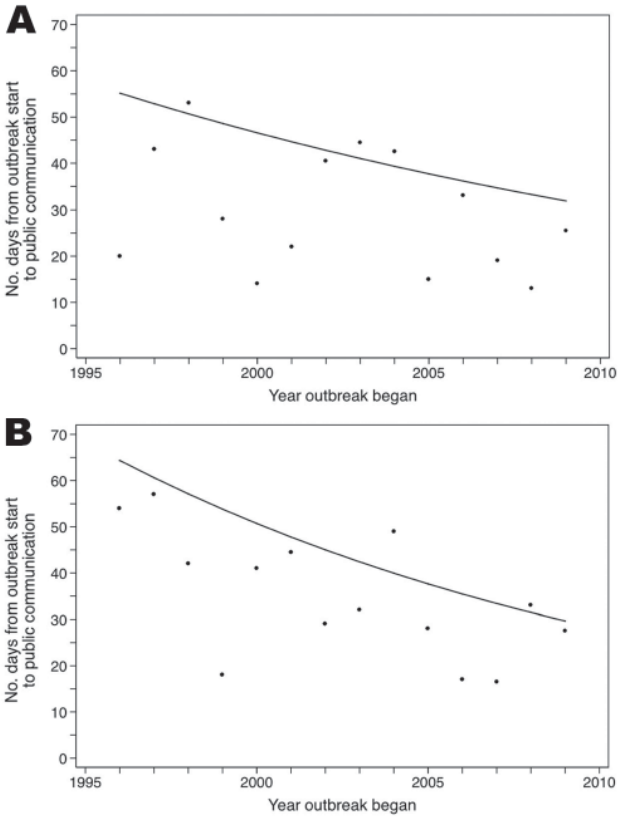

Figure 2. Median time (days) from the estimated start of an outbreak to its public communication for outbreaks reported by nongovernmental sources (A) and governmental sources (B), 1996–2009. Trendlines show average improvements over the study period.

This study has potential limitations. We encountered difficulty in selecting and consistently applying criteria to determine the initial source of public communication from ProMED-mail reports, which could have resulted in misclassification bias. Although other reporting systems that use informal information exist, they either lack a publicly available archive (for example, Global Public Health Intelligence Network) (*14*) or their database did not cover the entire study period (for example, HealthMap) (*15*). According to Heymann, et al., 65% of outbreaks recognized by WHO are first identified by informal sources (*4*), a proportion we did not find. Some outbreak reports were excluded because of missing data. We were able to internally validate the data that remained, but these exclusions limited the study's statistical power. Finally, use of outbreak reports collected from the WHO Disease Outbreak News might limit the generalizability of our findings to all infectious disease outbreaks. Despite these limitations, our data highlight the value of nongovernmental sources as an integral resource for providing timely information about global infectious disease threats, and demonstrate the significant improvements in the timeliness of outbreak reporting made by governmental sources.

This research was supported by a grant from Google.org and by the National Institutes of Health grants R01 LM010812 and G08 LM009776.

Mr Mondor is a research assistant in The Surveillance Lab at McGill University's Clinical and Health Informatics Research Group. His research interests include timely disease detection, infectious disease epidemiology, and health care data analysis.

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Address for correspondence: Timothy F. Brewer, 1020 Pine Ave West, Montreal, Quebec, H3A 1A2, Canada; email: timothy.brewer@mcgill.ca

Role of Birds in Dispersal of Etiologic Agents of Tick-borne Zoonoses, Spain, 2009

Ana M. Palomar, Paula Santibáñez, David Mazuelas, Lidia Roncero, Sonia Santibáñez, Aránzazu Portillo, and José A. Oteo

We amplified gene sequences from *Anaplasma phagocytophilum*, *Borrelia garinii*, *B. valaisiana*, *B. turdi*, *Rickettsia monacensis*, *R. helvetica*, *R. sibirica sibirica*, and *Rickettsia* spp. (including *Candidatus* Rickettsia vini) in ticks removed from birds in Spain. The findings support the role of passerine birds as possible dispersers of these tick-borne pathogens.

Hard ticks are a major vector of infectious diseases in industrialized countries. Several tick-borne bacterial diseases, such as Lyme disease, Mediterranean spotted fever, and tick-borne lymphadenopathy (also called *Dermacentor*-borne necrosis erythema and lymphadenopathy), are endemic to Spain. Furthermore, a few cases of human anaplasmosis and *Rickettsia monacensis* infection in humans have been diagnosed in Spain (*1*–*3*).

Birds are the preferred host for some tick species. As carriers of infected ticks, birds could be responsible for the spread of tick-borne bacteria that cause human anaplasmosis, Lyme disease, rickettsioses, and other diseases (*4*). Multiple studies support the conclusion or propose the hypothesis that birds play a role as reservoirs of *Anaplasma phagocytophilum*, *Borrelia burgdorferi*, and *Rickettsia* spp. (*4*–*6*). Because the Iberian Peninsula plays a major role in the migratory routes of birds, we aimed to determine the presence and prevalence of *A. phagocytophilum*, *B. burgdorferi* sensu lato, and *Rickettsia* spp. in ticks removed from birds captured in northern Spain.

Author affiliations: Hospital San Pedro–Centro de Investigación Biomédica de La Rioja, Logroño, Spain (A.M. Palomar, P. Santibáñez, S. Santibáñez, A. Portillo, J.A. Oteo); and Environment Resources Inc., Logroño (D. Mazuelas, L. Roncero)

DOI: http://dx.doi.org/10.3201/eid1807.111777

The Study

During April–October 2009, bird bandings were conducted in the protected area of Finca Ribavellosa in La Rioja, Spain (42°14′N, 2°54′W). Ticks were collected from birds and classified through taxonomic keys (*7*) and molecular methods (*8*). DNA was individually extracted by using 2 incubations of 20 minutes each with ammonium hydroxide (1 mL of 25% ammonia and 19 mL of Milli-Q water that had been autoclaved) at 100°C and 90°C.

DNA extracts were used as templates for PCRs targeting fragment genes for tick classification and for bacteria detection (Table 1). Two negative controls, 1 containing water instead of template DNA and the other with template DNA but without primers, and a positive control (a tick extract, *A. phagocytophilum*, *B. burgdorferi* sensu stricto, or *R. slovaca*) were included in all PCRs. Amplification products were sequenced, and nucleotide sequences were compared with those available in GenBank by using a BLAST search (www.ncbi.nlm.nih.gov/blast/Blast.cgi). Phylogenetic and molecular evolutionary analyses were conducted by using MEGA4 (*16* in online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1777-Techapp1.pdf).

A total of 222 ticks belonging to the species *Haemaphysalis punctata* (n = 1), *Ixodes frontalis* (n = 7), *I. arboricola* (n = 26), *I. ricinus* (n = 181), and other *Ixodes* spp. (n = 7) were collected from 97 passerine birds. Two nucleotide sequences for the 16S rRNA fragment gene of *I. arboricola* ticks were recorded (GenBank accession nos. JF791812 and JF791813) (Table 2).

A. phagocytophilum was detected only in 1 larva of an *I. ricinus* tick (0.5%). Twenty-nine (13.1%) samples tested positive for *B. burgdorferi* s.l. The most prevalent genospecies was *B. garinii* (n = 19), which was detected in *I. ricinus* (n = 16), *H. punctata* (n = 1), *I. frontalis* (n = 1), and *Ixodes* sp. (n = 1) ticks. *B. valaisiana* was amplified in 9 samples (8 *I. ricinus* and 1 *Ixodes* sp. ticks). *B. turdi* was found in 1 *I. frontalis* tick. *Rickettsia* infection was detected in 39 (17.6%) ticks. *R. monacensis* (n = 1), *R. helvetica* (n = 1), *R. sibirica sibirica* (n = 1), and *Rickettsia* spp. (n = 9) were detected in 12 *I. ricinus* ticks. Furthermore, according to *gltA*, *ompA*, and *ompB* sequence analysis, a possible new *Rickettsia* sp. was found in 25 *I. arboricola* ticks and 2 *I. ricinus* ticks. For these 27 samples, highest identities with *R. heilongjiangensis* (97.1%) and *R. japonica* (99.1%) were found for *ompA* (GenBank accession no. JF758828) and *ompB* (GenBank accession no. JF758826) nucleotide sequences, respectively, whereas *gltA* nucleotide sequences were identical to those from both *Rickettsia* spp. According to multilocus sequence typing (data not shown) and genetic criteria agreed on by experts, a *Candidatus* status could be assigned. We named it *Candidatus* Rickettsia vini (*17* in online Technical Appendix) (Table 2). The phylogenetic

Table 1. PCR primer pairs used in study of the role of birds in dispersal of etiologic agents of tick-borne zoonoses, Spain, 2009*

Bacteria	Gene target	Primer name	Primer sequence, 5′ → 3′	Amplified fragment, bp	Annealing temp., °C	Ref.
Anaplasma spp.	16S rRNA, nested	ge3a	CACATGCAAGTCGAACGGATTATTC	932	55	(*9*)
		ge10r	TTCCGTTAAGAAGGAT CTAATCTCC			
		ge9f	AACGGATTATTCTTTATAGCTTGCT	546	55	(*9*)
		ge2	GGCAGTATTAAAAGCAGCTCCAGG			
	msp	msp3F	CCAGCGTTTAGCAAGATAAGAG	334	56	(*10*)
		msp3R	GCCCAGTAACAACATCATAAGC			
Borrelia spp.	*flaB*, nested†	Outer 1	AARGAATTGGCAGTTCAATC	497	52	(*11*)
		Outer 2	GCATTTTCWATTTTAGCAAGTGATG			
		Inner 1	ACATATTCAGATGCAGACAGAGGTTCTA	389	55	(*11*)
		Inner 2	GAAGGTGCTGTAGCAGGTGCTGGCTGT			
	5S-23S intergenic spacer, nested	23SC1	TAAGCTGACTAATACTAATTACCC	380	52	(*12*)
		23SN1	ACCATAGACTCTTATTACTTTGAC			
		5SCB	GAGAGTAGGTTATTGCCAGGG	226	55	(*12*)
		23SN2	ACCATAGACTCTTATTACTTTGACCA			
Rickettsia spp.	*ompA*, seminested	Rr190.70p	ATGGCGAATATTTCTCCAAAA	631	46	(*13,14*)
		Rr190.701n	GTTCCGTTAATGGCAGCATCT			
		Rr190.70p	ATGGCGAATATTTCTCCAAAA	532	48	(*14*)
		Rr190.602n	AGTGCAGCATTCGCTCCCCCT			
	ompB, nested	rompB OF	GTAACCGGAAGTAATCGTTTCGTAA	511	54	(*15*)
		rompB OR	GCTTTATAACCAGCTAAACCACC			
		rompB SFG IF	GTTTAATACGTGCTGCTAACCAA	420	56	(*15*)
		rompB SFG/TG IR	GGTTTGGCCCATATACCATAAG			
	gltA central region, nested	RpCS.877p	GGGGGCCTGCTCACGGCGG	381	48	(*14*)
		RpCS1258n	ATTGCAAAAAGTACAGTGAACA			
		RpCS.896p	GGCTAATGAAGCAGTGATAA	337	54	(*15*)
		RpCS.1233n	GCGACGGTATACCCATAGC			

*Temp., temperature; ref., reference; *msp*, p44 major surface protein gene; *flaB*, flagellin gene; *ompB*, 120-kDa genus common antigen gene; *ompA*, 190-kDa protein antigen gene; *gltA*, citrate synthase gene.
†R = A/G; W = A/T.

Table 2. *Anaplasma phagocytophilum*, *Borrelia burgdorferi* s.l., and *Rickettsia* spp. detected in ticks removed from birds, Spain, 2009*

Bacteria	Tick		Bird species (no. specimens)	Gene targets
	Species	Stage		
A. phagocytophilum	*Ixodes ricinus*	1 L	*Turdus merula* (1)	*msp*
B. garinii	*I. ricinus*	4 L, 2 N	*T. merula* (9)	*flaB*, 5–23S is
		3 L, 4 N		*flaB* or 5–23S is
		1 L	*Erithacus rubecula* (1)	*flaB*
		1 L	*T. philomelos* (1)	*flaB*, 5–23S is
		1 L	*Troglodytes troglodytes* (1)	*flaB*, 5–23S is
	I. frontalis	1 F	*T. philomelos* (1)	*flaB*, 5–23S is
	Ixodes spp.	1 L	*E. rubecula* (1)	5–23S is
	Haemaphysalis punctata	1 L	*T. merula* (1)	*flaB*, 5–23S is
B. valaisiana	*Ixodes* spp.	1 L	*T. merula* (1)	*flaB*, 5–23S is
	I. ricinus	1 L, 1 N	*T. merula* (3)	*flaB*, 5–23S is
		2 L		*flaB*
		1 L, 1 N	*T. philomelos* (2)	*flaB*, 5–23S is
		1 L	*E. rubecula* (1)	*flaB*, 5–23S is
		1 L	*Garrulus glandarius* (1)	*flaB*
B. turdi	*I. frontalis*	1 F	*T. merula (1)*	*flaB*, 5–23S is
R. monacensis	*I. ricinus*	1 N	*Sylvia atricapilla* (1)	*ompA*
R. helvetica	*I. ricinus*	1 N	*G. glandarius* (1)	*gltA*
R. sibirica sibirica	*I. ricinus*	1 L	*S. atricapilla* (1)	*ompA*
Rickettsia spp.†	*I. ricinus*	1 N, 1 L	*T. philomelos* (1)	*ompB* or *gltA*
	I. ricinus	4 L	*E. rubecula* (4)	*ompB* or *gltA*
		2 N	*T. merula* (2)	*gltA*
		1 L	*Tr. troglodytes* (1)	*gltA*
Candidatus Rickettsia vini	*I. arboricola*	20 N	*Cyanistes caeruleus* (1)	*ompA*, *ompB*, *gltA*
		5 L	*Parus major* (1)	*ompA*, *ompB*, *gltA*
	I. ricinus	2 L	*E. rubecula* (2)	*ompA*, *ompB*, *gltA*

*L, larva; *msp*, p44 major surface protein gene; N, nymph; *flaB*, flagellin gene; 5S-23S is, 5S-23S rRNA intergenic spacer; *ompB*, 120-kDa genus common antigen gene; *ompA*, 190-kDa protein antigen gene; *gltA*, citrate synthase gene.
†Same identity with >1 validly published *Rickettsia* species.

tree based on *ompA* gene shows the nearest relationships among *Rickettsia* spp. (Figure).

Two *I. ricinus* larvae showed co-infection with *B. garinii* and *Rickettsia* sp. One nymph was co-infected with *B. valaisiana* and *Rickettsia* sp.

Conclusions

The presence of *Anaplasma*, *Borrelia*, and *Rickettsia* species in ticks removed from passerine birds corroborates the role of these vertebrates in the epidemiology and dispersion of tick-borne pathogens in Spain and in other zones of the planet. Some of the parasitized birds in our study, such as the European robin (*Erithacus rubecula*) or Eurasian blackcap (*Sylvia atricapilla*), are considered migratory or partial migratory birds. In addition, these species share an ecologic niche and ectoparasites (horizontal transmission) with other migratory birds that cover long distances from Africa to the Eurasian region.

Figure. The phylogenetic position of *Candidatus* Rickettsia vini based on the *ompA* nucleotide sequences in a study of the role of birds in dispersal of etiologic agents of tick-borne zoonoses, Spain, 2009. The evolutionary history was inferred by using the neighbor-joining method. The optimal tree with the sum of branch length = 1.09961140 is shown. The percentage of replicate trees in which the associated taxa clustered in the bootstrap test (1,000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed by using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset. A total of 563 positions were in the final dataset. Phylogenetic analyses were conducted in MEGA4 (*16* in online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1777-Techapp1.pdf).

Except for *I. arboricola*, the tick species captured in this study previously had been found on birds in Spain (*18* in online Technical Appendix). Nevertheless, *I. arboricola* ticks are commonly hosted by birds. The high prevalence of *I. ricinus* ticks was expected because it is the most frequent tick in this area, and the immature stages of this tick frequently parasitize birds.

I. ricinus ticks are the main vectors of *A. phagocytophilum* in Europe, and this microorganism has been detected on vegetation in the studied area (*1*). However, the low prevalence (0.5%) of *A. phagocytophilum* in the ticks in our study corroborates data from other studies (*19*,*20* in online Technical Appendix). The presence of *A. phagocytophilum* in a larva in our study supports the role of birds as reservoirs of *A. phagocytophilum*.

The prevalence (13.1%) of *B. burgdorferi* in our samples is similar to prevalences reported in other studies in Europe in which *I. ricinus* is the main species of tick captured from birds (*19* in online Technical Appendix). In Spain, *B. garinii*, *B. valaisiana*, and *B. afzelii* have been detected in ticks from birds (*18* in online Technical Appendix). According to our data, the human pathogen *B. garinii* was the most prevalent species, as reported in birds from Europe (*21* in online Technical Appendix). *B. turdi* was discovered in Asia. Although it has been recently detected in ticks from birds in Norway (*22* in online Technical Appendix), its finding in Spain was unexpected.

Regarding *Rickettsia* species, *R. monacensis* and *R. helvetica* are among the human pathogens detected in our study. Both species have been identified in ticks from birds in Europe (*19*,*20*,*23* in online Technical Appendix). On the contrary, *Candidatus* Rickettsia vini, a potential new *Rickettsia* species, also detected in our study, has not been related to human disease (*17* in online Technical Appendix). Several genospecies closely related to *R. heilongjiangensis* and *R. japonica* have been identified in *Ixodes* spp. ticks removed from birds (*23* in online Technical Appendix). *R. sibirica sibirica*, responsible for Siberian tick typhus in western People's Republic of China and in Siberia, was also amplified in an *I. ricinus* larva in this study.

Our data confirm the involvement of birds in the cycle of human tick-borne diseases. The findings confirm that birds can disperse vectors and microorganisms.

Acknowledgments

We thank Agustín Estrada-Peña for classifying the *I. arboricola* ticks and Óscar Gutiérrez for providing tick samples.

This study was presented in part at the XIV Congreso SEIMC (Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica) (Spain) in May 2010, the GEPE Scientific

Meeting from the XV Congreso SEIMC (Spain) in June 2011, and the 6th International Meeting on Rickettsiae and Rickettsial Diseases (Greece) in June 2011.

Fundación Rioja Salud awarded a grant (FRS/PIF-01/10) to A.M.P. Financial support was provided in part by a grant from "Instituto de Salud Carlos III" (EMER 07/033), Ministerio de Ciencia e Innovación (Spain).

Dr Palomar has worked in the Center of Rickettsiosis and Arthropod-borne Diseases at the Infectious Diseases Area, Hospital San Pedro–Center for Biomedical Research of La Rioja since March 2009. Her research interests are the taxonomy of ticks and their associated pathogens.

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Address for correspondence: José A. Oteo, Área de Enfermedades Infecciosas, Hospital San Pedro-CIBIR, C/Piqueras 98-7ª NE, 26006 Logroño (La Rioja), Spain; email: jaoteo@riojasalud.es

Calicivirus from Novel Recovirus Genogroup in Human Diarrhea, Bangladesh

Saskia L. Smits, Mustafizur Rahman, Claudia M.E. Schapendonk, Marije van Leeuwen, Abu S.G. Faruque, Bart L. Haagmans, Hubert P. Endtz, and Albert D.M.E. Osterhaus

To identify unknown human viruses in the enteric tract, we examined 105 stool specimens from patients with diarrhea in Bangladesh. A novel calicivirus was identified in a sample from 1 patient and subsequently found in samples from 5 other patients. Phylogenetic analyses classified this virus within the proposed genus Recovirus.

Diarrhea, characterized by frequent liquid or loose stools, commonly results from gastroenteritis caused by infection with bacteria, parasites, or viruses. Patients with mild diarrhea do not require medical attention; the illness is typically self-limited, and disease symptoms usually resolve quickly. However, diarrheal diseases can result in severe illness and death worldwide and are the second leading cause of death around the world in children <5 years of age, particularly in low- and middle-income countries (*1*). For many cases of diarrhea in humans, no causative agent is identified.

In recent years, many novel viruses have been identified in human and animal blood, respiratory secretions, and fecal material through viral metagenomic studies consisting of random amplification in combination with next-generation sequencing methods (*2*–*5*). To identify unknown human viruses in the enteric tracts of persons with diarrhea, we performed sequence-independent amplification on purified viral nucleic acid from fecal samples obtained from patients with diarrhea in Bangladesh (*6*,*7*). We identified a novel calicivirus and classified it in the proposed genus Recovirus. Caliciviruses, which are nonenveloped, positive-stranded RNA viruses with a polyadenylated genome of ≈6.4–8.4 kb, cause illness in animals and humans (*8*,*9*), including gastroenteritis in humans. The family *Caliciviridae* consists of 5 genera, *Norovirus*, *Sapovirus*, *Lagovirus*, *Vesivirus*, and *Nebovirus*, and 3 proposed genera, Recovirus, Valovirus, and chicken calicivirus (*8*–*10*).

Author affiliations: Erasmus Medical Center, Rotterdam, the Netherlands (S.L. Smits, C.M.E. Schapendonk, B.L. Haagmans, H.P. Endtz, A.D.M.E. Osterhaus); Viroclinics Biosciences B.V., Rotterdam (S.L. Smits, M. van Leeuwen, A.D.M.E. Osterhaus); and International Centre for Diarrhoeal Disease Research, Bangladesh, Dhaka, Bangladesh (M. Rahman, A.S.G. Faruque, H.P. Endtz)

DOI: http://dx.doi.org/10.3201/eid1807.120344

The Study

Each year, >100,000 diarrhea patients are admitted to the Dhaka hospital of the International Centre for Diarrheal Disease Research, Bangladesh (ICDDR,B). Fecal samples from 2% of these patients are collected and examined as part of systematic routine surveillance system for the presence of enteric pathogens (*11*). All procedures were performed in compliance with relevant laws and institutional guidelines and in accordance with the Declaration of Helsinki.

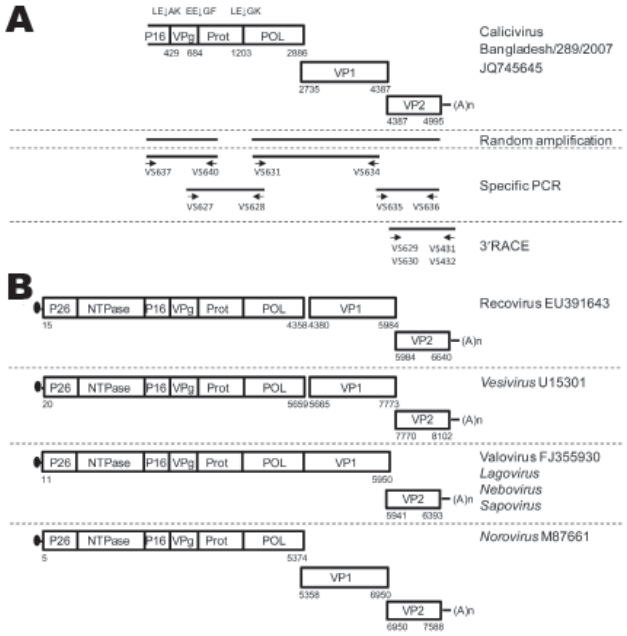

Figure 1. Schematic outline of the strategies used for PCR amplification of calicivirus Bangladesh/289/2007. A) Schematic representation of the calicivirus Bangladesh/289/2007 genome. Boxes represent the open reading frames encoding the calicivirus proteins. Indicated are the poly(A)-tail (A_n); putative cleavage sites indicated by XX↓XX. The 5′ end of the genome was not obtained. The bottom of the panel shows a schematic outline of the reverse transcription PCRs employed to amplify calicivirus Bangladesh/289/2007 sequences by using random amplification, degenerate PCR, and 3′ rapid amplification of cDNA ends (RACE) PCR. The orientations and positions of the oligonucleotides on the calicivirus genome are depicted and sequences shown in online Technical Appendix Table 2 (wwwnc.cdc.gov/EID/pdfs/12-0344-Techapp.pdf). B) Genome organization of caliciviruses in the genera *Vesivirus*, *Nebovirus*, *Norovirus*, *Sapovirus*, and *Lagovirus* and proposed genera Valovirus and Recovirus, for comparison with the new calicivirus Bangladesh/289/2007. The 5′ end of the genome is shown with a Vpg protein (black dots). Numbers indicate the nucleotide positions according to the virus genome for which the GenBank accession number is indicated.

Table 1. Percentage amino acid identity of calicivirus Bangladesh/289/2007 with representative caliciviruses of other genera*

Calicivirus genus	Strain	GenBank accession no.	NS polyprotein†	VP1	VP2
Norovirus	Norwalk	M87661	27.7	30.4	24.7
	Southampton	L07418	29.0	31.2	23.3
Sapovirus	Manchester	X86560	20.1	18.6	4.1
	PEC Cowden	AF182760	18.3	18.1	6.8
Vesivirus	FCV CFI68	U13992	18.4	16.3	15.1
	SMSV 1	U15301	19.0	19.3	5.5
Lagovirus	RHDV FRG	M67473	18.0	19.6	9.6
	EBHSV GD	Z69620	17.3	17.8	5.5
Nebovirus	BEC NB	AY082891	18.7	16.8	11.0
	Newbury 1	DQ013304	19.0	16.8	11.0
Recovirus‡	Tulane	EU391643	57.7	49.5	53.1
Valovirus‡	AB104	FJ355930	35.8	43.5	26.0

*NS, nonstructural; VP, viral protein.
†All polyprotein sequences were aligned without the capsid protein encoding sequences.
‡Tentative genus not yet accepted by the International Committee on Taxonomy of Viruses.

Stool specimens from a subset of patients from routine surveillance during 2007–2009 (1,614 samples total) were available for further studies. These specimens were prescreened for adenovirus and rotavirus A by using TaqMan EZ RT-PCR Core Reagents (Applied Biosystems, Foster City, CA, USA), rotavirus primers RVNSP3R and RVNSP3F and probe 5′-FAM-AGTTAAAAGCTAACACTGTCAAA-TAMRA-3′ (*12*), and TaqMan Universal Mastermix (Applied Biosystems) (*13*). Sequence-independent nucleic acid amplification and next-generation sequencing were performed on 105 stool specimens from diarrhea patients enrolled during 2007 by using a 454 GS Junior Instrument (Roche, Indianapolis, IN, USA) as described (*6*,*7*). More than 725,000 trimmed reads were assembled by using de novo assembly and analyzed according to BLAST searches (online Technical Appendix Figure 1 and Table 1, wwwnc.cdc.gov/EID/pdfs/12-0344-Techapp.pdf) (*6*,*7*). Sequences were classified on the basis of the taxonomic origin of the best-hit sequence (*6*,*7*). An E (expect) value of 0.001 was used as cutoff value of significant virus hits. The largest proportion of virus-related sequences in human diarrhea samples from Bangladesh in 2007 was related to known bacteriophages and mammalian viruses (online Technical Appendix Figure 1).

One sample, no. 289, yielded a novel mammalian virus from the family *Caliciviridae* that we further characterized by near full-length genome sequencing using random amplification with next-generation sequencing, specific reverse transcription PCRs, and 3′ rapid amplification of cDNA ends PCR (Figure 1, panel A) as described (*6*,*7*). We named the virus isolate calicivirus Bangladesh/289/2007 (GenBank accession no. JQ745645).

The *Caliciviridae* genome encodes a polyprotein precursor for nonstructural proteins, and 2 structural capsid proteins, viral protein (VP) 1 and VP2, from 2 or 3 open reading frames (ORFs) (Figure 1, panel B) (*8*,*9*). The genome organization of Bangladesh/289/2007 is most closely related to that of viruses in the genus *Norovirus*, with ORF2 encoding VP1 overlapping with ORF1 (Figure 1). The par-

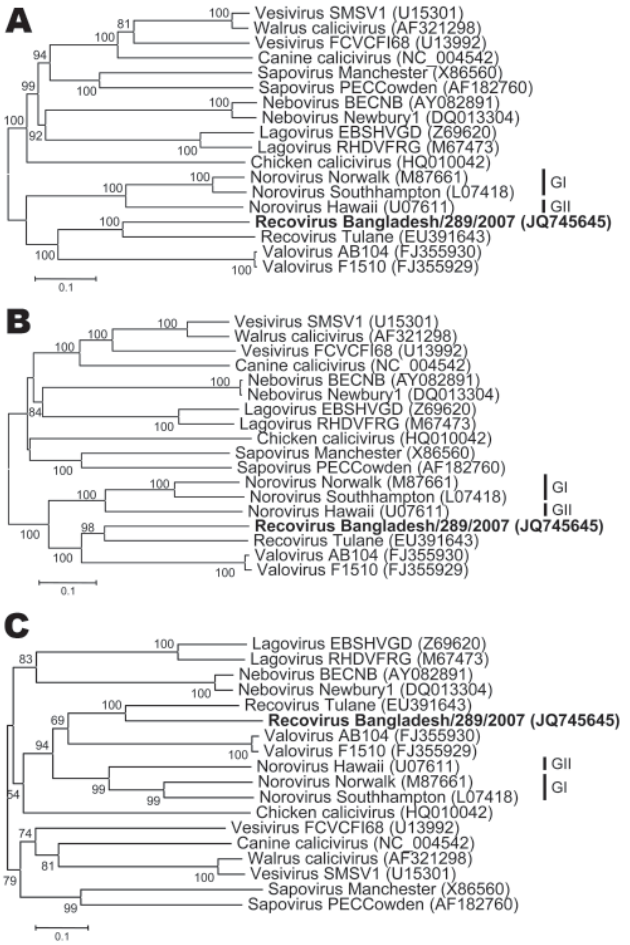

Figure 2. Neighbor-joining phylogenetic trees of the amino acid sequences of the partial polyprotein sequence (A), viral protein (VP) 1 (B), and VP2 (C) capsid proteins of selected representative caliciviruses and the newly identified Recovirus Bangladesh/289/2007 (indicated in **boldface**). Phylograms were generated by using MEGA5 (www.megasoftware.net) with p-distance and 1,000 bootstrap replicates. Significant bootstrap values and GenBank accession numbers are shown. Scale bars indicate amino acid substitutions per site.

tial polyprotein precursor and complete VP1 and VP2 proteins were aligned with corresponding sequences of representative caliciviruses. Divergence analysis demonstrated that the calicivirus Bangladesh/289/2007 showed 57.7%, 49.5%, and 53.1% amino acid identity in the polyprotein, VP1, and VP2, respectively, to its closest relative, Recovirus Tulane (GenBank accession no. EU391643), which was identified from rhesus macaques (Table 1; Figure 2).

Additionally, on the basis of the identity profiles of noroviruses (*14*)—strains, genotypes, and genogroups showed 85.9%–100%, 56.2%–85.7%, and 38.6%–55.1% identity, respectively—Bangladesh/289/2007 may represent a new genogroup in the proposed genus Recovirus. This conclusion was corroborated by comparison of a partial RNA-dependent RNA polymerase (RdRp) sequence of calicivirus Bangladesh/289/2007 to known recovirus RdRp sequences (online Technical Appendix Figure 2).

We performed a diagnostic real-time recovirus PCR targeting the RdRp of all 1,614 available samples from patients with diarrhea (*11*). Reverse transcription PCR–grade viral nucleic acid was extracted by using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche) and amplified by using reverse transcription PCR with primers VS665 and VS666 and probe VS664 (online Technical Appendix Table 2) and TaqMan EZ RT-PCR Core Reagents (Applied Biosystems). The cycling program consisted of 50°C for 2 min, 60°C for 30 min, 95°C for 5 min, and 50 cycles of 95°C for 20 s and 59°C for 1 min, resulting in a 164-bp amplicon.

In addition to sample 289, 5 other human diarrhea samples (Table 2) were sequence-confirmed to be positive for a recovirus, with high homology (>98%) to calicivirus Bangladesh/289/2007; this finding indicates that recovirus Bangladesh infects humans. Clinical data indicate that all patients with a recovirus present in their feces had 6 to >21 watery stools in the first 24 hours after illness onset (Table 2); 4 patients experienced vomiting and 2 patients had fever. Patient ages ranged from 3 months to 50 years. Three recovirus-positive patients showed evidence of coinfection with other pathogens that are known to cause diarrhea in humans, such as rotavirus A, adenovirus, *Vibrio cholerae*, or *Salmonella* spp.; the other 3 patients did not. Although viruses such as norovirus, sapovirus, and astrovirus were not detected in the recovirus-positive samples by sequence-independent amplification assays, all samples were not analyzed for all known enteric pathogens. Of 514 diarrhea samples gathered by the Diagnostic Unit, Department of Virology, Erasmus Medical Center, Rotterdam, the Netherlands, for diagnosis of gastrointestinal infections during 2007 and 2009, none was positive for Recovirus Bangladesh (data not shown).

Conclusions

For a large proportion of human diarrhea cases, no etiologic agent can be identified, despite multiple metagenomic studies of viruses in human stool aimed at identifying new etiologic agents (3–5). In addition, it cannot be known when and where emerging and reemerging viruses will appear in the human population. To identify potential etiologic agents of diarrhea in humans, we performed a metagenomic viral inventory in diarrhea samples from Bangladesh, which led to the identification of a novel calicivirus.

Although no species demarcation criteria have been defined for the family *Caliciviridae* by the International Committee on the Taxonomy of Viruses, we classified calicivirus Bangladesh/289/2007 in the proposed genus Recovirus, primarily on the basis of phylogenetic analyses (*8*). Bangladesh/289/2007 may also be considered a new genogroup of the genus Recovirus for the following reasons: 1) the genetic distance between Recovirus Tulane and Bangladesh/289/2007 VP1 is similar to that of VP1 capsids of noroviruses belonging to different genogroups (*14*); 2) the genetic distances between macaque recoviruses and Recovirus Bangladesh/289/2007 RdRP are similar to that of RdRP sequences of recoviruses belonging to different genogroups (*15*); 3) the genome organization of Recovirus Tulane and Bangladesh/289/2007 differs; and 4) Recovirus Tulane and Bangladesh/289/2007 were identified in different host species.

Table 2. Clinical data for patients positive for Recovirus Bangladesh/289/2007, Bangladesh*

Patient no.	Age, y/sex	Year of sample collection	Fever	Disease duration, d	Bowel movements†	Vomiting†	Abdominal pain	Patient condition	Other pathogens‡
201	23/M	2007	Yes	<1	11–15	<10	Yes	Lethargic	None
289§	25/M	2007	No	<1	6–10	<10	Yes	Lethargic	None
445	20/M	2007	No	1–3	11–15	0	Yes	Normal	None
507	50/F	2007	No	<1	6–10	<10	No	Drowsy	*Vibrio cholerae*
809	0/M	2008	No	4–6	>21	<10	No	Lethargic	Rotavirus A
1084	1/M	2008	Yes	1–3	6–10	0	Yes	Normal	Adenovirus/ *Salmonella* spp.

*All patients had watery diarrhea.
†Number of events in 24 h before sample collection.
‡The pathogens that were tested for were *Shigella* spp., *Aeromonas* spp., *Vibrio cholerae*, *Campylobacter* spp., and *Salmonella* spp. In addition, the samples were screened for *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., *Ascaris lumbricoides*, *Cyclospora cayetanensis*, *Isospora belli*, adenoviruses, and group A rotaviruses.
§The virus from patient 289 is the initially described calicivirus Bangladesh/289/2007 from this study.

In conclusion, this identification of a novel calicivirus, classified as Recovirus Bangladesh/289/2007, from human diarrhea samples provides PCR-based evidence that recoviruses can infect humans. A previous study found high prevalence of virus-neutralizing antibodies against a closely related calicivirus, Recovirus Tulane, in serum samples from animal caretakers (*15*). Larger epidemiologic studies using genetic and serologic screening will be necessary to provide more insight into the distribution and pathogenic potential of recoviruses in humans.

This work was partially funded by the European Community's Seventh Framework Program (FP7/2007–2013) under the project "European Management Platform for Emerging and Reemerging Infectious disease Entities" European Commission agreement no. 223498 and the Virgo Consortium. This research study was funded in part by ICDDR,B and its donors which provide unrestricted support to ICDDR,B for its operations and research. Current donors providing unrestricted support include the following: Australian Agency for International Development (AusAID), Government of the People's Republic of Bangladesh; Canadian International Development Agency (CIDA), Swedish International Development Cooperation Agency (SIDA), and the Department for International Development, UK (DFID).

Dr Smits works at the Virology Department, Erasmus Medical Center, and Viroclinics Biosciences BV, Rotterdam, the Netherlands. Her research interests are hepatitis C virus and severe acute respiratory syndrome coronavirus.

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Address for correspondence: Saskia Smits, Department of Virology, Erasmus Medical Center/Viroclinics Biosciences BV, PO Box 2040, 3000 CA Rotterdam, The Netherlands; email: smits@viroclinics.com

Tracking the Vector of *Onchocerca lupi* in a Rural Area of Greece

Domenico Otranto, Filipe Dantas-Torres, Elias Papadopoulos, Dušan Petrić, Aleksandra Ignjatović Ćupina, and Odile Bain

During a hot Mediterranean summer, an expedition brought parasitologists from Brazil, France, Greece, Italy, and Serbia to a wooded area near Xanthi, Thrace, northeastern Greece, near the Turkish border, on the track of the vector of the little-known nematode *Onchocerca lupi*. The scientific purposes of the expedition blended then with stories of humans, animals, and parasites in this rural area.

The Beginnings: What's that Worm in the Turkish Blue Eye?

In the early months of 2010, Nermin Sakru, a microbiologist from the Medical School of Trakya University, Erdine, Turkey, contacted one of the authors (D.O.) seeking advice on how to identify a nematode extracted from the eye of an 18-year-old girl who had never traveled out of her native Trakya. Nematodes that might have caused such an infestation were many (*1*), including *Thelazia callipaeda*. This helminth, which infests carnivores and humans, has been studied for more than a decade at the University of Bari in southern Italy (*2*–*5*); this research was what led the Turkish colleague to establish original contact. The patient complained of pain and redness in the left eye and reported being bitten by a fly on the left eyelid in the evening (around 5:00 PM), ≈30 days before onset of symptoms. Pain caused by a biting insect was suggestive of infestation other than by *T. callipaeda*, which is transmitted by *Phortica variegata* (Diptera, Drosophilidae), an insect that gently feeds on the ocular secretions of its hosts during the pleasantly warm Mediterranean summers (*6*).

Some days later, the nematode was morphologically and molecularly identified as a little spirurid, *Onchocerca lupi*, known to infest dog eyes, inducing an acute or chronic ocular disease characterized by conjunctivitis, photophobia, lacrimation, discharge, and exophthalmia. At that time, this helminth infestation had never been reported in dogs in Turkey, and information on the biologic features of the nematode was still meager, despite its wide distribution in Greece, Germany, Hungary, Portugal, and Switzerland and the increasing number of reported cases (*7*). *O. lupi* nematode infestation in humans (*8*) and its biologic and pathogenic affinity with *Onchocerca volvulus*, the agent of river blindness, heightened the interest of D.O. and F.D.T. in the life cycle of this nematode. The idea of investigating the biologic features of *O. lupi* soon began to move across the convoluted pathways of their brains like larvae of *Oestrus ovis* (the nasal bot fly) migrating toward the central nervous system, the main decisional center of all animals!

Organizing the Scientific Expedition: Paris and Antwerp

In the early autumn of 2010, D.O. and F.D.T. had the opportunity to work at O.B.'s laboratories at the Natural History Museum in Paris to morphologically describe dermal microfilariae of a filarioid of the genus *Cercopithifilaria,* isolated some months earlier from a dog in Sicily (*9*,*10*). Those days were short in sunlight, as fall reached Paris much earlier than southern Italy, making the laboratory not the coziest place for late shifts. The helminthology laboratory of the museum is a historically rich place where such eminent scientists as Alain G. Chabaud had described numerous nematodes of medical and veterinary concern for more than 30 years, (*11*,*12*). Working in that small laboratory with simple, dated, yet handy, equipment led to inspiring discussions about prospective studies. By dealing with the zoonotic infestation by *O. lupi* nematodes, they realized how important this parasite species could be, even as a model for better understanding of *O. volvulus* pathogenesis. A major gap in the knowledge of this parasite species was with regard to its vector.

D.O., F.D.T., and O.B. supposed that, as in several other *Onchocerca* species, the potential vector of *O. lupi* could be a black fly (Diptera, Simuliidae) (*13*) or even a

Author affiliations: Università degli Studi di Bari, Valenzano, Italy (D. Otranto, F. Dantas-Torres); Aggeu Magalhães Research Centre Oswaldo Cruz Foundation, Recife, Brazil (F. Dantas-Torres); Faculty of Veterinary Medicine, Thessaloniki, Greece (E. Papadopoulos); University of Novi Sad Faculty of Agriculture, Novi Sad, Serbia (D. Petrić, A. Ignjatović Ćupina); and Muséum National d'Histoire Naturelle, UMR 7205 CNRS, Paris, France (O. Bain)

DOI: http://dx.doi.org/10.3201/eid1807.AD1807

biting midge (Diptera, Ceratopogonidae) (*14*), so they decided to carry out a field study in an area where this parasite species is endemic. The choice for the best places to look for animal cases was not easily made as this infestation had never been reported in Italy, Brazil, or France. However, O.B. recalled that canine onchocercosis caused by *O. lupi* infestation was reported in the Chalkidiki peninsula, province of Thessaloniki, Greece (*15*), where E.P. has been active for 2 decades in veterinary parasitology.

Months later, during the annual meeting of the European Network for Arthropod Vector Surveillance for Human Public Health (Antwerp, April 2011), 2 Serbian entomologists (D.P. and A.I.C.) with expertise on black fly taxonomy and biology were hearing about this new parasite and the enthusiastic plans of an Italian researcher (D.O.) keen on studying its vector. The hypothesis of this scientific expedition blended then with stories of researchers and parasitologists in the years of the Yugoslav Wars (1991–1995) (*16*). Scientists around the table agreed that, sometimes, research activities do soothe physical and mental pains, helping to get wars out of people's minds. Once back in Novi Sad (Serbia), D.P. and A.I.C. decided they would take part in the expedition with O.B., F.D.T., D.O., and E.P.

In Thessaloniki on the Way to Xanthi

In June 2011, participants of the expedition team from the National History Museum in Paris (O.B.), the School of Veterinary Medicine of Bari (D.O. and F.D.T.), the School of Veterinary Medicine of Thessaloniki (E.P. and Socrates Ptochos), and the Faculty of Agriculture, University of Novi Sad in Serbia (D.P. and A.I.C.) received the first message in preparation for the expedition:

> "The main aim of our expedition is to study the occurrence of *O. lupi* infested dogs and to identify the vector of this nematode. It will be a fieldwork whose protocol might require some adjustments according to the preliminary results. Skin and blood samples will be collected from dogs living in areas around rivers to diagnose *O. lupi* infestation. In the meantime, adult black flies shall be collected by dry ice baited traps and drop nets directly on dogs. During the study, samples will be processed and examined at night in accordance with the field activities."

The expedition was partially funded by a pharmaceutical company (see Acknowledgments) and with a budget saved from a former (and different) project of the University of Bari and made available for this research. Because of economic constraints and the numerous commitments dictating the life of any academic, the project duration was fixed at 9 days—a terribly short time for such an ambitious task.

The first step toward retrieval of the developmental infective larvae in a putative vector collected in the field was to identify an area of *O. lupi* infestation. The first meeting took place in a clean, pretty hotel in the vicinity of Thessaloniki airport. While some of us (D.O., F.D.T., O.B.) arrived by plane, others (D.P., A.I.C.) arrived from Serbia after a many hours of driving. Almost nobody knew each other, and the first dinner, in spite of the tiredness, was the real kick-off meeting for the discussion and planning of the field activities. At the end of the first day, all team members were focused on their own duties and commitments in the expedition.

Early in the morning of the second day, after we had a chat over a coffee with A. Komnenou for better defining the localities where *O. lupi* infestations had been reported, the expedition moved to a wooded area near Xanthi. In ancient times, Thrace was considered the fourth continent, after Europe, Asia, and Africa, because of its great difference from the rest of Greece. Geographically, it belongs to the Balkans, with 5 major river systems and few safe anchorages. In this area, until late in the 20th century, population centers were formed in the foothills of the valleys, far from the mosquito- and pirate-infested marshy coastal lowlands.

Our hotel was located just outside Xanthi, on the way to Mount Koula near the small river Kosinthos. Most roads were constantly crossed by turtles (*Testudo graeca*) that carried ticks (*Hyalomma aegyptium*). This place was chosen for the small stream in a large, stony valley and for the overall environmental characteristics of the surroundings—bushes and oaks representing the optimal biotope for black flies (putative vectors of *O. lupi*).

We were the only guests at the hotel, and the owner allowed us to use a large dining room as our makeshift laboratory (Figure 1). We set up 3 optical microscopes and a stereomicroscope on 4 tables, together with slides, entomologic forceps, and ethanol necessary for checking, at night, samples collected during daytime.

Kalimera, Xanthi: The Frontier Land

The expedition team members soon learned that Xanthi was the hometown of Democritus (c. 460 BCE–370 BCE), the ancient Greek philosopher who formulated the atomic theory for the cosmos. With a population of ≈55,500, this region is a frontier land. The main sampling area of the expedition was located around the boundaries of the small village (≈1,000 inhabitants) Amaxades (longitude 25°04′27″E, latitude 41°07′12″N, altitude 56 m), between Xanthi and Komotini. This is a traditionally agricultural area, with soil particularly suitable for tobacco cultivation. Most samples were taken at the cabin of veterinarian Triantaphyllos Papavasiliou, who had been a fellow student

Figure 1. Expedition headquarters in large dining room of a hotel near Xanthi, Greece. Expedition team members (left–right): seated, Aleksandra Ignjatović Ćupina and Odile Bain; standing, Filipe Dantas-Torres, Dušan Petrić, Elias Papadopoulos, Socrates Ptochos, and Domenico Otranto.

of E.P. at the University of Thessaloniki. As soon as the rumor of foreign academics visiting the village of Xanthi in search of some odd parasites in dogs' eyes spread, a queue of old and young locals soon formed outside the cabin, with many more joining in the evening of the first day of field work. These were working people, tough in their faces as well in their hands, with deep, authentic eyes, not so different from those of southern Italians, or Serbians, or any others living where hard work shapes life paths. They were people suspended between their history and their present, and hardly the future, such as for their language, which is a mixture of Greek and Turkish. Nearly the entire Muslim population of Greece is concentrated in these villages, earning its living from a combination of livestock breeding (especially small ruminants) and agriculture. Animals are reared according to a semi-extensive system—grazing during the day and being corralled within stalls at night—with substandard hygiene and husbandry in most of the rural properties we had the opportunity to visit. Sheep and goats are kept for dairy purposes and are milked twice a day. Wolves represent such a threat in the area that farmers usually keep a number of shepherd dogs on their properties. Like their owners, dogs are usually malnourished and have a variety of diseases, most commonly parasitic (sarcoptic mange; tick, flea, and filarial worm infestations; gastrointestinal helminthiasis). We knew that dogs commonly feed on sheep and goat carcasses, a practice resulting in the transmission of pathogens that cause diseases such as coenurosis, hydatidosis, and toxoplasmosis. Human hydatidosis is one of the most prevalent zoonoses in this part of Greece (*17*,*18*).

While we collected samples from dogs, we also collected hematophagous insects by carbon dioxide (dry ice, NS-2 type) installed near the river stream close to our hotel, and by sweep nets, drop nets, and aspirators. Eventually, black flies, and other hematophagous insects of the *Culicidae*, *Psychodidae*, *Ceratopogonidae*, and *Tabanidae* families, were collected. Skin and blood samples were also collected from 21 animals of different ages and sexes. Their owners collaborated with the sample collection, providing information on where their animals were kept and their habits.

Six dog samples were positive for *Dirofilaria immitis* microfilariae, which fit with the occurrence of potential vectors (*Ochlerotatus caspius*, *Culex pipiens*, *Anopheles maculipenis* s.l.) of this filarioid in this area. On the fourth day of sampling, with only 2 days of work left, no dog samples were positive for *O. lupi*. Nonetheless, black flies were captured early in the morning and in the evening by netting directly on some horses grazing around the hotel and by dry ice–baited traps on the riverbanks. At noon, when everything seemed to be lost, E.P. received a phone call from Dr. Papavasiliou, who was about to depart to an Aegean island for holidays. A farmer had just called him complaining of blindness in his dog, and Dr. Papavasiliou promptly suggested that he visit the expedition's headquarters for a clinical check and sample collection; he managed this just a few seconds before being advised by the flight crew that "all mobile phones must be switched off during takeoff."

In *Onchocerca lupi*'s Lair

The dog's owner, a shepherd, arrived at the hotel accompanied by his son and carrying a small, febrile animal. After the clinical check, skin and blood samples were collected and processed within an hour at the makeshift laboratory. The skin sample was positive for *O. lupi* first-stage larvae, prompting the researchers to immediately visit the farm where the animal lived. It was a roasting hot afternoon (June 24, 2011) when the expedition team reached the rural area close to the village of Komotini to take samples from other dogs living in proximity to the animal with the positive sample. The shepherd's family seemed pleased to host us and very eager to assist with research activities. The sheep flock was watched over by both father and son. In the same farm, another animal, with visibly impaired eyesight, keratitis, and uveitis, was found positive for *O. lupi* (Figure 2).This seemed the right time (the only time left) for exposing the collected black flies to feed on the 2 infested dogs in World Health Organization cages (used for insecticide resistance testing). Small glass tubes of entomologic aspirators were used.

Therefore, 4 glass tubes with ≈20–30 black flies each were held on the shaven skin of the head of the positive dogs for 30 minutes to allow them to feed. Out of 4 black flies species, 3 (*Simulium velutinum*, *S. reptans*, and *S. pseudequinum*) fed on the dogs. Afterwards, the blood-fed black flies were kept in the same glass tubes with proper cotton tap impregnated with sugar water solution, ready to be brought to France for dissection and examination for developing larvae of *O. lupi* at +7, +14, and +21 days. Only time would tell the success of the expedition. In heavy rain, the team drove back to Thessaloniki to rest before taking the plane home.

Epilogue

Some weeks after the end of our field activities, O.B. wrote to the expedition team that no developing larvae were detected in the dissected black flies. This should have been expected because of the low number of fed black flies dissected (n = 11). From the beginning of their adventure, researchers knew the inherent difficulties, essentially due to the short time available to look for *O. lupi*–infested dogs, to collect black flies, and to try and feed them on infested animals. It was a challenging task to be accomplished in 9 days. However, their motivation remained high throughout the entire expedition. It was something between irrationality and strong will to learn the life cycle of this scarcely known parasite. The uniqueness of the experience was linked to the fact that, because of the crisis affecting the economies mostly in Greece and Italy, and over the past 20 years, in Serbia, funds for basic research have diminished and are only used for applied research on parasites with major impact on human and animal health. Although acknowledged as a parasite of zoonotic importance in Turkey (*8*) and Tunisia (*19*), *O. lupi* so far has minimal relevance for human health. Nonetheless, the closely related *O. volvulus*, causative agent of human river blindness, is an important cause of visual impairment, affecting >17.7 million globally and remains of major public health importance, especially in developing countries (*20*).

Figure 2. Dog with keratitis and uveitis that was found to be positive for *Onchocerca lupi* nematodes by parasitologic examination.

During those days, we learned that the originality of basic research should reside in walking any possible pathway, even the most narrow, tortuous tracks that could lead to medical science discoveries. This principle, beyond any other rational reason, should be the trigger of scientific research.

Acknowledgments

We thank Vincenzo Lorusso for the revision of the text and his contribution on the style of the article. D.O. and F.D.T. wish to express their gratitude to Alma Unifi for his absolute empathy in their daily lives.

Funding was provided by Lénaïg Halos (Merial, France).

This article is dedicated to all researchers who have been lucky enough to establish lasting friendship with other colleagues throughout their daily working activities, and to those who are still looking for such a chance.

Dr Otranto is a full professor at the Faculty of Veterinary Medicine, University of Bari, Italy. His research interests include biology and control of arthropod vector-borne diseases of animals and humans.

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Address for correspondence: Domenico Otranto, Dipartimento di Sanità Pubblica e Zootecnia, Facoltà di Medicina Veterinaria, Università degli Studi di Bari, Strada Provinciale per Casamassima km 3, 70010 Valenzano, Bari, Italy; email: d.otranto@veterinaria.uniba.it

Treatment Duration for Patients with Drug-Resistant Tuberculosis, United States

To the Editor: In the United States, almost 80% of tuberculosis (TB) cases are diagnosed on the basis of positive culture results for *Mycobacterium tuberculosis*, and >90% of initial isolates are tested for drug susceptibilities (*1*,*2*). Recommended treatment durations are 6–9 months for patients with isoniazid- and rifampin-susceptible TB; ≤18 months for patients with rifampin-monoresistant TB; and, following culture conversion, 18–24 months for patients with isoniazid- and rifampin-resistant TB (*3*). Appropriately completed TB treatment maximizes patient and public health benefits and minimizes adverse events and costs (*3*). We examined treatment duration by drug resistance pattern among a national cohort of case-patients with TB diagnosed in the United States.

We analyzed routinely collected data from the Centers for Disease Control and Prevention's National TB Surveillance System. To ensure that all patients had at least 3 years of follow-up, we examined cases of culture-positive TB verified in 2006. We calculated treatment duration for patients who were alive and had initiated TB therapy at diagnosis and who had results for initial drug susceptibility testing. Treatment duration was calculated by subtracting the therapy start date from the therapy end date. The 15th day of the month was assigned as the day treatment started or ended if that information was missing. Patients who did not complete therapy were censored as of the last known follow-up. We categorized cases as isoniazid monoresistant; rifampin monoresistant; multidrug resistant (MDR), defined as resistant to at least isoniazid and rifampin; or drug susceptible, defined as susceptible to isoniazid, rifampin, and ethambutol and with no known resistance to pyrazinamide (i.e., pyrazinamide susceptible or missing test results). Survival distributions by drug-resistance pattern were estimated by using Kaplan-Meier analysis and compared by using log-rank test statistics. Patient characteristics were compared by using χ^2 tests or, when cell sizes were <5, Fisher exact tests.

Of 13,734 TB cases reported in 2006, 10,747 (78.3%) were confirmed by culture. Of the 10,747 patients with culture-confirmed TB, 10,120 (94.2%) were alive and had initiated TB therapy at diagnosis and had start and end therapy dates and initial drug susceptibility results. Duration of treatment was calculated for 9,734 (96.2%) cases, of which, 8,973 (92.2%) were classified as drug-susceptible, 618 (6.3%) as isoniazid-monoresistant, 24 (0.2%) as rifampin-monoresistant, and 119 (1.2%) as MDR TB. The remaining 386 (3.8%) cases were excluded from analysis because the patients had pyrazinamide-monoresistant TB, suggestive of *Mycobacterium bovis* infection (165), or they were missing susceptibility testing results for isoniazid, rifampin, or ethambutol (112) or had other resistance patterns (109).

At 12 months, the cumulative completion of therapy among patients with drug-susceptible, isoniazid-monoresistant, rifampin-monoresistant, or MDR TB was 87.6%, 81.0%, 17.4%, and 1.9%, respectively (Figure). At 24 months, 73.9% of patients with rifampin-monoresistant TB and 40.2% with MDR TB had completed treatment. Treatment duration was shortest for patients with drug-susceptible TB (median 252 days), compared with a median of 274, 555, and 766 days for patients with isoniazid-monoresistant, rifampin-monoresistant, and MDR TB, respectively. Differences in treatment duration based on drug susceptibility were significant ($p<0.001$) for all comparisons. The MDR TB group included 4 extensively drug-resistant cases (also resistant to any fluoroquinolone and ≥1 of the injectable drugs capreomycin,

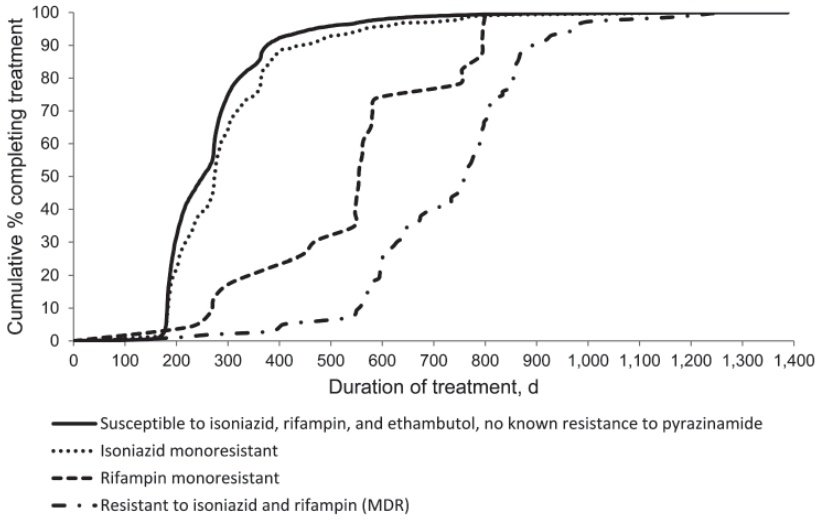

Figure. Treatment duration, by drug-resistance pattern, among reported tuberculosis case-patients who completed treatment, United States, 2006. Cases were among patients who were alive and initiated therapy at diagnosis and who had start and end therapy dates as well as results for initial drug susceptibility testing to isoniazid, rifampin, and ethambutol. Susceptibility testing was conducted on culture-positive *Mycobacterium tuberculosis* isolates from any specimen type. A color version of this figure is available online (wwwnc.cdc.gov/EID/article/18/7/12-0261-F.htm).

kanamycin, or amikacin) (*4*); no remarkable change in duration of treatment resulted when those 4 cases were removed from analysis.

The surveillance system captures only the initial treatment regimen; thus, we could not assess changes to treatment regimens in response to drug susceptibility test results or treatment nonadherence. We observed no difference in history of prior TB; HIV infection; or miliary, meningeal, pediatric, or bone and joint TB among case-patients with isoniazid-resistant versus drug-susceptible TB ($p \geq 0.12$ for all comparisons). TB treatment recommendations in the United States emphasize completion within 12 months of initiating therapy, with exceptions for rifampin-resistant TB, meningeal TB, and disseminated disease in pediatric patients (children <15 years of age) (*1*,*5*). We found no change in treatment duration by drug-resistance pattern after removing cases of meningeal TB or cases in children from analysis.

The length of TB treatment duration in the United States has improved since therapy outcomes were first recorded in the National TB Surveillance System in 1993. In our study, 90% of case-patients with drug-susceptible TB completed therapy within 373 days, compared with 671 days in 1993 (*6*), and 90% of patients with isoniazid-monoresistant TB completed therapy within 432 days. Although the percentage of MDR TB cases in the United States has declined since 1993, drug resistance remains a serious concern because the percentage of isoniazid-monoresistant TB cases has remained stable (*7*). Our analysis suggests that despite the effectiveness of rifampin-containing regimens and an apparent lack of clinical differences to justify extending therapy, longer treatment durations persist among patients with isoniazid-monoresistant TB (*8*). In our cohort study, <75% of patients with rifampin-monoresistant TB and 40% with MDR TB completed therapy within 24 months, suggesting no improvement since 1993 in the length of treatment duration for rifampin-resistant TB strains (*6*).

Acknowledgments

We acknowledge the state and local health department personnel who treat TB patients and collect and report the data used for these analyses. We thank Thomas Navin for comments on earlier versions of this letter.

Carla A. Winston and Kiren Mitruka

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

DOI: http://dx.doi.org/10.3201/eid1807.120261

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Address for correspondence: Carla A. Winston, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E91, Atlanta, GA 30333, USA; email: cwinston@cdc.gov

Exposure of US Travelers to Rabid Zebra, Kenya, 2011

To the Editor: Rabies is an acute progressive encephalitis caused by infection with a lyssavirus (genus *Lyssavirus*, family *Rhabdoviridae*) (*1*). Most human infections are caused by bites from rabid animals, but the virus also can be transmitted by contact of open wounds or mucous membranes with animal saliva (*1*,*2*). Prompt administration of postexposure prophylaxis (PEP) is recommended to prevent rabies (*3*). Canids are common sources of human exposures in many regions of Africa, Asia, and Latin America (*4*). However, all mammals are susceptible, including herbivores such as horses, cattle, and antelope (*5*–*7*).

Approximately 16–200 rabies virus exposures occur per 100,000 international travelers (*2*). Travelers might be unaware of exposure risks from less commonly affected species because prevention guidelines focus on avoiding contact with feral and wild carnivores (primarily dogs) and bats (*2*). After travelers at a safari lodge in Kenya were exposed to a rabid zebra, the Centers for Disease Control and Prevention (CDC) and international partners conducted

a contact investigation to ensure affected travelers received timely exposure assessments and appropriate PEP recommendations.

In January 2011, an orphaned zebra foal was taken to a safari lodge for care. Tourists were permitted to view, pet, and feed the zebra. A dog suspected of being rabid bit the zebra on July 31. Attempts to capture the dog for testing were unsuccessful. The zebra became ill around August 24 and died on August 26 (Figure).

Rabies was suspected because of neurologic signs and was diagnosed in the zebra after detection of rabies virus antigens by direct fluorescent antibody testing at the Kenya Central Veterinary Laboratory. Lodge staff received results on August 30 and immediately communicated the information to travelers who had visited during July 24–August 26 by email through booking travel agents (because lodge staff did not have traveler contact information). This email conveyed the diagnosis and information about rabies virus transmission and vaccine and advised travelers to consult their physicians if they believed they were at risk.

On September 1, after receiving the email, several US travelers reported contact with the zebra's mouth and saliva to state health officials. State health officials notified CDC that same day. CDC initiated a contact investigation of US travelers; the World Health Organization International Health Regulations Office coordinated contact investigation for non-US travelers. The Kenya Ministry of Public Health and Sanitation, Field Epidemiology and Laboratory Training Program, and the Kenya Wildlife Service performed environmental assessments, evaluated lodge staff and animal exposures, and reviewed bite surveillance and preparedness in the surrounding district. CDC Rabies Program staff corroborated the rabies diagnosis and genotyped the variant as one associated with dogs in Africa, supporting the presumed transmission through dog bite.

On September 8, lodge staff provided CDC with travelers' surnames, number of travelers per group, countries of citizenship and residence, and travel agent contact information. Of 243 travelers, 136 (56%) were US residents from 14 states (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/pdfs/12-0081-Techapp.pdf). The remaining 107 travelers were residents of 16 countries, primarily in Europe (online Technical Appendix Figure). CDC obtained traveler contact information from travel agents. State health officials contacted US travelers by telephone or email.

Viral shedding duration for rabid zebras is unknown. An infectious period was estimated as the 14 days from the foal's illness until its death (August 10–26) (*8*). Of 136 US travelers, 77 (57%) visited the lodge during this period. The remaining 59 US travelers who visited during July 24–August 9 also were contacted to document medical assistance received and to provide rabies education.

Twenty-eight (21%) US travelers had already initiated PEP when interviewed by state public health officials. Exposure risk categories based on Advisory Committee on Immunization Practices recommendations were developed to address the unique circumstances of this investigation, i.e., the period and nature of travelers' exposures to the zebra (online Technical Appendix Table 2) (*3*). None reported high-risk exposures; 2 reported moderate-risk exposures; and 26 reported low- or no-risk exposures, for which PEP would not have been recommended. CDC has not received any reports of human rabies in travelers exposed to the zebra in this incident.

Initial exposure notifications to travelers were delivered by travel agents, rather than public health officials. Public health intervention was delayed while traveler contact information was obtained. During this delay, travelers sought care from private physicians who made time-sensitive PEP decisions with incomplete information, resulting in unnecessary PEP administration according to published standards (*3*). Unnecessary PEP should be avoided because rabies biologicals are expensive (averaging $4,000/patient [*9*]), and rabies PEP entails small but real risk for adverse events (*3*). Inclusion of a health provision in travel agency privacy agreements to permit release of traveler contact information for public health use would improve response times for similar events.

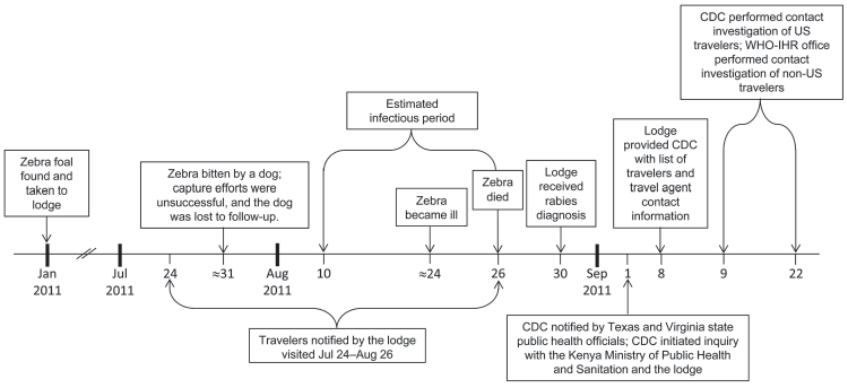

Figure. Timeline of events for traveler exposures to a rabid zebra and subsequent contact investigation of US travelers, Kenya, January 2011–September 2011. CDC, Centers for Disease Control and Prevention; WHO-IHR, World Health Organization's International Health Regulations Office.

Travelers to rabies-endemic regions should avoid contact with wild and feral animals, even in seemingly safe captive settings (*2*). Any mammal can be rabid, and infectious animals might appear healthy for several days before illness onset; avoiding all wild and feral animals while traveling is the ideal preventive measure. All animal bites and scratches should be washed thoroughly with soap and water and receive immediate medical attention (*2*).

Acknowledgments

We thank affected travelers for participating in this investigation and the safari lodge staff and travel agents for their assistance in reaching travelers. We are also grateful to the Kenya Central Veterinary Laboratory and to Michael Niezgoda, Ivan Kuzmin, and the staff of the CDC Rabies Program for laboratory support. Finally, we thank all collaborators at the Department for Disease Surveillance and Response and Field Epidemiology and Laboratory Training Program at the Kenya Ministry of Public Health and Sanitation, Kenya Wildlife Service, Kenya Ministry of Forestry, Kenya Ministry of Livestock Development, the World Health Organization's International Health Regulations Office, and all US state public health partners for their support and partnership during this investigation.

Emily W. Lankau, Joel M. Montgomery, Danielle M. Tack, Mark Obonyo, Samuel Kadivane, Jesse D. Blanton, Wences Arvelo, Emily S. Jentes, Nicole J. Cohen, Gary W. Brunette, Nina Marano, and Charles E. Rupprecht

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (E.W. Lankau, D.M. Tack, J.D. Blanton, E.S. Jentes, N.J. Cohen, G.W. Brunette, N. Marano, C.E. Rupprecht); Centers for Disease Control and Prevention, Nairobi, Kenya (J.M. Montgomery, W. Arvelo); and Ministry of Public Health and Sanitation, Nairobi (M. Obonyo, S. Kadivane)

DOI: http://dx.doi.org/10.3201/eid1807.120081

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Address for correspondence: Emily W. Lankau, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop C01, Atlanta, GA 30333, USA; email: vij3@cdc.gov

Culicoids as Vectors of Schmallenberg Virus

To the Editor: In autumn 2011, an unidentified disease of livestock was reported on both sides of the Dutch–Germany border. By using metagenomics, the etiologic agent of this disease was identified as a novel orthobunyavirus and named Schmallenberg virus (SBV) (*1*). Other members of the genus *Orthobunyavirus* (e.g., Akabane virus) are widespread in Africa and Asia; biting midges (*Culicoides* spp.) and mosquitoes are responsible for transmitting these viruses. Hence, we reasonably assumed that European culicoids might be responsible for transmitting SBV within Europe. We present evidence that culicoids captured October 2011 in Denmark contained SBV RNA and most likely are vectors for this agent.

In autumn 2011, culicoids were collected from several sites within Denmark. One site, a chicken farm in Hokkerup (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/7/12-0385-FA1.htm), was selected for study because of its location close (6 km) to the German border and proximity (<10 km) to an SBV-infected sheep farm in Germany, as reported on March 9, 2012, by the Friedrich Loeffler Institute surveillance website (www.fli.bund.de). The culicoids were collected during October 14–16 by using a Mosquito Magnet Independence trap (Mosquito Magnet, Lititz, PA, USA) baited with carbon dioxide and octenol. Midges were sorted manually into 91 specimens of the *C. obsoletus* group (comprising *C. obsoletus*, *C. chiopterus*, *C. dewulfi*, and *C. scoticus*) and 17 of the *C. punctatus* sensu stricto group, then stored at –20°C.

Pools of culicoids were homogenized in water (100 μL) by

using a 3-mm stainless steel bead (Dejay Distribution Ltd., Launceston, UK) in a TissueLyser II (QIAGEN, Hilden, Germany) for 1 min at 25 Hz (*2*). After homogenization, additional water (100 μL) was added to the samples, and then the mixture was centrifuged at 3,000 × g for 5 min. Nucleic acids were extracted from the supernatant (100 μL) by using a MagNA pure LC Total Nucleic Acid Isolation Kit on a MagNA pure LC (Roche Diagnostics, Basel, Switzerland) and eluted in water (50 μL).

Two separate 1-step reverse transcription quantitative PCRs (RT-qPCRs), targeting the L segment and the S segment of SBV RNA, were performed according to protocols provided by the Friedrich Loeffler Institute in Germany (*1*) on the extracted nucleic acids by using a Mx3005p qPCR system (Agilent Technologies, Palo Alto, CA, USA). Another RT-qPCR targeting ruminant β-actin mRNA was performed as an internal endogenous control (*3*).

Two of 22 pools tested strongly positive for the large (L) and small (S) segments of SBV RNA. Each positive sample was derived from 5 midges of the *C. obsoletus* group. One pool produced cycle threshold (C_t) values of 26.4 and 24.5 (in the L segment– and S segment–specific assays, respectively), whereas the second positive pool gave C_t values of 28.8 (L segment) and 27.6 (S segment). These pools were negative for the internal endogenous control that targeted the bovine/ovine β-actin mRNA. This result makes it unlikely that the detection of SBV RNA within the midges resulted from recent blood meals from infected animals remaining within the culicoids and suggests the virus has replicated within the midges. The PCR amplicons (145 bp; Figure) from the L segment–specific RT-qPCR were sequenced by using BigDye 1.1 chemistry on an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequences of 80 bp from the amplicons, excluding the primer sequences, had 100% identity with the expected region of the SBV segment L (*1*).

Reported C_t values generated by using the same assays from blood of naturally infected cattle were 24–35 (*1*). Usually, ≈100 μL of bovine/ovine blood is used for virus detection, whereas <1 μL of blood remains in a midge after a blood meal. This uptake of blood should therefore lead to a C_t value that is at least 6–7 units higher (≈100-fold lower level of RNA) when a single midge is tested by RT-qPCR (*4*). Thus, even if all 5 culicoids in a pool had recently taken a blood meal from a viremic animal, the C_t values observed here strongly suggest replication of SBV within the *C. obsoletus* group midges. However, in principle, other hosts of SBV could have a much higher level of viremia than cattle and could provide the levels of SBV RNA detected. *C. punctatus* s.s. midges cannot be ruled out as a possible vector of SBV because of the limited number of insects tested.

Our study demonstrates the presence of SBV RNA in *C. obsoletus* group midges caught in Denmark during October 2011. The low C_t values (i.e., high SBV RNA levels) and the absence of ruminant β-actin mRNA in these samples strongly suggest that SBV replicates in these midges and hence that the *C. obsoletus* group midges are natural vectors for this virus.

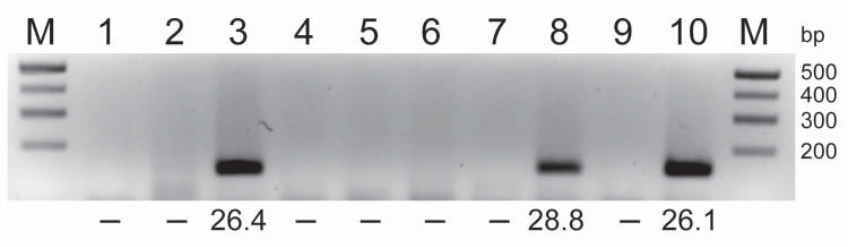

Figure. RNA extracted from pools of *Culicoides obsoletus* group midges was tested in 1-step reverse transcription quantitative PCRs (RT-qPCRs) for the Schmallenberg virus large segment, and the products were analyzed by agarose gel electrophoresis. Lanes 1–8, *C. obsoletus* group midge pools 1–8; lanes 9–10; negative and positive controls, respectively. Numbers below lanes are cycle threshold values from RT-qPCRs; –, no value. M, size marker. Amplicons (145 bp) from positive pools were extracted and sequenced.

Acknowledgments

We gratefully acknowledge the provision of protocols for the RT-qPCRs for SBV RNA by Martin Beer and Bernd Hoffmann. We also thank Helle Rasmussen and Janne Holm Hansen for excellent technical assistance.

Vector collections were funded by the Danish Veterinary and Food Administration as part of a national arthropod mapping project, and the studies were supported within a Green Development and Demonstration Programme and the European Research Area Network Emerging and Major Infectious Diseases of Livestock–funded project by The Ministry of Food, Agriculture and Fisheries.

Lasse Dam Rasmussen, Birgit Kristensen, Carsten Kirkeby, Thomas Bruun Rasmussen, Graham J. Belsham, René Bødker, and Anette Bøtner

Author affiliations: Technical University of Denmark, Kalvehave, Denmark (L.D. Rasmussen, T.B. Rasmussen, G.J. Belsham, A. Bøtner); and Technical University of Denmark, Copenhagen, Denmark (B. Kristensen, C. Kirkeby, R. Bødker)

DOI: http://dx.doi.org/10.3201/eid1807.120385

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Address for correspondence: Anette Bøtner, DTU National Veterinary Institute, Lindholm, DK-4771 Kalvehave, Denmark; email: aneb@vet.dtu.dk

Buruli Ulcer in Gabon, 2001–2010

To the Editor: Worldwide, Buruli ulcer is the third most common mycobacterial infection, following only tuberculosis and leprosy (*1*,*2*). It has been identified in 30 countries, including 12 African countries (*1*–*3*). For Gabon, the first report of a case consistent with Buruli ulcer was published in 1961 (*4*). The patient was a European woman who sought care at a hospital in Lambaréné for a painless upper arm nodule, which evolved into a plaque and then an extensive ulcer. The only other Buruli ulcer reports available for Gabon are a case report from 1968 and a case-series report from 1986 (*5*,*6*). We report data for Buruli ulcer in this sub-Saharan African country for 2001–2010, including prevalence within a hospital population and clinical presentation of the cases. These data can be used to assess long-term developments in the number of cases of Buruli ulcer in this region.

In Gabon, the major focus of Buruli ulcer is the area around Lambaréné (population ≈25,000), the capital of Moyen Ogooué Province (population ≈35,000). It is located near the equator in the central African rainforest. Lambaréné lies near the confluence of 2 major rivers, Ogooué and Ngounié, and is the starting point for one of the largest river deltas in Africa. Numerous lakes are present throughout the region.

The Albert Schweitzer Hospital in Lambaréné serves the entire province. At this hospital, Buruli ulcer is diagnosed on the basis of clinical presentation. In addition, tissue samples are sent to the Prince Leopold Institute of Tropical Medicine in Belgium for PCR analysis. All cases are treated surgically, and since 2006, patients have received rifampin and streptomycin as well. Since 2007, patient information has been recorded on a BU-02 form, designed by the World Health Organization to register and report cases of Buruli ulcer (*1*).

We reviewed cases of Buruli ulcer at the Albert Schweitzer Hospital. We checked the hospital registry and patient records from 2001 through 2010 to identify probable cases of Buruli ulcer on the basis of clinical appearance and response to treatment. We also gathered information from BU-02 forms from 2007 through 2010.

During 2001–2010, the number of patients admitted to surgical wards because of suspected Buruli ulcer ranged from 5 to 40 per year (average 25 patients/year) (Figure). Despite moderate variability from year to year, the number of cases over the years increased (χ^2 for trend, $p = 0.003$), which could be associated with increased awareness of the disease. The variability was not caused by changes in the number of patients hospitalized.

During 2007–2010, detailed clinical information from BU-02

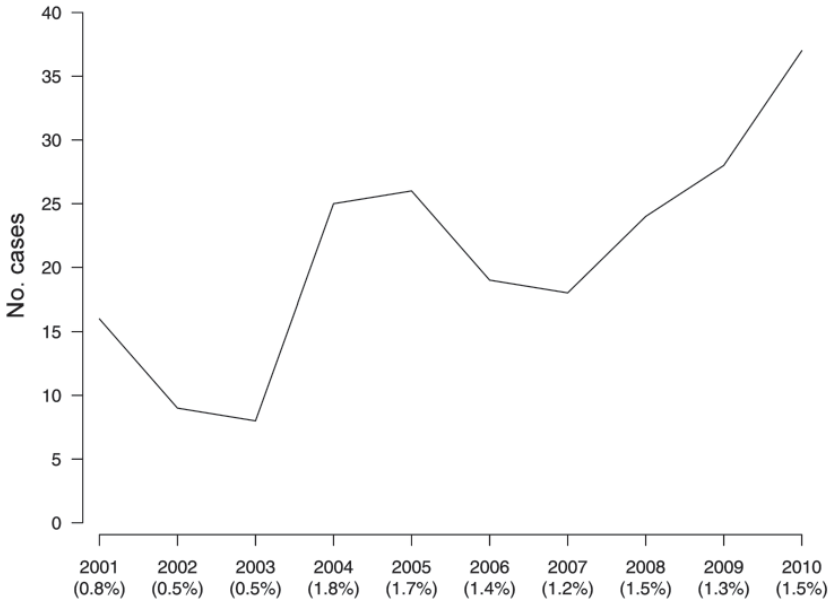

Figure. Number (line) and prevalence (in parentheses) of Buruli ulcer cases, Gabon, 2001–2010.

forms was available for 77 patients. PCR results were available for 57 patients and confirmed the diagnosis for 39. Patient ages ranged from 2 to 72 years; 40 (52%) patients were <15 years of age. The male/female ratio was 0.83. For 44 (57%) of the patients, Buruli ulcer was a new diagnosis. In addition, 56 (73%) patients had an ulcerative lesion, and 21 (37%) of these had lesions >5 cm. The lesions were located on the lower arm for 41 (53%) patients, upper arm for 28 (36%) patients, chest and/or back for 7 (9%) patients, and perineal region for 1 (1%) patient.

Depending on the type of lesion, the length of hospitalization ranged from 1 to 352 days (median 31 days). The longest hospitalization was almost 1 year; the patient was a child who had severe lesions and lived in conditions in which adequate wound care and follow-up after hospital discharge were unlikely.

In Gabon, the available data on Buruli ulcer come mainly from surgical wards in areas where prevalence is high. A national survey of hospital registration data in 2005 detected 3 cases in Ngounie Province in southern Gabon and 5 cases in Woleu-Ntem Province in northern Gabon. All cases are thought to have been acquired locally, thus establishing the existence of 2 previously unknown foci (U. Ateba Ngoa et al., unpub. data).

Buruli ulcer has a strong economic effect on the community and health facilities. For example, in 2010, management of the disease at the Albert Schweitzer Hospital cost an estimated 554–1,660 euros per person, not including drug costs (*7*). In 2009, African countries where Buruli ulcer is endemic, including Gabon, signed the Cotonou Declaration (*8*). According to this declaration, these countries have committed themselves to fight Buruli ulcer by several measures, including assessing the magnitude of the disease and conducting surveillance.

U.A.N. and A.A.A. were supported by the "Deutsch-Afrikanische Kooperationsprojekte in der Infektiologie," grant no. KR 1150/6-1.

Ulysse Ateba Ngoa, Gregoire K. Adzoda, Bayonne Manou Louis, Ayola Akim Adegnika, and Bertrand Lell

Author affiliations: Albert Schweitzer Hospital, Lambaréné, Gabon (U. Ateba Ngoa, G.K. Adzoda, A.A. Adegnika, B. Lell); Ministry of Health, Libreville, Gabon (B.M. Louis); University of Tübingen, Tübingen, Germany (U. Ateba Ngoa, A.A. Adegnika, B. Lell); and Leiden University Medical Center, Leiden, the Netherlands (A.A. Adegnika)

DOI: http://dx.doi.org/10.3201/eid1807.110613

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Address for correspondence: Bertrand Lell, Medical Research Unit, Albert Schweitzer Hospital, BP 118, Lambaréné, Gabon; email: bertrand.lell@uni-tuebingen.de

Ebola Virus Antibodies in Fruit Bats, Ghana, West Africa

To the Editor: Fruit bats are the presumptive reservoir hosts of Ebola viruses (EBOVs) (genus *Ebolavirus*, family *Filoviridae*). When transmitted to humans and nonhuman primates, EBOVs can cause hemorrhagic fevers with high case-fatality rates. In 2008, we detected Zaire EBOV (ZEBOV) antibodies in a single migratory fruit bat (*Eidolon helvum*) from a roost in Accra, Ghana (*1*). This bat is common in sub-Saharan Africa and lives in large colonies, often in cities. The flight of an individual *E. helvum* bat during migration has been recorded as >2,500 km (*2*).

To understand whether the single seropositive *Eidolon helvum* bat was evidence of EBOV circulation in the Greater Accra Region or elsewhere in sub-Saharan Africa, we tested serum of 88 nonmigratory fruit bats from the surrounding region of Ghana. Serum samples had been collected, as previously described (*3*,*4*), during May–June 2007 from fruit bats in woodland and tropical forest habitats in southern Ghana within 180 km of Accra. Initial screening for EBOV antibodies was conducted by using ELISA with a 1:1 mixture of recombinant nucleoprotein (NP) of ZEBOV and Reston EBOV (REBOV). Proteins were expressed in an *Escherichia coli* expression vector with a polyhistadine tag (*1*,*5*). Samples with optical density (OD)

readings 3-fold above the mean OD of 2 negative control serum samples were considered EBOV-positive by ELISA. ELISA-positive samples were tested separately (at a dilution of 1:50) for reactivity against ZEBOV and REBOV NPs by using ELISA and Western blot (WB) as described (*1*). Each sample with positive results from both assays was retested at increasing dilutions to determine the highest dilution (endpoint titer) at which it remained positive (>3-fold above the OD for EBOV-negative sera).

We detected EBOV antibodies (1:1 mixture of both NP antigens, OD>0.7) in serum samples from 32 of 88 bats (10/27 *Epomops franqueti*, 14/37 *Epomophorus gambianus*, 7/16 *Hypsignathus monstrosus,* 1/4 *Nanonycteris veldkampii,* and 0/1 *Epomops buettikoferi*). When tested against an individual NP, 13 of the 32 EBOV-positive serum samples were positive for EBOV (OD >0.50). Of those 13 EBOV-positive samples, 9 were ZEBOV-positive only (from 3 *E. franqueti*, 4 *E. gambianus,* and 2 *H. monstrosus* bats), 3 were REBOV-positive only (from 2 *E. gambianus* and 1 *H. monstrosus* bats), and 1 sample from an *E. gambianus* bat was positive for both ZEBOV and REBOV. Seven samples that the EBOV NP ELISA identified as positive were also positive by WB (Table). Each WB-positive serum sample was positive for the EBOV antigen that it had been most reactive against in the ELISA: 5 WB test results were positive for ZEBOV (2 of those samples also bound to REBOV), and 2 bound to REBOV only. Serum samples with positive OD values at endpoint dilutions >1:50 were definitively positive by WB; whereas those with positive OD values at and endpoint dilution of 1:50 only could be positive, negative, or equivocal by WB (Table).

Previous serum and viral antigen tests indicated the presence of EBOV among 2 of these bat species (*E. franqueti* and *H. monstrosus*) in Gabon, located in central Africa (*6*). Two others (*E. gambianus* and *N. veldkampii*) were not previously identified as potential reservoirs. Because these are nonmigratory fruit bats, our findings demonstrate that at least 1 serotype of EBOV circulates in bats in the Upper Guinean forest ecosystem in West Africa. These data might provide evidence that Taï Forest EBOV (TEBOV), formerly known as Côte d'Ivoire EBOV, circulates in this ecosystem among bats native to West Africa (*7*). EBOV antibody titers are highly correlated (*8*), but using TEBOV antigen might increase seroprevalence if TEBOV is the circulating virus. However, geographic location does not necessarily determine EBOV genetic relationships (*9*), and lack of cross-reactivity between serum samples positive for REBOV and ZEBOV in our study might indicate that divergent viruses circulate regionally, given phylogenetic and antigenic relationships between EBOV species (*7–10*).

We detected a relatively high proportion of EBOV-seropositive fruit bats in a relatively small sample size of mixed species. We suggest

Table. ZEBOV- and REBOV-specific results of ELISA and Western blot analysis of serum from Ebola virus–positive fruit bats, Ghana*

Location, date, fruit bat species	Sex	Age	ELISA OD (endpoint titer dilution)		Western blot†	
			REBOV	ZEBOV	REBOV	ZEBOV
Sagyimase						
May 29, 2007						
Epomops franqueti	F	A	0.11 (1:50)	0.53 (1:200)	–	+
E. franqueti	F	SI	0.08 (1:50)	0.62 (1:100)	+	+
Hypsignathus monstrosus	F	A	0.12 (1:50)	1.08 (1:200)	+	+
H. monstrosus	F	SI	1.11 (1:200)	0.17 (1:50)	+	–
H. monstrosus	F	A	0.15	0.71	–	–
H. monstrosus‡	M	SI	0.05	0.11	–	–
May 30, 2007						
E. franqueti	F	A	0.1§	0.53§	–	+
Adoagyiri						
May 31, 2007						
Epomophorus gambianus	M	A	0.59	0.67	–	–
E. franqueti§	F	A	0.05	0.16	–	–
E. gambianus	F	A	0.19	0.55	–	–
E. gambianus	F	A	0.13 (1:50)	1.24 (1:800)	–	+
E. gambianus	F	SI	0.65 (1:200)	0.36 (1:50)	+	–
Oyibi						
June 2, 2007						
E. gambianus	M	A	0.51	0.63	–	–
Negative control (RAB691/d0)			0.19	0.27		
Positive control (RAB691/EBOV-N)			1.39	1.42	+	+

*ZEBOV, Zaire Ebola virus; REBOV, Reston Ebola virus; OD, optical density; A, adult; SI, sexually immature.
†Only serum samples with ZEBOV- or REBOV- positive ELISA results (optical density >0.50) were tested.
‡Negative-control field serum samples.
§Additional serum was not available for endpoint titer dilution determination.

that the prevalence of EBOV in these tested bat species is greater than that previously detected in *E. helvum* bats (1/262 serum samples) (*1*). The higher estimated prevalence in these species occurred despite the fact that *E. helvum* bats live in large colonies comprising several million animals, which make the species an ideal host for acute RNA virus infections. The relatively low seroprevalence of EBOV among *E. helvum* bats compared with that among sympatric species is contrary to our findings for a lyssavirus and an uncharacterized henipavirus (*3*,*4*). Our results, therefore, lead us to question what factors (e.g., host, ecologic) limit EBOV circulation in straw-colored fruit bats. Virus isolation is required to characterize EBOVs circulating among fruit bats in Ghana, and additional testing, including longitudinal sampling of bats, is required to further investigate the epidemiology of EBOV in West Africa. Possible public health threats should also be investigated and addressed. These initial findings, however, suggest that the risk for human infection with EBOV might be greater from bat-human contact in rural and forest settings than from urban-roosting *E. helvum* bats.

Acknowledgments

We thank the Wildlife Division of the Forestry Commission, Ghana, and the Veterinary Services Directorate for their continued support for this study, and 2 anonymous reviewers for their comments.

This study was funded by the Wellcome Trust (to D.T.S.H.), UK Department for Environment, Food and Rural Affairs (grant VT0105), and the Research and Policy for Infectious Disease Dynamics program (RAPIDD [managed by the US Department of Homeland Security and the Fogarty International Center, National Institutes of Health]). A.A.C. is supported by a Royal Society Wolfson Research Merit award.

David T.S. Hayman, Meng Yu, Gary Crameri, Lin-Fa Wang, Richard Suu-Ire, James L.N. Wood, and Andrew A. Cunningham

Author affiliations: University of Cambridge, Cambridge, UK (D.T.S. Hayman, J.L.N. Wood); Zoological Society of London, London, UK (D.T.S. Hayman, A.A. Cunningham); Animal Health and Veterinary Laboratories Agency, Weybridge, UK (D.T.S. Hayman); Colorado State University, Fort Collins, CO, USA (D.T.S. Hayman); CSIRO Livestock Industries, Geelong, Victoria, Australia (M. Yu, G. Crameri, L.-F. Wang); and Ghana Forestry Commission, Accra, Ghana (R. Suu-Ire)

DOI: http://dx.doi.org/10.3201/eid1807.111654

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Address for correspondence: David T.S. Hayman, University of Cambridge, Madingley Rd, Cambridge, CB3 0ES, UK; email: davidtshayman@gmail.com

Outbreak-associated Novel Duck Reovirus, China, 2011

To the Editor: In 2011, an unidentified disease in Pekin ducks (*Anas platyrhynchos*) was reported in People's Republic of China. The infection caused death in 40% of ducks of various age and 35%–40% mortality in different flocks. Clinical signs included unstable gait, weakness in legs, and diarrhea. At necropsy, large necrotic foci were observed in the spleens. All classical endemic and emerging viruses, such as duck enteritis virus, duck hepatitis virus, duck flavivirus, duck parvovirus, and avian influenza virus, could be excluded as the causative agent by PCR and serologic methods. To identify the cause of the disease, we tested tissue from affected ducks and subsequently isolated a novel duck-pathogenic orthoreovirus from the livers of affected ducks.

Avian orthoreoviruses (ARVs) belong to the family *Reoviridae*, genus *Orthoreovirus* (*1*). The virions are nonenveloped, with icosahedral symmetry and a double capsid containing 10 double-stranded RNA segments that can be separated by polyacrylamide gel electrophoresis into 3 size classes: large (L1–L3), medium (M1–M3), and small (S1–S4) (*2*,*3*). ARVs cause a range of diseases in chicken, including viral arthritis/tenosynovitis, and are associated with respiratory disease, enteric disease, inclusion body hepatitis, hydropericardium, runting stunting syndrome, malabsorption syndrome, and sudden death. ARVs also have been isolated from the Muscovy duck (*Cairina moschata*). Muscovy duck reovirus infection caused illness in 30% and death in 20% of ducks on poultry farms in Israel (*4*). In China, reovirus infection has been reported in Muscovy ducklings, with a resulting death rate of 10%–30% since 1997 (*5*). The isolated reovirus was highly pathogenic to 1-day-old Muscovy ducklings by experimental infection. However, the Muscovy duck reovirus isolate was nonpathogenic for Pekin ducks when inoculated subcutaneously (*4*).

Since 2007, three isolates of orthoreovirus were confirmed in Pekin ducks from several duck farms in China. However, experiment infection with the isolates did not cause death (*6*). In 2011, farmers and veterinarians in China reported to the Animal Health Services and National Research Institutes an unidentified disease in ducks that spread rapidly around the county. We conducted further investigation to identify the causative agent of this disease. The diseased ducks showed depression and leg weakness. Large necrotic foci were observed in the spleens of the dead ducks. Histopathologic examination showed necrotic foci and granulomas in the spleen. Focal hepatic necrosis and proliferation of bile ducts were seen in the liver. Virus isolation from liver homogenate was conducted in duck embryo fibroblast cultures. At 48 hours after infection, a strong cytopathic effect was observed, including syncytium formation. All duck embryos experimentally infected with the isolate died within 48–72 hours after infection. The dead embryos showed swollen livers with petechial hemorrhages. Spherical, spiked virus particles, consistent with those of members of the family *Reoviridae*, were observed by electron microscopy. As reported (*7*), the diameter of the particles was ≈85 nm (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/7/12-0190-FA1.htm). The RNA extracted from DRV-infected duck embryo fibroblast cultures showed 10 dsRNA segments in 3 size classes (L1–3, M1–3, and S1–4) on polyacrylamide gel electrophoresis. The isolate was designated as novel duck reovirus, DRV-TH11. The pathogenicity of DRV-TH11 was tested by infecting 10-day-old Pekin ducks subcutaneously at a dose of $4 \times 10^{4.5}$ 50% tissue culture infective dose. Experimental infection caused death on day 3 after infection. The clinical signs and histopathologic examination show the same features as the naturally infected ducks.

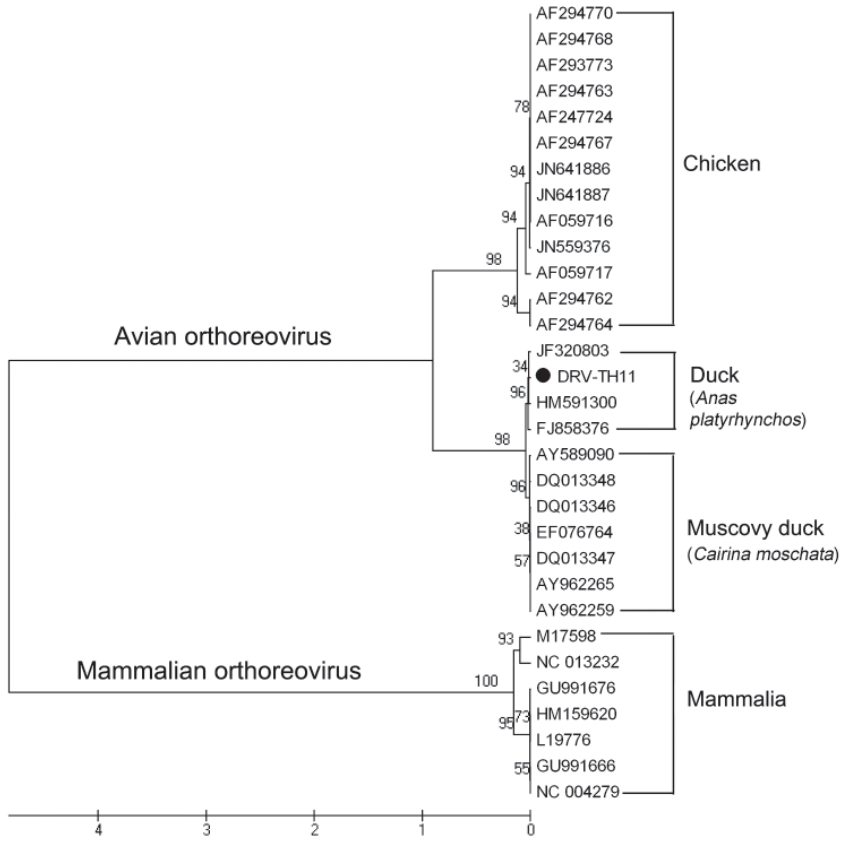

Figure. Phylogenetic relationship between DRV-TH11 isolate and orthoreovirus of the avian orthoreovirus (ARV) and mammalian orthoreovirus (MRV). ARV includes chicken reovirus, Muscovy duck reovirus, and Pekin duck reovirus. GenBank accession numbers of the sequences in the analysis are indicated in the tree. The neighbor-joining tree is based on the complete sequence of s2 gene (1,251 nt). Numbers at nodes represent the percentage of 1,000 bootstrap replicates (values <50 are not shown). Scale bar indicates a branch length corresponding to 100 character-state changes.

For phylogenetic analyses, the S2 gene was amplified by reverse transcription PCR with avian reovirus–specific primers. The complete sequence of the S2 gene (GenBank accession no. JQ664689) was aligned

with 30 published orthoreovirus sequences, including data on all 3 newly obtained sequences from Pekin duck reovirus in China in 2008 and 2011. Phylogenetic relationship was assessed by using the neighbor-joining method based on a Tamura 3-parameter model and bootstrap analysis (1,000 replicates) as implemented in MEGA5 (*8*). The phylogenetic tree shows that the complete sequence of S2 gene is distinct but clusters closely with sequences from all 3 Pekin duck isolates within the ARVs serogroup, which suggests that the novel virus is an ARV-like virus within the genus *Orthoreovirus* (Figure).

In summary, we isolated a novel duck-pathogenic orthoreovirus from the liver of affected Pekin ducks. The regression test in its natural host animal showed that the newly isolated virus caused their deaths. This finding highlights the need to prevent and control this highly transmissible infectious agent. Further study is needed to determine what role the newly isolated DRV played in the 2011 outbreaks on many of the duck farms in China.

This work was supported by the Fundamental Research Funds for the Central Institutes program (no.2011JB06, 2011JB13), Special Fund for Agro-scientific Research in the Public Interest (no. 201003012), the Chinese Natural Sciences Foundation (31101848), and the National Advanced Technology Research and Development Program of China (863 Program) (no. 2011AA10A200).

Zongyan Chen, Yinqi Zhu, Chuanfeng Li, and Guangqing Liu

Author affiliation: Shanghai Veterinary Research Institute–Chinese Academy of Agricultural Sciences, Shanghai, People's Republic of China

DOI: http://dx.doi.org/10.3201/eid1807.120190

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Address for correspondence: Guangqing Liu, Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences, No. 518 Ziyue Rd, Minhang District, Shanghai 200241, People's Republic of China; email: zychen@shvri.ac.cn

Considerations for Oral Cholera Vaccine Use during Outbreak after Earthquake in Haiti, 2010–2011

To the Editor: We wish to thank Date et al. for their clear discussion of the arguments against the use of oral cholera vaccines (OCVs) in Haiti in 2010–11 (*1*). The epidemic curve in their article suggests that the control activities had an effect on mortality rates, resulting in a decrease in case-fatality rates to <1%. This finding is a remarkable success not achieved during the recent cholera outbreak in Zimbabwe that affected 98,531 persons, of whom 4,282 (4.3%) died (*2*). However, the article does not discuss the lack of effect of the control measures in Haiti on the spread of the epidemic. Considering the failure of containment, it would have been interesting to read how the authors judge the recommendation not to vaccinate, with the benefit of hindsight.

The authors list a catalog of arguments against the use of OCVs in outbreaks. These included the priority of water provision and cholera treatment measures, how modeling data provided no convincing justification for vaccination campaigns, how mobile populations cannot be trusted to take 2 doses, the time for a 2-dose vaccine to generate immunity, the logistic challenges in a setting of inadequate infrastructure and human resources, the cold chain requirements, the difficulty in transport of bulky vaccine, clean water requirements for the buffer, civil

unrest, and an unpredictable response from the public.

Overall, we agree entirely that a mass cholera vaccination campaign is a massive logistic challenge. We do, however, question whether logistic challenges of similar size would stop vaccination campaigns against, e.g., influenza in Hong Kong, People's Republic of China. We are convinced that citizens of Hong Kong and their advocates would not tolerate such arguments regarding challenges. Is it because the at-risk population in Haiti is perceived to have few, if any, powerful advocates that such arguments listed by the Centers for Disease Control and Prevention and the Pan American Health Organization could be applied unchallenged?

A much stronger argument against vaccinations is the limited availability of an appropriate licensed vaccine prequalified for purchase by United Nations agencies. At the start of the outbreak October 2010, only 1 OCV, Dukoral (Crucell, Leiden, the Netherlands), was licensed and prequalified. However, not even 300,000 doses of Dukoral were available at the start of the outbreak. A second OCV, Shanchol (Shantha Biotechnics Ltd., Basheerbagh, Hyderabad, India), was licensed but was prequalified only in September 2011. The bigger question is why the international agencies failed to ensure an appropriate vaccine supply following the catastrophic cholera outbreak in Zimbabwe in 2008–2009. Highly effective OCVs have been licensed since 1991 and are marketed to affluent tourists who are at little, if any, risk of being exposed to cholera. The neglect of OCVs as a public health tool during the past 20 years represents a failure of the cholera experts and policymakers alike. Again, such a failure would be unthinkable for a disease affecting more privileged population groups.

The authors write that the lack of data proving that reactive vaccination campaigns are effective was an argument against the use of OCVs in Haiti. We are in agreement that it is unknown whether a reactive mass cholera vaccination campaign would result in adequate vaccine coverage to provide protection and contain further spread. There are simply no data. It is surprising that the Centers for Disease Control and Prevention and Pan American Health Organization experts did not recognize and use the unique opportunity in Haiti to conduct mass vaccination campaigns for the purpose of collecting such vital data.

Finally, the argument of questionable cost-effectiveness is mentioned by the authors. Indeed, data are lacking on the economic benefits of using OCVs in severe outbreaks, although their cost-effectiveness in cholera-endemic situations has been demonstrated (*3*). We believe that anyone who has lived through the agonizing indignities of a cholera attack, especially during a cholera outbreak, would dismiss the economic argument out of hand. No one should have to suffer, much less to die from a vaccine-preventable and quickly curable disease. Using the argument that vaccinations could be too expensive is morally questionable, if not to say revolting.

We have arrived at the conclusion that the withholding of cholera vaccines during the outbreak in Haiti in 2010–2011 was a judgment error and missed opportunity to collect useful data. We wonder whether this article was written to justify what turns out to be an unsound decision, considering the move by other agencies to proceed with a pilot cholera vaccination campaign (*4*). We believe that persistent neglect of OCV as a public health tool is based on the shortcomings of the current generation of cholera experts and policy makers. The long list of technical reasons provided by the authors regarding why the implementation of mass vaccinations was impossible in Haiti are plausible excuses. However, the true reason that cholera vaccines have not been used in Haiti 20 years after they have been licensed and shown to be effective is the fact that populations affected by cholera outbreaks are underprivileged, even by the standards of impoverished populations. It will take decision makers who are less risk-averse and more compassionate to contain the next cholera outbreak. We hope that future decisions will not be biased by previous untrue dogma that vaccination and other measures such as sanitation and effective treatment would oppose each other when the opposite is true. A more enlightened environment would enable more widespread use of OCVs.

Lorenz von Seidlein and Jacqueline L. Deen

Author affilation: Menzies School of Health Research, Casuarina, Northern Territory, Australia

DOI: http://dx.doi.org/10.3201/eid1807.120071

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Address for correspondence: Lorenz von Seidlein, Menzies School of Health Research, John Mathews Building (Bldg 58), PO Box 41096, Casuarina, Northern Territory 0810, Australia; email: lseidlein@gmail.com

In Response: Drs. von Seidlein and Deen criticized decisions regarding oral cholera vaccine (OCV) use in Haiti, but acknowledged that there are no data showing that a reactive mass OCV campaign would contain further disease spread (*1*). They agreed that such a campaign is a massive logistic challenge and asserted that the limited supply of World Health Organization (WHO)–prequalified OCV available during the first 11 months of the epidemic in Haiti was an even stronger argument against vaccination. They then asserted that: 1) by "withholding" "highly effective" OCVs during the outbreak in Haiti, an opportunity "to collect data" was missed; 2) the decision not to vaccinate against cholera "was tolerated" because, like other economically disadvantaged populations, "Haitians have few powerful advocates;" and, 3) the limited OCV supply represents a "failure of the current generation of cholera experts and policymakers."

In our institutions' efforts to support national authorities, the welfare of the Haitian people was and remains our primary concern. Our publication describes considerations during the initial response to an expanding epidemic, when the focus was on saving lives; recommendations were revisited after immediate cholera treatment and prevention efforts were successfully established.

As documented in our report (*2*) and in the media (*3*,*4*), the decision at the peak of the epidemic to not use the available doses of WHO-prequalified OCV to vaccinate 150,000 persons (1.5% of the Haitian population) was made by the Haitian government, in the setting of well-publicized differences of opinion among experts. Although this decision may have resulted in "data not being collected," decisions by sovereign governments are rarely overruled by international organizations, scientists or policymakers. Vaccine use without government approval would have raised questions about the appropriateness of using the outbreak to pilot a large-scale reactive cholera vaccination campaign without documented effectiveness.

OCV effectiveness is moderate when compared with measles and rubella vaccines. Although vaccine-preventable diseases, e.g., measles and influenza, are primarily prevented through vaccination, cholera can be prevented and controlled through other means.

It is true that underprivileged, impoverished populations are disproportionately affected by epidemic cholera, as they have been for centuries. This is not for lack of access to OCVs (which are also unavailable in the United States), but because of lack of access to potable water and adequate sanitation. OCVs do have a place in cholera prevention and response, but a greater public health deficit underlies the spread of cholera in Haiti and other countries where it remains endemic or epidemic. Ensuring universal access to safe water and sanitation, beyond recent progress toward meeting the Millennium Development Goals (*5*), is vital for global cholera control. The Pan American Health Organization (PAHO), the United Nations Children's Fund, and the Centers for Disease Control and Prevention (CDC) have called upon the international community to assist Haiti in this effort (*6*).

The issue of OCV availability is being addressed by various public health organizations. In September 2011, WHO convened an expert consultation to discuss the strategic framework for an OCV stockpile (*7*); the second follow-up meeting was planned for April 26–27, 2012, for further action. Recently, the Coalition for Cholera Prevention and Control, funded by the Bill & Melinda Gates Foundation, held an inaugural meeting of cholera and immunization experts and policymakers to develop comprehensive cholera prevention and control strategies that include appropriate use of OCVs in endemic and epidemic settings (*8*). Cholera outbreaks are unpredictable; increased demand from endemic countries can ultimately drive vaccine production, and help maintain a stockpile for outbreak use.

During the past 20 years, a substantial effort has been made by CDC, WHO, PAHO, and private and public partners working with governments to provide existing vaccines in an equitable manner to some of the world's most disadvantaged populations, and to ensure that these populations have equal opportunities to receive new vaccines, such as rotavirus and pneumococcal vaccines (*9*,*10*). New vaccines require greater investments than in the past; criteria such as preventable burden, cost-effectiveness, and sustainability are key to systematic, evidence-based vaccine introductions (*11*,*12*). CDC and PAHO are providing technical and financial assistance to the Haitian government for improving the national vaccine cold chain capacity; launching a measles, rubella and polio catch-up campaign; and introducing pentavalent (diphtheria + tetanus + pertussis + *Haemophilus influenzae* type b + hepatitis B), rotavirus, and pneumococcal vaccines. With approval of the Haitian government, CDC and PAHO have provided technical assistance to 2 organizations implementing small-scale OCV campaigns in Haiti.

Contrary to the authors' suggestion of "unquestioned dogma," the current generation of cholera and immunization experts and policymakers are engaged in developing an evidence-based, integrated approach to cholera prevention and control that will optimize OCV use without neglecting either primary prevention through improvements in water, sanitation, and hygiene, or prevention of cholera-related deaths through improved access

to life-saving treatment. All cholera prevention and control measures for populations at highest risk need the continued support of powerful advocates in the scientific, political, and policy-making spheres.

Kashmira Date, Terri Hyde, Eric Mintz, Andrea Vicari, M. Carolina Danovaro-Holliday, Cuauhtemoc Ruiz-Matus, Ariel Henry, Jon Andrus, and Vance Dietz

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (K. Date, T. Hyde, E. Mintz., V. Dietz); Pan American Health Organization, Washington DC, USA (A. Vicari, M.C. Danovaro-Holliday, C. Ruiz-Matus, J. Andrus); and Formerly, Haitian Ministry of Population and Public Health, Port-au-Prince, Haiti (A. Henry).

DOI: http://dx.doi.org/10.3201/eid1807.120408

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Address for correspondence: Kashmira Date, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop A04, Atlanta, GA 30333, USA; email: kdate@cdc.gov

The Origins of AIDS

Jacques Pepin

Cambridge University Press, Cambridge, UK, 2011
ISBN-13: 978-0521186377 (paperback)
Pages: 310; Price: US $28.99

This excellent but frustrating book is essential reading for anyone deeply interested in the early history and dissemination of HIV/AIDS. Interest must be deep because the author spares few details about colonial medical systems in the former French and Belgian territories, Congolese politics around independence, and a host of other obscure matters.

The book's strengths include clear explanations of complex themes, such as the molecular evolution of simian and human retroviruses, and a comprehensive review of early events in the pandemic. Many of the book's sections are engaging. For nonspecialists, the book provides some of the most intelligible analyses of molecular epidemiology and the early history of HIV/AIDS, including consideration of different explanations of the origin of HIV. For example, the author usefully examines and dismisses the hypothesis still promulgated by Edward Hooper that HIV originated in eastern Congo during the 1950s after material grown in monkey or chimpanzee cell cultures was used for mass vaccination against polio. Nonetheless, readers will cover a lot of material that could have been omitted or skip sections not essential to the core theme.

Fascinating insights and anecdotes are scattered throughout the text. The reader will find commentaries on early tropical researchers and public health officials, as well as description of a cryptic wasting illness in the 1930s referred to as "Cachexie du Mayombe." The clinical description of patients with this syndrome is eerily reminiscent of patients with AIDS: "an assembly of bones held together by skin… whose only life lay in their gaze." There is also an incidental but valuable discussion of the late Jonathan Mann, founding Director of the World Health Organization's Special (later Global) Programme on AIDS, who deserves his own full historical biography.

This book represents a personal mission for Jacques Pepin, a Canadian infectious disease specialist and epidemiologist with broad African experience who developed an abiding interest in human African trypanosomiasis (sleeping sickness). Pepin's thesis regarding HIV derived from findings from retrospective studies of HIV-2 that he then applied to HIV-1. He proposes that during the colonial era in central and western Africa, the extensive re-use of needles and syringes in medical practice and campaigns against endemic tropical diseases amplified the early spread of HIV-1 after cross-species transmission from chimpanzees. Kinshasa became the early HIV epicenter from which subsequent global dissemination occurred, principally through sexual transmission.

The evidence offered by Pepin for the iatrogenic hypothesis is probably better presented than ever before. Nevertheless, the evidence is speculative because it is based on circumstantial and ecologic associations, such as those between earlier medical practices and trends in hepatitis C virus infection. Despite excessive speculation in parts of the book, such as that concerning the role of the trade in plasma from Haiti, this work is still a "tour de force" and deserves widespread recognition.

Kevin M. De Cock

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

DOI: http://dx.doi.org/10.3201/eid1807.120461

Address for correspondence: Kevin M. De Cock, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D69, Atlanta, GA, 30333, USA; email: kdecock@cdc.gov

Eradication: Ridding the World of Diseases Forever?

Nancy Leys Stepan

Cornell University Press, Ithaca, NY, USA, 2011
ISBN-10: 0801450586
ISBN-13: 978-0801450587
Pages: 272; Price: US $35.00

Public health, like any dynamic field filled with social reformers, scientists, and passionate believers, generates conflicting views, approaches, and goals. Thus, on domestic and global fronts, public health advocates compete for priority and resources for vertical (single-disease) versus horizontal (infrastructure or systems) programs; infectious diseases versus noncommunicable diseases; targeting diseases to improve health versus emphasizing the role of economic development or social determinants; and primary health care versus eradicating diseases.

Eradication: Ridding the World of Diseases Forever? by Nancy Leys Stepan provides a rich context for the role of eradication historically and conceptually in public health and, along the way, touches on many of the fault lines that stress and enrich public health. The depth and breadth of the author's approach also enrich her book and broaden its appeal to readers whose interests go beyond the topic of disease eradication and include public health history, governance, leadership,

philosophy, and dependence on multiple disciplines.

The book's introduction and first chapter alone would provide a fine primer to begin the exploration of "what makes a population get healthier?" After this concise and clear context of eradication and its pursuit (eradicationism), the text then focuses specifically on eradication efforts and some key disease eradicators. Particular emphasis is given to a major 20th century public health leader and proponent of disease eradication, Fred Lowe Soper, and his role with the Rockefeller Foundation, his successful efforts in Brazil and other countries, and his global influence as director of the Pan American Health Organization. He targeted yellow fever and malaria, primarily through vector control (mosquito eradication), and became a champion for use of DDT. Stepan uses the colorful and compelling personality and strengths of Soper, the political complexities of international work, and the unforeseen conflict of insecticidal vector control with the advent of environmentalism to illustrate the considerable hurdles involved in any program of disease eradication, no matter how initially successful and promising. She continues with detailed examples of the successful program of smallpox eradication.

After a description of the guinea worm eradication program, which has made extraordinary progress, the book seems to end a bit abruptly. Only a handful of pages are devoted to the world's major current disease eradication program, polio, and there is little mention of measles. The book relies for information and opinion on distinguished leaders in eradication efforts, but almost all of them are American or live in the United States. Are European views different? What about having more insights from public health figures in the involved nations in Africa, southern Asia, and South America? The result feels somewhat parochial and incomplete.

Nevertheless, this book provides an interesting and useful perspective on a major public health movement and is suitable for students beginning their public health studies as well as for their professors of epidemiology and public policy. Veterans of eradication efforts will enjoy reading it. Those currently involved in eradication campaigns and those considering joining them would be wise to read this book and absorb its lessons.

Jeffrey P. Koplan

Author affiliation: Emory University, Atlanta, Georgia, USA

DOI: http://dx.doi.org/10.3201/eid1807.120474

Address for correspondence: Jeffrey P. Koplan, Emory University Woodruff Health Sciences Center, 1440 Clifton Rd NE, Suite 410, Atlanta, GA 30322, USA; email: jkoplan@emory.edu

Infectious Disease: A Geographic Guide and Atlas of Human Infectious Diseases

Eskild Petersen, Lin H. Chen, and Patricia Schlangenhauf, editors

Infectious Disease: A Geographic Guide, Wiley-Blackwell, Oxford, UK, 2011
ISBN: 978-0-470-65529-0
Pages: 480; Price: US $84.95

Heiman F.L. Wertheim, Peter Horby, and John P. Woodall, editors

Atlas of Human Infectious Diseases, Wiley-Blackwell, Oxford, UK, 2012
ISBN: 978-1-4051-8440-3
Pages: 306; Price: US $130.00

Infectious Disease: A Geographic Guide and Atlas of Human Infectious Diseases, 2 books recently published by Wiley-Blackwell, deliver to the global medicine bookshelf diagnostic adjuncts for expatriate clinicians and those who see immigrants or returning travelers, while also serving as pretravel references on regional disease risk and authoritative sources for anyone needing infectious diseases information. Mary Wilson, who contributed to the first book and wrote the foreword for the second, filled a similar need in 1991 with A World Guide to Infections. Now these new books remind us that even in the age of near–real-time, electronic references, a printed volume to hold in one's hands can be an unmatched resource.

Infectious Disease: A Geographic Guide, edited by Eskild Petersen, Lin Chen, and Patricia Schlagenhauf, uses United Nations regions as an organizational basis, which achieves the objective of maintaining relevance with respect to by-country travel while reflecting the fact that pathogens do not recognize political borders. The regions are still country groupings, but the way this book cuts up the world integrates how transmission varies by topography, geoclimatic factors, and the fauna that include pertinent disease reservoirs and vectors. Well-written chapters also review background regional histories, evolving global disease patterns, and the impacts of migration, climate change, and public health interventions. Extensively published physicians who have experience in geographic medicine contributed to all of the book's clinical content. Fifteen of the 22 region-specific chapters include authorship from within that region. Nicely organized tables dominate over paragraphs of text. Occasional inconsistencies occur in the use of a unique font that sets off headings and subheadings, but this is a relatively minor side effect of a first printing.

The sequence that reliably characterizes nearly all of the region chapters is by organ system, with diseases then addressed categorically by du-

ration of symptoms, using a 4-week cutoff point. Additional sections cover adenopathy, fever without focal symptoms, eosinophilia with elevated IgE, antimicrobial drug resistance, vaccine-preventable diseases, and statistical summaries addressing economics, demographics, and mortality. However, some authors added sections with a syndromic, taxonomic, transmission-based, or incubation-based approach, and 4 of the chapters have more than a slight departure from the essential scheme. Nevertheless, the quality of the content is consistent and, in fact, is enhanced by the variations.

Overall, the Geographic Guide is an outstanding, quick reference for clinicians. The book provides a link between a patient's history or travel itinerary on one hand and a differential diagnosis or guidance for preventive measures on the other.

From its title and external appearance, Atlas of Human Infectious Disease could be mistaken for a cytology or histology text. Once open, a geography book appears, and quickly enough, its pages reveal an essential visual almanac for anyone whose work confronts, or whose interests include, infectious diseases. This book shows the pictures we often seek but have difficulty finding: those that answer the question, "Where?" For a less common disease, where has it been reported? For a more common one, where is it not controlled? Part atlas and part disease manual, this work reflects an intensive effort by 120 contributors and reviewers, assuring the user of a broad, collective expertise. Oxford tropical disease researchers Heiman F.L. Wertheim and Peter Horby and ProMED mail co-founder John P. Woodall are the lead editors. The book provides taxonomic consistency with widely available sources such as the Control of Communicable Diseases Manual, and scientific articles specific to each topic were used extensively.

The first 40 of its 273 pages offer mapping of factors that influence disease transmission, clinical penetrance, and control. This portion could easily stand alone as a reference for a broad range of readers interested in any of numerous topics, from urbanization to climate to the global use of antibiotics and vaccines. The bulk of the book is a compendium of 2-page, clinical–epidemiologic summaries of human infections, each having the same left-page map and right-page text layout. An equal-area world map is the usual template for incidence and endemicity displays, whereas regional maps and insets are used as needed; however, no section or entry on geographically diverse, health care–associated bacterial infections is included. The entries do include selected opportunistic infections, but apart from agents of general public health importance such as bloodborne viruses, health care–associated infections are not given particular attention. On the other hand, doing so could easily have doubled the volume of the book.

Overlays on the maps are simple and usually contrast well with the core scheme to readily show relationships. For example, the outlining of vector distributions does not interfere with the use of solid colors to map disease occurrence. Where overlays would not work as well, the page includes one or more parallel maps, which may show inverse relationships such as immunization coverage versus disease incidence. The book indirectly begs for better surveillance by depicting large gray areas marked "No Data"; even the most developed countries often fail to escape this distinction. In fact, the type of passive reporting that supplied map data for some diseases leaves one wondering: is it "no data" or "no disease"?

Clever adjustments for reporting bias were made in some cases, though the nature of source data too often prohibits any valid attempt. Likewise, dependence on political borders to outline geographic distribution usually prohibits depiction of spatial density. When a disease has worldwide distribution, that fact is not usually evident in the map, which instead focuses on high-risk areas. This method is appropriate and reinforces the need to use the map and text pages together.

Purchasers are provided a code to download the book in various digital formats. Downloading is a fast and easy process, as is using the electronic version of the book itself. A single click on any topic or figure in the navigation pane takes the reader directly to the page desired. The resolution is excellent, and one can either scroll or page up and down through each entry. (An electronic book version of Infectious Disease: A Geographic Guide is also available from the publisher, but as a separate purchase from the print edition.)

The Atlas clearly sets a new standard as a geographic medicine reference and is certain to become an indispensable tool for epidemiologists and infectious diseases specialists. The editors hope it will also encourage the reporting of infectious diseases worldwide, which may well become its most important role.

Bruno P. Petruccelli

Author affiliation: Retired, Medical Corps, US Army

DOI: http://dx.doi.org/10.3201/eid1807.120604

Address for correspondence: Bruno P. Petruccelli; email: petru94@verizon.net

Gene Davis (1920–1985) *Niagara Knife* (1967) Acrylic on canvas (294.6 cm × 546.1 cm) High Museum of Art, Atlanta, Georgia, USA. Gift of Turner Broadcasting System, Inc.

Health Threats of All Stripes

Polyxeni Potter

"I just decided to do a stripe painting, just to be outrageous," Gene Davis said, pondering the origins of his iconic works. "Let's see if I can't do something that goes in the opposite direction from painterly abstraction." This decision "to get away from painterliness" and "move somewhere else" was at the heart of his art. "It's something, you know, that shakes them up. It's not a painting of a bouquet of flowers."

"I've never been a realist artist…. I haven't gone through the usual classical training at all. I just bypassed the entire issue. And I'm not sorry." Davis admitted reluctance to being bossed or instructed and professed being a free operator. All the same and despite the absence of academic training, he came to art early in life. "When I was 8, 9 years old, somewhere in that vicinity, I used to do little childlike drawings and send them in to the Washington Post 'children's page'… and they thought enough of them to publish them…. And then I took… a drawing course in high school." Later in his career he taught art at the Corcoran School of Art and Design and for a time at American University, Skidmore College, and the University of Virginia.

A native Washingtonian, Davis frequented art venues, particularly the Phillips Collection. "The small masterpieces of Paul Klee… made an unforgettable impression on me, and I can remember being equally smitten with the complex color harmonies of Bonnard." But his interest did not peak until his late 20s, "Because during my early 20s, I was a very happy newspaper man. I covered the White House for 5 years." This career included stints as sports writer for the now defunct Washington Daily News and work for United Press International and the New York Times—as a copy boy "a real elitist job to have, because it was a stepping stone to the reportorial." "I earned my living as a writer for something like 35 years before I really was successful enough as an artist to quit my job and to paint full time. And that took place in 1968."

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

DOI: http://dx.doi.org/10.3201/eid1807.AC1807

Davis did not "jump into the art stream" until 1949. "I started after having read an article in the New York Times about van Gogh that turned me on." He did not join the local art scene until 1950 when he met noted Washington artist and curator Jacob Kainen, who became his mentor and introduced him to Morris Louis and Kenneth Nolan…. "In those days, the big issue was whether you were going to be a realist or an abstractionist…. I leaped right in as an abstractionist." "What really impressed me about the abstract expressionists [Jackson Pollock and his circle] was the degree to which you could deemphasize skill and still say something that had tremendous intensity…. It's the 'what' of it more than the 'how' of it."

But soon "All the art departments—college art departments—were grinding out little de Koonings and Pollocks…. So in that climate, it seemed that… there was no place to go." Young painters, Davis among them, were looking for change. "Frank Stella, Noland, myself. There's a whole group of them —Ellsworth Kelly." Their new direction was soon labeled "post-painterly abstraction," and a group of artists who had not intended to band together began to be referred to as the Washington Color School, their bold work anticipating later movements. "I'd be the last person in the world to claim that Washington's art influenced Pop Art but I think things were in the air. And they had bright, brazen colors just like we did. There was something, a common denominator that went through the '60s. It was an exciting period. The Kennedy era, optimism was in the air, excitement, campus rebellion—all that stuff was all—you can't isolate any of it."

"What you see is what you see," proclaimed Frank Stella, expressing the period's preoccupation with art concerned only with the direct experience of color and form. Davis also elaborated on the content of color and form. "I have very, very strong subject matter in my work, which is stripes." Invested with enough intensity, "A stripe is just as real as a… flower…. There's no such thing as a painting about nothing…. For example, if you look at 17-th century Dutch art, you'll see that there are endless numbers of painters who painted the same subject matter as Rembrandt—these middle class people with their big hats

and their long collars and all that. So, Rembrandt's great, and most of those people are eminently forgettable. What makes the difference? It isn't the subject matter, obviously. It's the form."

Niagara Knife, on this month's cover, is one of Davis' stripe paintings, a hallmark of his work for 20 years—the stripe as form. He painted mostly vertical stripes, because horizontal ones "carry the illusion of landscape." He painted color stripes on the street leading to the Philadelphia Museum of Art and a parking lot in Lewiston, New York, turning them into massive works of art. He never planned more than a few stripes ahead. He improvised, allowing each color to inspire the next.

"I play by eye in the same way that a jazz musician plays by ear." In addition to form, color was of great interest to Davis. Color and interval—the distance between things, as in music. "Music is an art of sound interval, time interval, and painting—my painting—is an art of space intervals. One is time, one is space." A frustrated musician, he often referred to music as a way of discussing his work. "If you have a painting which has all half-inch stripes in it, multi-color, and you put a bright red over here, and another bright red over there, no more of those bright reds in the entire painting, there's an interval established between the two reds. Because all the other colors in the painting will be something else. But these two relate."

"I paint to surprise myself." Davis believed that shocking or even offending the viewer had an energizing effect. "Ambiguity interests me." This could be created by the contrast of opposites. "It's a little like Mozart, who was a master of ambiguity in that his works can often be regarded as little tinkling, felicitous things, but there's a strong note of melancholy running throughout. You get that melancholy plus felicity and it creates ambiguity."

The breadth of a line, the distance between colors, and the interaction of colors create an optic and kinetic effect and an architectural complexity in Davis' work that appear analytical, mathematical. Yet, it is all "intuitive and romantic." "I'm a real shoot-from-the-hip artist." The work invites personal interpretation, teasing the eye and challenging it to grasp the total, vibration and all.

In that each stripe is individually executed to be viewed at once alone and in conjunction with the others, Davis' *Niagara Knife* is not unlike the effort addressed in this month's issue of Emerging Infectious Diseases: public health at the global level. Each laboriously painted thin or thick stripe, each narrow or wide interval, each lyrical color combination a nation; marching bands of color a dazzling array of diversity and separateness; and altogether as Davis intended them, a bright ensemble, a symphony of color, a public health collaboration as spectacular as any bouquet of flowers.

"Painting stripe paintings is a vigorous kind of thing. I've got at least to be down on my hands and knees," Davis explained to those curious about his craft. Laboriously, line by line, the painting becomes an integrated total. The same rigor certainly applies to drafting any international regulations, including those intended to protect public health. Outbreak by outbreak, experience with Public Health Emergencies of International Concern delineates what requires international reporting to improve global health and emergency response. Because, while individual stripes are drawn on the canvas of global health as nations agree to report to the World Health Organization those health events of concern to international health, the total picture is harder to assemble. Distinguishing which health events pose international health threats is at times as ambiguous as a Davis painting, and therefore, implementation of the International Health Regulations has not yet realized its full potential.

While ambiguity can energize a work of art, it can upset a regulatory document. Generally avoided for its capacity to introduce uncertainty, ambiguity represents the human factor, addressed in the regulations by a decision instrument to guide subjective judgment. Improving the validity of the instrument, along with clarifying measurable goals and progress indicators, promises to overcome some of the ambiguity, pulling individual stripes into bands and the bands into a full form the colors of international health.

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Address for correspondence: Polyxeni Potter, EID Journal, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D61, Atlanta, GA 30333, USA; email: pmp1@cdc.gov

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To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to **www.medscape.org/journal/eid**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Assessment of Public Health Events through International Health Regulations, United States, 2007–2011

CME Questions

1. Based on the report by Dr. Kohl and colleagues, which of the following statements about International Health Regulations (IHR) and overall potential public health emergencies of international concern (PHEIC) from states parties posted by the World Health Organization (WHO) on a secure Web portal between July 2007 and December 2011 is most likely correct?

A. Nearly all states parties posted at least 1 PHEIC
B. states parties are obligated to notify WHO of PHEIC within 72 hours of becoming aware of an event
C. Less than 1% of posted PHEIC were from the United States
D. states parties are only required to report natural release of biological materials

2. Based on the report by Dr. Kohl and colleagues, which of the following statements about events reported by the United States and posted by WHO on a secure Web portal between July 2007 and December 2011 is most likely correct?

A. 5% of US events involved human influenza caused by a new subtype
B. One of the US PHEIC involved anthrax
C. All of the US PHEIC involved infectious diseases
D. The only PHEIC determined by the WHO Director-General to date was the first report of the 2009 pandemic virus

3. You are a public health official consulting to the United States about the benefits of and procedures for complying with the 2005 IHR. Based on the report by Dr. Kohl and colleagues, which of the following statements would most likely appear in your report?

A. The United States is unlikely to be notified by another country's National Focal Point regarding potential public health threats
B. Notifications from other countries are unlikely to affect US public health decisions
C. The IHR facilitates rapid information exchange among WHO and its Member States, improving situational awareness of emerging threats and coordinated and transparent global response
D. Current strategies for recognizing events to be reported to WHO are well developed and highly standardized

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

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Article Title

Low Pathogenic Avian Influenza A (H7N2) Virus Infection in Immunocompromised Adult, New York, USA, 2003

CME Questions

1. You are seeing a 48-year-old man who complains of 2 weeks of severe malaise, tactile fever, cough, and weight loss.
You consider whether this patient has influenza. Which of the following statements best characterizes the majority of low pathogenic avian influenza (LPAI) viral infections?

A. The mortality rate approaches 50%
B. Almost all cases are diagnosed in the hospital
C. Most infections are mild in nature
D. They are characterized by higher rates of secondary pneumonia compared with other influenza infections

2. You want to offer targeted antimicrobial therapy for this patient. What was the patient in the current case study treated for originally?

A. Community-acquired pneumonia
B. Primary influenza infection
C. Primary HIV infection
D. Tuberculosis

3. The patient is confirmed to have infection with LPAI. How did the patient in the current case study acquire LPAI?

A. Keeping pigeons above his home
B. Working in a poultry processing plant
C. Direct contact with infected coworkers
D. Unknown

4. What else should you consider regarding the current case study as you treat this patient with LPAI infection?

A. The presence of conjunctivitis suggests something different from infection with H7 subtype viruses
B. Concomitant HIV infection has been definitively associated with an increased risk of lower respiratory tract infection with H7N2 virus
C. The case patient recovered without specific treatment for influenza
D. The influenza A isolate grew rapidly in culture

Activity Evaluation

1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
4. The activity was presented objectively and free of commercial bias.				
Strongly Disagree				Strongly Agree
1	2	3	4	5

EMERGING INFECTIOUS DISEASES

Upcoming Issue

Vaccination of Health Care Workers for Protecting Patients at Risk for Acute Respiratory Disease

Resistance to Ciprofloxacin and Other Antimicrobial Drugs in *Neisseria gonorrhoeae*, United States

Molecular Epidemiologic Investigation of Anthrax Outbreak in Heroin Users, Europe

Hepatitis E Virus Strains in Rabbits and Evidence of a Closely Related Strain in Humans, France

Enzootic Risk Measures to Predict Urban West Nile Disease, Los Angeles, California, 2004–2010

Hepatitis E Virus in Pork Production Chain, Czech Republic, Italy, and Spain

Solid Organ Transplant–associated Lymphocytic Choriomeningitis, United States, 2011

ESBL–producing *Klebsiella oxytoca* and Contaminated Hand-washing Sinks

Population Diversity among *Bordetella pertussis* Isolates, United States, 1935–2009

VIM-2–producing Multidrug-Resistant *Pseudomonas aeruginosa* Clone ST175, Spain

Hepatitis E Virus Genotype 3 in Wild Rats, United States

Paragonimus kellicotti in Missouri

New Variants of Porcine Epidemic Diarrhea Virus, China, 2011

Severe Human Granulocytic Anaplasmosis Transmitted by Blood Transfusion

Autochthonous Infections with Hepatitis E Virus Genotype 4, France

Klebsiella pneumoniae in Gastrointestinal Tract and Pyogenic Liver Abscess

Escherichia coli O104 Associated with Human Diarrhea, South Africa, 2004–2011

Rickettsia felis in Fleas, Southern Ethiopia

Complete list of articles in the August issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

July 22–27 2012
XIX International AIDS Conference (AIDS 2012)
Washington, DC, USA
http://www.aids2012.org/Default.aspx

August 25–29, 2012
2012 Infectious Disease Board Review Course
Ritz-Carlton, Tysons Corner
McLean, VA, USA
http://www.IDBoardReview.com

September 5–8, 2012
Incidence, Severity, and Impact Conference
Munich, Germany
http://www.isirv.org

September 9–14, 2012
XVIIIth International Pathogenic Neisseria Conference (IPNC) 2012
Maritim Hotel, Würzburg, Germany
http://www.ipnc2012.de

October 17–21, 2012
IDWeek Annual Meeting
San Diego, CA, USA
http://www.IDWeek.org

October 27–31, 2012
APHA 140th Annual Meeting & Expo
San Francisco, CA, USA
http://www.apha.org/meetings/AnnualMeeting

November 11–15, 2012
ASTMH 61st Annual Meeting
Atlanta Marriot Marquis
Atlanta, GA, USA
http://www.astmh.org

2013
September 5–10, 2013
Options for the Control of Influenza VIII
Cape Town, South Africa
http://www.isirv.org

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). 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.

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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://wwwnc.cdc.gov/eid/pages/author-resource-center.htm.

Emerging Infectious Diseases is published in English. To expedite publication, we post some articles online ahead of print. Partial translations of the journal are available in Japanese (print only), Chinese, French, and Spanish (http://wwwnc.cdc.gov/eid/pages/translations.htm).

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Manuscript Submission. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

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Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

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. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit figures as separate files, in the native format when possible (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpg or .tif files. Other file formats may be acceptable; contact fue7@cdc.gov for guidance. Figures should not be embedded in the manuscript file. Use color only as needed. Use Arial font for figure lettering. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced to print size. Large figures may be made available online only. Place figure keys within the figure; figure legends should be provided at the end of the manuscript file.

Videos. Submit as AVI, MOV, MPG, MPEG, or WMV. Videos should not exceed 5 minutes and should include an audio description and complete captioning. If audio is not available, provide a description of the action in the video as a separate Word file. Published or copyrighted material (e.g., music) is discouraged and must be accompanied by written release. If video is part of a manuscript, files must be uploaded with manuscript submission. When uploading, choose "Video" file. Include a brief video legend in the manuscript file.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may 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.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

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