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On the Cover

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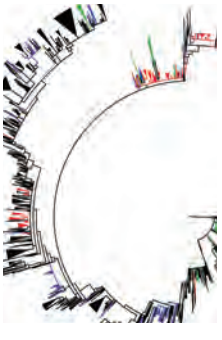

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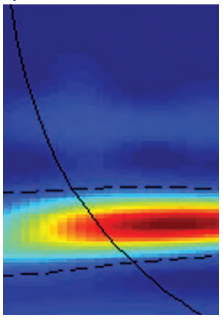

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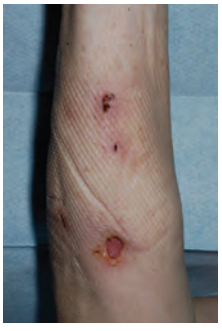

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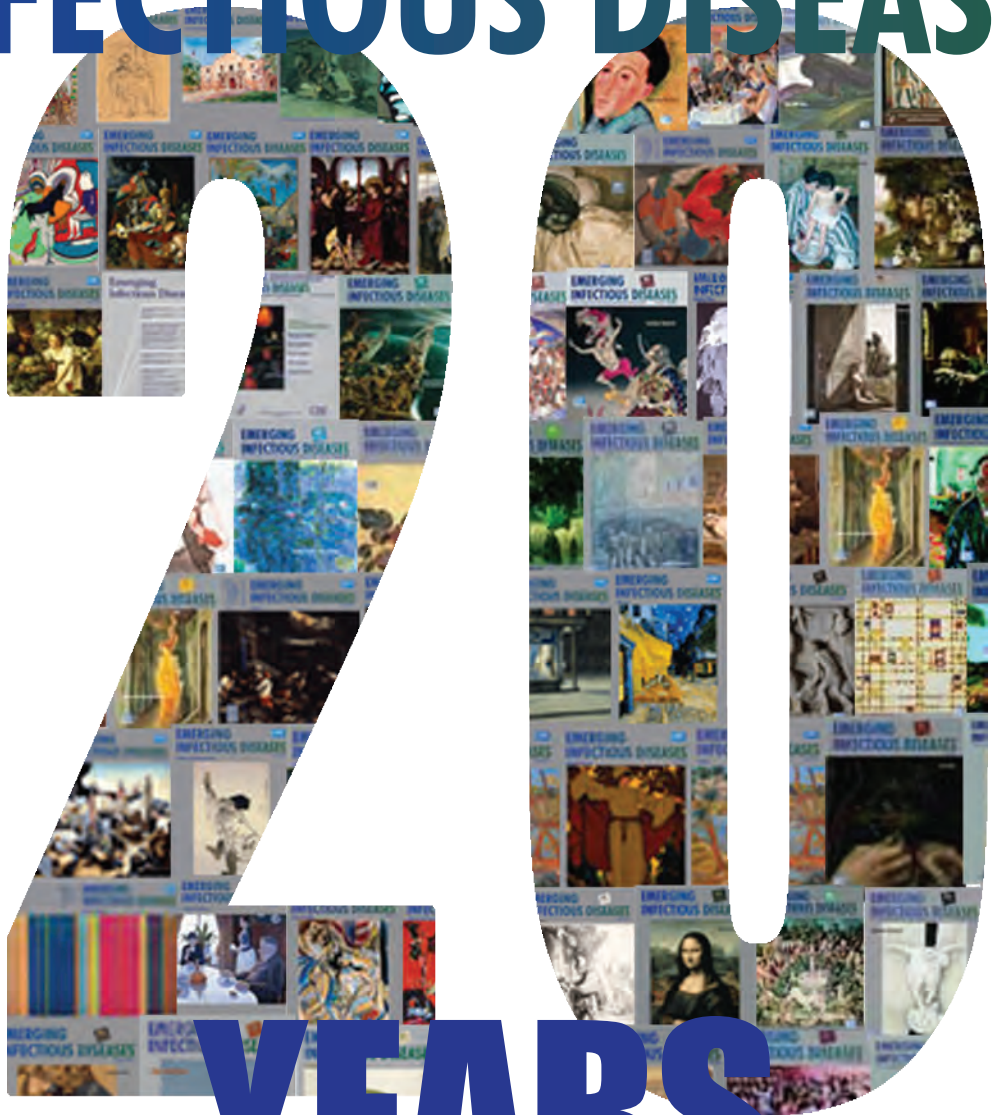

20 YEARS

Presenting the ongoing challenges that emerging microbial threats pose to global health

Drivers of Emerging Infectious Disease Events as a Framework for Digital Detection

Sarah H. Olson,[1] Corey M. Benedum,[1] Sumiko R. Mekaru, Nicholas D. Preston, Jonna A.K. Mazet, Damien O. Joly,[2] John S. Brownstein[2]

The growing field of digital disease detection, or epidemic intelligence, attempts to improve timely detection and awareness of infectious disease (ID) events. Early detection remains an important priority; thus, the next frontier for ID surveillance is to improve the recognition and monitoring of drivers (antecedent conditions) of ID emergence for signals that precede disease events. These data could help alert public health officials to indicators of elevated ID risk, thereby triggering targeted active surveillance and interventions. We believe that ID emergence risks can be anticipated through surveillance of their drivers, just as successful warning systems of climate-based, meteorologically sensitive diseases are supported by improved temperature and precipitation data. We present approaches to driver surveillance, gaps in the current literature, and a scientific framework for the creation of a digital warning system. Fulfilling the promise of driver surveillance will require concerted action to expand the collection of appropriate digital driver data.

Unusual infectious disease (ID) events occur when an underlying mix of antecedent epidemiologic drivers provide the necessary conditions for a pathogen to emerge in susceptible populations. These conditions may be driving emergence through a wide variety of mechanisms, such as climate change, industrial development, ecosystem change, and social inequality (*1*). Public health policy has traditionally targeted well-described socioeconomic drivers, such as lack of sanitation, lack of hygiene awareness, and poor access to health care and disease prevention services (e.g., bed nets, vaccinations, and treatments), but researchers have increasingly evaluated the complex interactions among drivers related to globalization, political issues, human susceptibility, and biophysical environmental change (*1*–*8*) (Table).

Whereas early ID research primarily focused on pathogen identification and specific disease ecologies (*9*), researchers are now exploring the multifactorial causes of emergence. No longer is the question "What causes Ebola?" but rather, "Why does an Ebola outbreak occur at a particular time or location?" As a result of this transition in research, global disease event data have grown. The World Health Organization Global Burden of Disease Reports, commissioned in 1992, first demonstrated the feasibility of measuring both global risk and disease occurrence data (*10*). Scientists built on such initial global platforms and began to develop tools to improve reporting and awareness of disease outbreaks among local and global health workers. In the 1990s, digital systems such as ProMED and Global Public Health Intelligence Network demonstrated the utility of real-time digital disease event detection (*11*,*12*). The emergence of these intelligence platforms was followed by a new generation of surveillance tools, such as HealthMap, Biocaster, and MedISys (*13*–*15*).

Behind the development of surveillance tools for ID events, growing research suggests that untapped driver signals could be quantified and monitored to anticipate emergence risk as a new form of epidemic intelligence. Temperature and precipitation data are already used to forecast meteorologically sensitive IDs, but more driver data could support improved predictive models covering a much broader range of IDs. For example, across 397 outbreaks of international concern, as classified by the World Health Organization, nearly 40% were attributed to 1 driver: lack of public health infrastructure (*2*). Further, changes in land use, another known driver, can produce animal–human interfaces ripe for spillover events (*3*,*7*). Indeed, between 1940 and 2005, 60% of emerging ID events were of zoonotic origin and showed a substantial positive correlation with wildlife abundance and diversity (*4*). The logical progression to further strengthen public health infrastructure is

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[2]These authors were co-senior authors of this article.

Table. Disease drivers identified in the literature and examples of data availability*

Driver theme (references)	Global data examples†	Regional data examples†
Human susceptibility to infection (*1,2,4*)	Vaccine rumor surveillance, product distribution data from manufacturers, self-reported immunization status	US influenza vaccination rates, measles vaccination rates from the Mozambique Health Information System
Climate and weather (*1,2,4*)	Numerous satellite products, National Oceanic and Atmospheric; Administration, Climatic Research Unit, Center for Sustainability and the Global Environment, vulnerability to climate change	Climate data, social media reports of climate and air pollution effects on Twitter and Sina Weibo
Human demographics and behavior (*1,2,4*)	Night time lights, Gridded population of the world, mobile phone operator data	National census data products, Twitter, world population
Economic development (*1,2,4*)	International Monetary Fund, World Bank	National departments of economics
Land use and ecosystem changes (*1,2,4*)	Global agricultural lands, Center for International Earth Science Information Network, Global Forest Change 2000–2012, Global Forest Watch, global livestock distribution densities	National departments of agriculture, croplands in western Africa, Africa mining digital news reports, IMAZON Deforestation Alert System
Technology and industry (*1,2,4*)	Digital news, United Nations Global Pulse	NA
Human wildlife interaction (*2,4*)	Species distribution grids, digital news reports	State-level hunting data
Breakdown of public health measures (*1,2,4*)	Natural disaster hotspots	News of impending natural disasters (i.e., predicted hurricane landfall)
Poverty and social inequality (*1*)	Center for International Earth Science Information Network, Global Observatory	National census data
War and famine (*1,2,4*)	Famine early warning system, digital news and social media	Syria Tracker
Lack of political will (*1*)	Historical records, Transparency International, Cline Center for Democracy	NA
International travel and commerce (*1,2,4*)	Flight and shipping data	Regional distribution data of food products

*The table is purposely not exhaustive but provides a survey of types of available digital data that are associated with different drivers. NA, not applicable.
†See online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/21/8/14-1156-Techapp1.pdf) for available references.

to expand surveillance to monitor the full spectrum of ID emergence drivers (Figure 1) (*2*,*16*,*17*).

In the historical case of bromeliad malaria (see Drivers in Action and Hindsight section), actions were taken only after astute clinicians recognized the pattern of an ID event, malarial symptoms around cacao farms. Had a driver surveillance system been available, the elevated risk for an outbreak might have been indicated by reports of agricultural land use changes and human movement in conjunction with the underlying poverty and lack of disease prevention practices. Ideally, a warning system would then trigger an active surveillance program, a preemptive investigation to reduce risk factors, or even control measures (Figure 1).

An integrated driver surveillance system has the potential to be an integral tool for analysts and decision makers at 2 stages. First, whereas standard surveillance methods were designed to detect an outbreak, a driver-centric system will provide situational awareness of potentially unhealthy conditions before and following an outbreak. At the pre-outbreak stage, decision makers will have a tool that summarizes risk across data streams for multiple drivers of IDs. Second, at the postoutbreak stage, driver surveillance could result in more efficient resource allocation. Interventions could be tailored to local needs and capacity based on the knowledge of underlying drivers that will vary over space and time (e.g., infrastructure, vaccine coverage, and public health capacity) (*18*).

A warning and response system will require improved understanding of the relationships among specific drivers and ID emergence events. Advancements must also be made in terms of driver data collection. Well-developed datasets regarding climate, land cover change, and population density already exist (e.g., Center for International Earth Science Information Network, Climate Research Unit, and Google Earth Engine), but well-developed datasets are not readily available for drivers associated with human behavior (e.g., open-source curated data on immunization coverage, public health breakdown, sanitation and hygiene, and vector control). Well-developed, readily available datasets are those that provide subcountry level data or higher resolution at regular and frequent intervals with documented and standardized tools that are published and freely available online. Through curation and accessibility, these data and an expanded knowledge of drivers could greatly enhance mathematical models that describe ID transmission and epidemic occurrence. Herein, we survey available digital resources, present a conceptual framework for such a digital disease driver surveillance platform, and discuss opportunities for and obstacles to its successful implementation.

Drivers in Action and Hindsight

In the 1940s, a malaria epidemic began when economic pressures and poverty led the agriculture sector of Trinidad

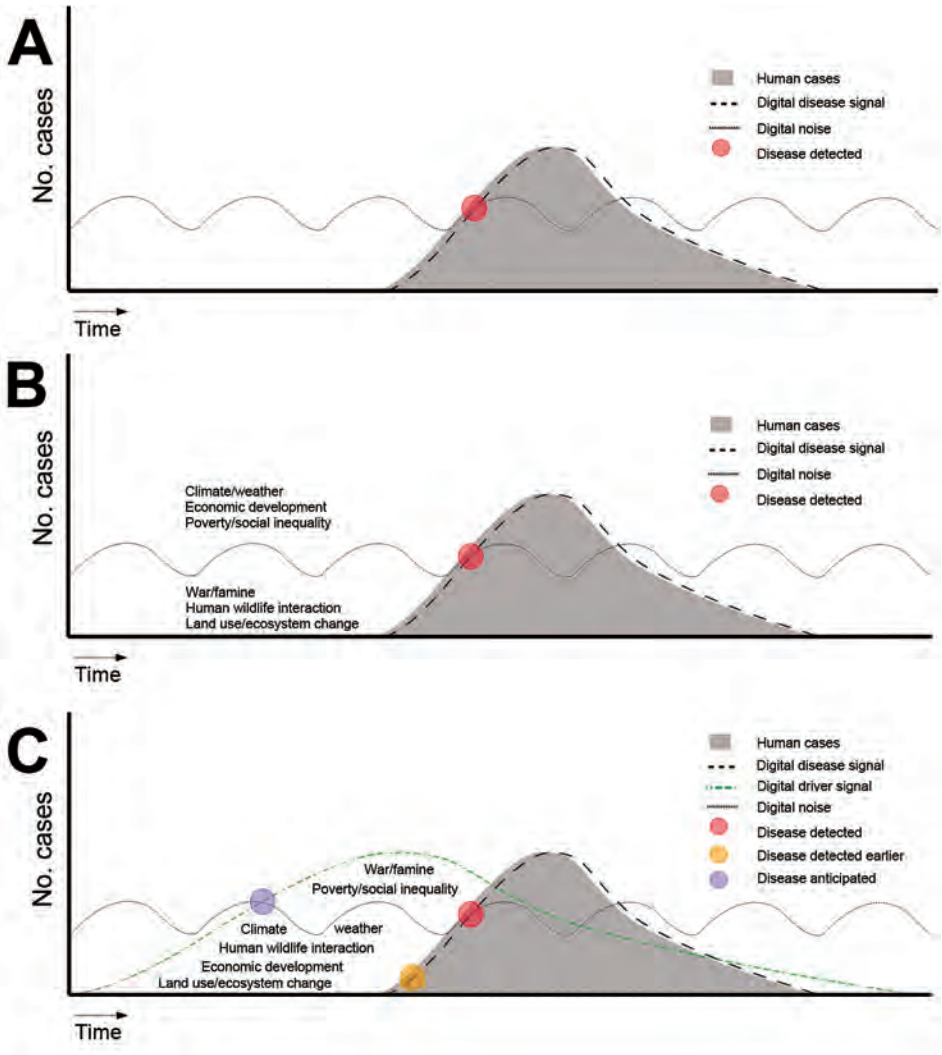

Figure 1. Surveillance and detection of disease by traditional (A, B) and digital (C) detection systems. A) Traditional disease detection, in which a close association exists between the number of cases and the digital disease signal. Disease is detected when the signal exceeds the noise. B) Disease emergence or outbreaks often occur following a driver. Examples of such drivers include climate and weather, economic development, poverty and social inequality, war and famine, human–wildlife interactions, land use and ecosystem changes. C) Detection of disease by using digital techniques. In this system, drivers of disease (not disease) are monitored, essentially to monitor for conditions suitable for disease emergence. Hypothetically, the careful surveillance of drivers that have been separated from digital noise could shorten the time to disease detection (as indicated by the orange dot).

into the cacao industry, an activity that requires a large human labor force and shade trees. These shade trees supported a bromeliad tank species, a family of plants that contain water-holding structures, creating an ideal breeding site for *Anopheles bellator* mosquitoes and new niches for different mosquitoes within the forest canopy (*19*,*20*). During the epidemic, medical doctors first noted an increase in the prevalence of splenomegaly, an indication of malaria, among schoolchildren correlated with areas cultivating cacao, and that *A. bellator* mosquitoes were only found near the cacao farms. Removal of the bromeliads reduced *A. bellator* mosquito populations and returned malaria rates to prior endemic levels (*20*). In hindsight, the outbreak resulted from the convergence of poverty, commerce, and agricultural and land-use changes; lack of malaria prevention and treatment services; and resulting shifts in local mosquito ecology. Building on our accumulating knowledge of drivers and the digital streams of data that now exist, the public health sector can be provided with early warning tools to recognize when conditions are ripe for disease emergence events, tools that could not have existed in the mid-20th century.

Identifying Existing Optimal Spatial Data System Components

Driver monitoring requires access to data from multiple domains at sufficient resolution and scale to correlate with known disease events and overcome potential biases. To build a driver surveillance system, issues with data availability and compatibility must be addressed in conjunction with temporal resolution and spatial scale.

Data availability categorized by drivers of interest is shown in Figure 2. The data were collected for the Health Scapes Project (http://healthscapes.io), an initiative launched in 2009 to assemble and collaboratively curate global open data of relevance to the global health research community. Of note, datasets were not readily found for certain drivers (i.e., human wildlife interaction and breakdown of public

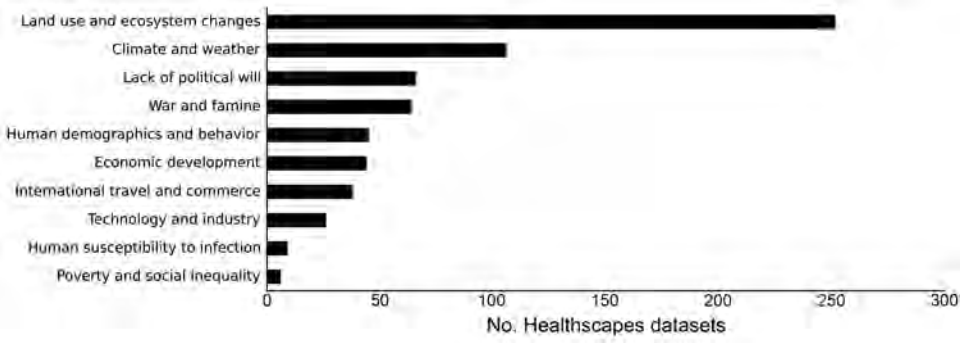

Figure 2. Number of datasets, by disease driver, available globally. The data were collected for the HealthScapes Project (http//:healthscapes.io).

health measures), and few datasets were found for other drivers (i.e., human susceptibility to infection and poverty and social inequality).

Availability

The global volume of data is doubling every 2 years (*21*). Although this trend presents an opportunity for analysts, much of the data are either unreduced big data that have become stored at major institutions or transient data that are not stored. Although potentially applicable to geographic, ecologic, and sociologic driver detection, only a fraction of these data have been processed for analysis on current research infrastructure, and even less is publicly accessible. Despite efforts to make data available, few providers have adopted provenance or metadata standards or leveraged mechanisms to facilitate collaboration, such as web services and application programming interfaces.

Compatibility

Technology trends, software development practices, and disciplinary preferences have resulted in a mosaic of data types and formats. Despite increasing interoperability, propriety obstacles persist, and innovations have isolated valuable legacy data in archaic formats.

Temporal Resolution

The distribution of data is generally skewed to the present because of increased digitization, the emergence of the Internet, and widespread instrumentation of systems. Research funding levels, political cycles, disruptive events, research trends, individual efforts, and the evolution of organizations all contribute to temporal variability in data resolution.

Spatial Scale

Few driver datasets of interest have complete global coverage. When considering data storage location as a proxy for spatial coverage, 50% is in North America, 30% in Europe, and 14% in the Asia–Pacific region; the Middle East, Latin America, India, and Africa account for the remaining 6% (*22*). This uneven distribution presents a challenge for monitoring disease in underdescribed regions. Furthermore, when attempting to grid, or rasterize, data at the global scale, researchers may codify incorrect assumptions through interpolation or by applying bias corrections for research effort. The distribution of data is evolving rapidly; if we consider submarine cable traffic rather than storage volume, intra-Asian connections have surpassed trans-Atlantic bandwidth (*23*). It is not yet determined how this shifting flow of information may influence driver coverage and effect digital media surveillance.

Leveraging Digital Media Reports

Digital media reports already provide a rich source of information on disease outbreaks (*24*) and, likewise, may be useful in monitoring disease drivers to identify periods of increased risk for outbreaks (e.g., news articles about breakdown of public health infrastructure, conflict, and vaccine programs). However, this driver surveillance resource presents a unique mix of opportunities and challenges. On the opportunity side, only a handful of languages account for most content on the Internet, resulting in a distilled set of global languages (*25*). If this pattern continues, only a subset of languages will need surveillance. Furthermore, the ability of natural language processing tools to parse reports for contextual data, such as location information, is improving. On the challenge side, digital media is fleeting and curated datasets are limited. For example, Google News, a popular digital media aggregator, does not provide a list of the sources used in its feed, and, thus, its content could change with time (*26*). Digital media can also miss nondigital disease-related news reports and details due to language or source (e.g., print and audio) limitations (*27*).

An objective evaluation of digital media for driver surveillance will ultimately require an unbiased dataset that links media reports of disease drivers to actual outbreaks that enable determination of the specificity and sensitivity of the system. Notably, it will be difficult to develop such a system because of the circular nature of these data (i.e., the system would be evaluated based on the system used to collect the data). In the absence of independently collected gold standard data, one cannot determine the degree to which unknown unknowns are missed. In summary, more

research is needed in evaluating digital media systems and the influence of these potential limitations.

New Data Technology Solutions for Drivers

Numerous projects have been established as hubs for collecting, cataloging, and sharing global data on a wide variety of potential drivers. Underlying these streams is an increasingly open-data ethic. Numerous repositories of existing data specialize in distributing accessible data formats (e.g., World Organisation for Animal Health's World Animal Health Information Database, Global Biodiversity Information Facility, Center for International Earth Science Information Network, Google Earth Engine, Gapminder World, European Union BioFresh project, and HealthScapes). Recent Digital Disease Detection conferences have highlighted numerous new data sources, including mobile phone and Internet technology that has enabled real-time syndromic and resource monitoring from social media (Twitter and Facebook), active surveillance (e.g., Flu Near You and Influenzanet), and participatory surveillance (Opendream, SMS for Life, and Saude Na Copa 2014). On the ground and at the source, data loggers and sensors are decreasing in cost and are supported by open-source platforms (e.g., Mataki, Arduino, and Onset HOBOs).

HealthMap is one example of emerging digital surveillance technology that uses a flexible architecture and an automated processing system to target ID events and the wildlife trade (*13*,*28*). This framework could be applied to identify any driver of interest as dictionaries for different languages and subject areas are used; the platform is fundamentally subject- and language-agnostic. This structure enables for inclusion of data not distributed digitally, resulting in the ability to expand overall media coverage. In the near future, developments in optical character and audio language recognition offer the potential for the rapid processing of nondigital media, such as photographs of print media and audio news sources.

Coinciding with the growing number of data sources has been an explosion of visualization, analytical, and hybrid tools, often fueled by the open-source community. Once data are collected, these tools will provide new ways for analysts to convey complex data and ideas, such as driver surveillance trends, to decision makers in a clear and logical format (e.g., Hans Rosling's Trendalyzer, online Technical Appendix Table 2, http://wwwnc.cdc.gov/EID/article/21/8/14-1156-Techapp1.pdf). Cloud-based platforms have evolved from desktop-based Esri software and leverage the open-source framework, social coding, and collaboration to provide researchers new opportunities to combine complex multisource big data with newly created virtual forums (e.g., Google Earth Engine and social modeling platforms). In addition to new analytic techniques, digital driver surveillance can build on advances in the way science is conducted. One example is the Open Science Framework, which has pioneered online project spaces that enable others to make changes or add on to the project without affecting the original (https://osf.io/). Another example is rOpenSci (https://ropensci.org), an effort funded by the Sloan Foundation to wrap scientific application programming interfaces in a manner that is readily accessible to analysts and researchers. This new approach sets the groundwork for collaborative, iterative, and adaptive management of ID driver surveillance models.

Application of Driver Surveillance for a Novel ID

The emergence of Nipah virus (NiV) on the global scene can be linked to several drivers, all of which could have been monitored and potentially recognized, resulting in quicker containment of outbreaks. The first human NiV cases were reported in 1998 in Malaysia. This initial spillover event has been linked to agricultural intensification where mango trees were planted in close proximity to pigsties (*16*,*29*). This change in practice led to increased contact between pigs and the natural reservoir for NiV, the flying fox, and subsequent introduction of NiV into the pig population (*29*). The continued contact between the bats and pigs resulted in NiV establishment within the pig population and increasing exposure of pig workers to NiV (*29*,*30*).

Perhaps NiV emergence could have been anticipated if a system existed that raised warnings about the coincident land-use change and agricultural intensification. Scientists were eventually able to match the unknown disease affecting humans with NiV and identified risk factors and modes of transmission, but the key connections might have been recognized sooner. Although an isolated change in agricultural practices would not typically warrant an intervention, the emergence of a new porcine respiratory disease in conjunction with the change could have led to closer monitoring. When the initial spillover into the human population occurred, the knowledge of a new porcine disease, changes in driver activity, and the identification of a new virus in bats all occurring within the same region could have individually triggered heightening alerts.

After emergence, driver surveillance would have provided valuable situational insight. In the early stages of the outbreak, there was a mass sell-off of pigs in response to the cluster of cases and the perceived link among farmers between the sick pigs and the cluster (*29*,*31*,*32*). This sell-off resulted in the spread of NiV to southern regions of Malaysia, where there is a dense pig population (*29*). As the outbreak spread southward, the international pig trade moved the virus to Singapore (*32*). Market monitoring could have potentially identified the increase in pig sales, as well as the regional sources and endpoints of traded pigs. If supply chains and the locations that sit at high-risk critical nodes could be identified, increased active surveillance

or even preemptive interventions, such as animal culling, could have been deployed.

Field Deployment as a Two-Way Communication Interface

A successful epidemic surveillance platform should leverage the latest web technologies to support global communication among health workers with near–real-time information on the drivers of disease emergence. It is important that the platform have the capability to capture ground-truthed data from field teams to effectively crowd-source data curation to amplify efforts in the global response to disease emergence. Such open data curation and provisioning services would further help democratize data access to those often most affected by IDs. Core features of the system would include the ability to identify relevant drivers for a disease or region of interest as well as the capacity to monitor spatiotemporal trends for these drivers. A user could use this information to make decisions about allocating funding for health control measures or prioritizing health effect assessments of development initiatives. In the field, a mobile interface to the platform could be used to identify future field sampling sites, intervention options, or changes in drivers. It is essential the platform have the capacity to monitor and overlay multiple drivers, in light of known interactions. The utility of the system would be enhanced by being able to set notifications on drivers of interest that could initiate a response, follow up, or further investigation. A truly dynamic web interface would enable the user to simulate different health outcomes under different scenarios, such as economic policies, control strategies, or climate futures. In anticipation of future ID outbreaks, a well-designed system could be used strategically by decision makers to allocate or predeploy limited human and material resources to risk hotspots. Globally, the system could be used to help orchestrate multinational responses to emerging ID threats, identify priorities, and raise awareness of the role of antecedent drivers in global health.

Moving toward a Prototype Platform

For a first-generation analysis platform to prove useful, it must provide insight regarding specific drivers and the drivers' subsequent effect upon current risk. We envision a country-based analysis framework that could comprise 3 components. The first component will measure a nation's risk for an emerging ID event by establishing driver baselines through the use of historical data or published ratings. The hotspots approach used by Jones et al. (*4*) illustrates the potential of this component, which could be extended from using historical events to using historical driver data to identify geographic locations at risk for ID emergence. Together these baselines would provide a Bayesian prior distribution of risk. The second component will introduce existing and new digital alerts as new evidence for a model. The addition of these alerts would build a risk map according to baseline levels; that is, a driver with a low baseline will not contribute much to changing the overall risk with the addition of a few alerts, but for a driver with a high baseline, the addition of a few driver alerts could push it into a higher risk category. By overlaying the digital disease alerts upon the baseline prior distribution of risk, we can then analyze these data for trends and biases to anticipate future emerging ID events. The third component will involve continually updating beliefs about the distribution of risk that is determined on the basis of new evidence.

Despite our positive outlook on digital driver surveillance, we note several caveats, such as the complexity of causal human–environment interactions (*33*) and the use of models to confront imperfect knowledge. For instance, false-positive signals, when a driver system identifies a strong signal but no disease occurs, are inevitable. Without appropriate user sensitization, this result could lead to misallocation of resources and erosion of trust. Conversely, some disease events might lack any warning signals, as may have been the case with the 2009 pandemic of influenza A(H1N1)pdm09 virus infections. Despite the existence of these problems, we believe they will be alleviated through a better understanding of the disease–driver and driver–driver associations. However, some problems will be unavoidable. For example, a potentially strong limitation would be that infrequent updating of driver data could result in either missing a brief signal or not identifying the signal until it is too late to implement control measures. Ultimately, we must recognize that digital surveillance is one tool among many that can help supplement, but not replace, traditional public health surveillance.

Conclusions

A digital driver surveillance platform that improves situational awareness by active monitoring of ID events and associated drivers is an obtainable goal for the public health community. We believe that future surveillance platforms will be able to not only describe driver activity in space and time but also indicate driver thresholds, severity, and likely interactions among drivers. Digital media reporting offers tremendous potential to contribute to existing datasets because of its automated ability to scrape news sources for alerts and provide real-time driver data. Channels of communication need to be opened between the data producers, who may not see the wider utility of what is collected, and data consumers, who may not understand barriers with data collection. In addition, funding agencies that support driver-associated data collection efforts need to require that recipients follow best data practices. We recognize the existence of obstacles to developing a digital driver surveillance platform, but as data become more available, compatible,

and refined, our ability to overcome systematic biases and sources of error to identify driver activity will become easier. This report is a call to action to improve collection of driver and ID event data to rapidly develop the science and our understanding of relationships between drivers and emerging ID events, and move toward driver-based ID surveillance systems.

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Escherichia coli O157 Outbreaks in the United States, 2003–2012

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Learning Objectives

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

- Identify the most common source of outbreaks of *Escherichia coli* O157 in the United States
- Identify the most common specific food source associated with foodborne outbreaks of *E. coli* O157 in the United States
- Evaluate epidemiologic variables associated with the severity of *E. coli* O157 outbreaks
- Assess other epidemiologic data from outbreaks of *E. coli* O157 in the United States

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Infections with the Shiga toxin–producing bacterium *Escherichia coli* O157 can cause severe illness and death. We summarized reported outbreaks of *E. coli* O157 infections in the United States during 2003–2012, including demographic characteristics of patients and epidemiologic findings by transmission mode and food category. We identified 390 outbreaks, which included 4,928 illnesses, 1,272 hospitalizations, and 33 deaths. Transmission was through food (255 outbreaks, 65%), person-to-person contact (39, 10%), indirect or direct contact with animals (39, 10%), and water (15, 4%); 42 (11%) had a different or unknown mode of transmission. Beef and leafy vegetables, combined, were the source of >25% of all reported *E. coli* outbreaks and of >40% of related illnesses. Outbreaks attributed to foods generally consumed raw caused higher hospitalization rates than those attributed to foods generally consumed cooked (35% vs. 28%). Most (87%) waterborne *E. coli* outbreaks occurred in states bordering the Mississippi River.

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Signs and symptoms of infection with Shiga toxin–producing *Escherichia coli* O157 can include diarrhea that is often bloody, severe stomach cramps, and vomiting; infection can progress to hemolytic uremic syndrome (HUS) and death (*1*). In the United States, these infections and related illnesses are estimated to cost >$405 million annually (*2*).

E. coli O157 can be transmitted to humans through contaminated food and water, directly between persons, and through contact with animals or their environment. The most common reservoir is cattle, and ground beef is the most frequently identified vehicle of transmission to humans. *E. coli* O157 was first recognized as a foodborne pathogen after outbreaks during 1982 were linked to ground beef consumption (*1*). Since then, many other sources have been identified (*3*), mostly through outbreak investigations. We describe the epidemiology of *E. coli* O157 outbreaks during 2003–2012.

Methods

Surveillance

We identified *E. coli* O157 outbreaks in the United States using these sources: the Foodborne Disease Outbreak Surveillance System (FDOSS) for outbreaks during 2003–2012; the Waterborne Disease Outbreak Surveillance System (WBDOSS) for outbreaks during 2003–2010 (data were not available after 2010); the National Outbreak Reporting System (NORS) for outbreaks facilitated by transmission between persons, animal contact, environmental contamination, or with an unknown transmission mode during 2009–2012 (*4*); reports to the Centers for Disease Control and Prevention of person-to-person and animal contact–associated outbreaks during 2003–2008; and PubMed using these search terms: *Escherichia coli* O157 outbreak, STEC O157 outbreak, *Escherichia coli* O157, STEC O157, and O157. Data reported for each outbreak, with varying degrees of completeness, included primary transmission mode; states where exposure occurred; number of illnesses (culture-confirmed and epidemiologically linked); hospitalizations; HUS cases (defined by physician diagnosis; no laboratory data were collected); deaths, age, and sex distribution of patients; month the outbreak began; and setting where the outbreak occurred or, for foodborne disease outbreaks, the place of food preparation. For foodborne disease outbreaks, the implicated food (if identified) was reported. For waterborne disease outbreaks, water source type (e.g., recreational, drinking, waste water) was reported.

Definitions

An *E. coli* O157 outbreak was defined as an event during which ≥2 persons had culture-confirmed *E. coli* O157:H7 or O157:NM infection resulting from a common exposure. Epidemiologically linked, clinically compatible cases without laboratory confirmation were included in the case count. States within the contiguous United States were categorized as "north" if they were located entirely north of parallel 37 north latitude and "south" if entirely below this latitude; states crossing this line (California and Nevada) were excluded from some analyses.

Implicated foods were classified into categories by using an existing classification scheme (*5*) with 2 modifications: fruits and nuts were classified separately and meat acquired by hunting was classified with other meat. Implicated foods were further classified by whether they were usually consumed cooked (beef, other meats, poultry) or raw (leafy vegetables, fruits, unpasteurized dairy, sprouts). Steak was considered tenderized if described as tenderized or injected. Settings were classified as home; restaurant (e.g., restaurant, delicatessen, or banquet facility); institution (e.g., childcare center, school, or prison); fair; petting zoo; recreational area (e.g., park or beach); and other (e.g., grocery store, commercial production facility, or farm animal show).

Analysis

We summarized demographic and epidemiologic findings by transmission mode and food categories for which ≥3 outbreaks were reported. Two periods, 2003–2007 and 2008–2012, were evaluated to assess changes over time. Severity was assessed by calculating hospitalization (no. hospitalized/total no. ill) and HUS rates (no. physician–diagnosed HUS cases/total no. ill). Culture-confirmation rates (no. culture-confirmed/total no. ill) were also calculated. Analyses of settings were limited to outbreaks that occurred in a single setting type. For transmission modes other than food, data for age group, sex, and culture confirmation were not available before 2009. We compared the median outbreak size and state outbreak rates (outbreaks with exposure occurring in a single state/sum of state populations during 2003–2012, from U.S. Census Bureau intercensal estimates) using the Kruskal-Wallis test, and performed categorical analyses using χ^2 and Fisher exact tests; 2-tailed p values <0.05 were considered significant.

Results

During 2003–2012, a total of 390 *E. coli* O157 outbreaks (353 O157:H7, 15 O157:NM, 1 O157:H7 and O157:NM, and 22 O157 with an unknown H antigen) were reported. These outbreaks resulted in 4,928 illnesses, 1,272 (26% of illnesses) hospitalizations, 299 (6%) physician-diagnosed HUS cases, and 33 (0.7%) deaths (Table 1). The median outbreak size was 6 illnesses (range 2–238). Primary transmission modes were foodborne (255 outbreaks, 65%), animal contact (39, 10%), person-to-person (39, 10%), waterborne

Table 1. Characteristics of *Escherichia coli* O157 outbreaks by transmission mode and food category, United States, 2003–2012*

Transmission source	Outbreaks (% of all outbreaks)	Illnesses (median outbreak size)	Hospitalizations (% of all illnesses)	Physician-diagnosed HUS (% of all illnesses)	Deaths (% of all illnesses)
Food	255 (65)	3,667 (6)	1,035 (29)	209 (6)	25 (0.7)
Beef	78 (20)	1,144 (7)	316 (28)	67 (6)	5 (0.4)
Poultry†	1 (0)	60	5 (8)	0	0
Other meat	7 (2)	39 (4)	12 (31)	4 (10)	0
Dairy	16 (4)	140 (6)	52 (37)	22 (16)	0
Leafy vegetables	29 (7)	922 (16)	321 (35)	53 (6)	7 (0.8)
Fruits	6 (2)	57 (8)	20 (35)	5 (9)	6 (10.5)
Sprouts	3 (1)	35 (13)	4 (11)	0	0
Nuts	1 (0)	8	3 (38)	0	0
Other foods‡	29 (7)	580 (11)	123 (21)	24 (4)	0
Food unknown	85 (22)	682 (5)	179 (26)	32 (5)	10 (1.5)
Animal contact	39 (10)	552 (6)	127 (23)	51 (9)	2 (0.4)
Person-to-person	39 (10)	322 (5)	45 (14)	24 (7)	2 (0.6)
Water	15 (4)	154 (6)	NA	NA	1 (0.6)
Other or unknown	42 (10)	233 (4)	65 (28)	15 (6)	3 (1.3)
Total	390 (100)	4,928 (6)	1,272 (27§)	299 (6§)	33 (0.7)

*HUS, hemolytic uremic syndrome; NA, not available.
†Poultry was thought to be cross-contaminated by ground beef (http://www.ct.gov/dph/lib/dph/infectious_diseases/ctepinews/vol29no5.pdf).
‡Outbreaks transmitted from other foods were 2 each of: guacamole, pico de gallo, salsa, and potato salad; and 1 each of: cookie dough; alfalfa sprouts and iceberg lettuce; baked beans and unknown fruit; meatballs, steak and green salad; cantaloupe and hamburger; lamb and beef; lettuce and green grapes; lime and bean dip; macaroni; Mexican wheat snack; sandwich; seafood; vegetable-based salad; pepperoni; jerky; multiple foods. Agencies sometimes report >1 food vehicle when epidemiologic evidence cannot distinguish between them even when it is likely that only one was the source.
§Excludes 154 illnesses from the denominator that were associated with waterborne disease outbreaks because no data was collected for hospitalization and physician-diagnosed HUS cases for this transmission mode.

(15, 4%), and other or unknown (42, 11%). Foodborne disease outbreaks caused the most illnesses (3,667, 74%), hospitalizations (1,035, 81%), physician-diagnosed HUS cases (209, 70%), and deaths (25, 70%). During 2009–2012, when data for culture-confirmed infections were collected for all outbreaks, the proportions of transmission were unknown modes (76%), foodborne (69%), person-to-person (62%), and animal contact (40%).

The median annual number of outbreaks reported during 2008–2012 was higher than during 2003–2007 (45 vs. 33, p = 0.12) (Figure 1). The median annual number of foodborne disease outbreaks did not change, although the number attributed to dairy (11 vs. 5) and fruits (5 vs. 2) more than doubled. The median annual number of outbreaks attributed to other modes of transmission did not change.

Vehicles

Of the 255 foodborne disease outbreak reports, 170 (67%) implicated a specific food, of which 141 (83%) could be classified into a single category. The implicated categories were beef (78 outbreaks, 55%), leafy vegetables (29, 21%), dairy (16, 11%), fruits (6, 4%), other meats (7, 5%), sprouts (3, 2%), nuts (1, 1%), and poultry (1, 1%) (Table 1). The types of beef implicated were ground beef (54 outbreaks, 69% of beef-associated outbreaks) and steak (10, 14%). The types of steak, reported in 5 outbreaks, were sirloin (4 outbreaks) and filet mignon (1). Steaks were mechanically tenderized in 5 outbreaks (2 sirloin, 1 filet mignon, 2 unknown). Other implicated meats were venison (3 outbreaks), lamb (2), and bison/buffalo (2).

In all 16 outbreaks attributed to dairy, unpasteurized products were implicated; 13 (81%) to unpasteurized milk

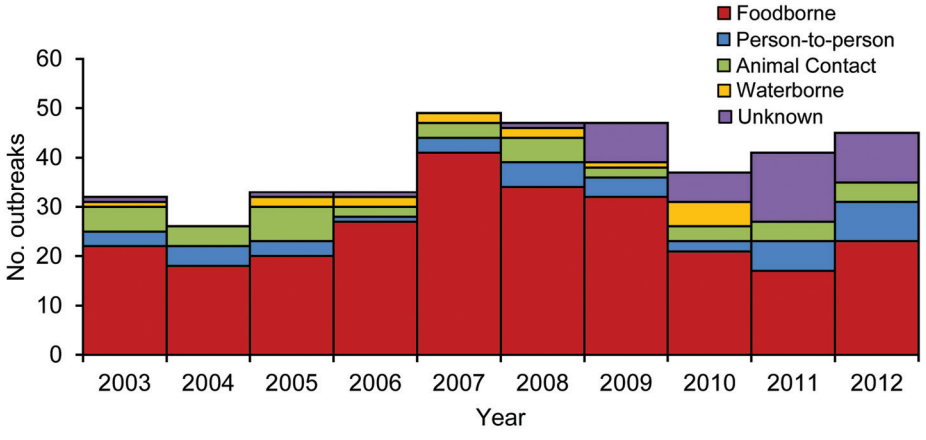

Figure 1. Number of *Escherichia coli* O157 outbreaks by transmission mode and year (n = 390), United States, 2003–2012.

and 3 (9%) to cheese made from unpasteurized milk. Most outbreaks attributed to leafy vegetables implicated lettuce (22 outbreaks, 76%), including romaine (3), iceberg (1), and mesclun mix (1); others in this category were spinach (4, 13%) and salads with unspecified types of greens (3,10%). Outbreaks attributed to fruit implicated unpasteurized apple cider (4 outbreaks), fruit salad (1), and strawberries (1), a newly identified food vehicle for this pathogen. Other newly identified food vehicles were hazelnuts sold in the shell (1 outbreak), packaged cookie dough (1), salsa (2), and pico de gallo (2). A total of 39 outbreaks were transmitted by contact with animals; ≥1 animal type was reported for 24 (62%), including cattle (15), goats (12), sheep (8), pigs (3), rabbits (3), chickens (2), and 1 each of elk, llama, alpaca, hedgehog, and dog. Every outbreak that identified a reported animal type included ≥1 ruminant animal. Although no specific animal types were listed for the remaining 14 outbreaks, livestock were reported for 2 and petting zoos or county fairs for 11.

Of the 15 waterborne disease outbreaks, 10 were attributed to recreational water (3 treated, 7 untreated), 3 to drinking water, and 1 possibly to wastewater. The water source was unknown for the remaining outbreak.

Severity

The severity of outbreaks differed by transmission mode and among foodborne disease outbreaks by food category (Table 1). Hospitalization rates were lower for person-to-person outbreaks compared with all other transmission modes (14% vs. 28%, p<0.0001). Physician-diagnosed HUS rates were higher for animal contact outbreaks than for all other modes (9% vs. 6%, p = 0.0005). The 33 deaths occurred in foodborne (25 deaths), person-to-person (2), animal contact (2), and waterborne (1) disease outbreaks; transmission mode was unknown for 3 deaths.

Among foodborne disease outbreaks, hospitalization rates were lower for outbreaks attributed to beef than for all other food vehicles (28% vs. 35%, p<0.001). Hospitalization rates were higher among outbreaks attributed to foods generally consumed raw than for cooked foods (35% vs. 28%, p = 0.0001). Deaths occurred most commonly in outbreaks attributed to leafy vegetables (7 deaths, 0.8% of illnesses in leafy vegetable outbreaks), fruits (6, 11%), and beef (5, 0.4%).

Demographic Characteristics

Age group distribution of cases varied by transmission mode and food category (Figure 2). Patient age group was available for 2,884 (81%) illnesses in foodborne outbreaks, 63 (90%) illnesses in waterborne outbreaks, and, during 2009–2012, 121 (96%) illnesses in person-to-person outbreaks and 189 (73%) illnesses in animal contact outbreaks. Most of the illnesses in person-to-person outbreaks (73, 60%) were among children <5 years old. The highest proportions of illnesses in waterborne and animal contact outbreaks (67% and 43%, respectively) were among persons 5–19 years of age. Foodborne disease occurred among persons in all age groups; only 8% of foodborne illnesses occurred among children <5 years of age, but >25% of dairy-associated illnesses were among children of this age group. The largest proportions of illnesses in outbreaks attributed to beef and dairy were among persons 5–19 years old (38% and 47%, respectively), whereas the largest proportion reported in outbreaks attributed to sprouts and leafy vegetables was among persons 20–49 years old (71% and 45%, respectively).

Overall, 55% of patients were women; the percentage did not vary significantly by transmission mode. Patient sex was available for 3,385 (92%) illnesses in foodborne disease outbreaks, 63 (90%) illnesses in waterborne outbreaks, and, from 2009–2012, 126 (97%) illnesses in person-to-person outbreaks and 193 (80%) illnesses in animal contact outbreaks. In foodborne disease outbreaks, the proportion of female patients was highest in outbreaks attributed to fruits (67%) and leafy vegetables (65%) and lowest in outbreaks attributed to meats other than beef (31%) (p<0.0001); cases were evenly distributed among women and men in outbreaks attributed to beef and dairy.

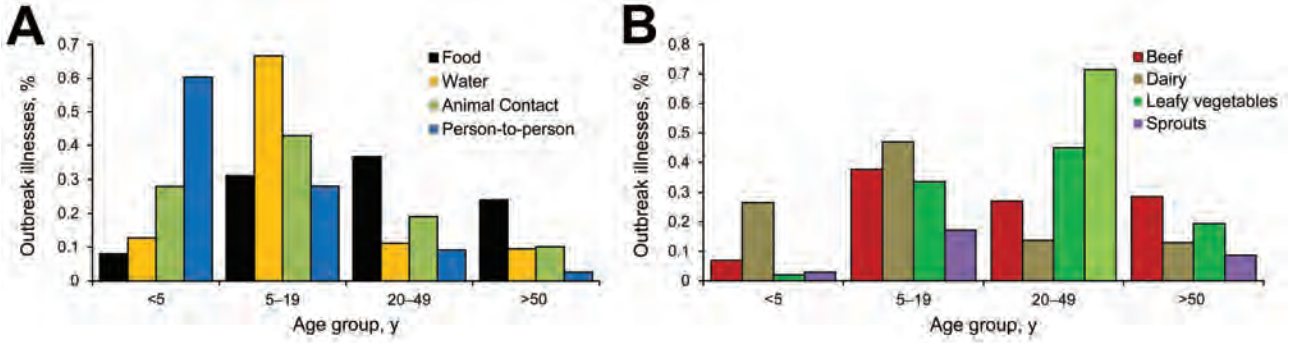

Figure 2. Percentage of *Escherichia coli* O157 outbreak illnesses by age group and A) transmission mode (n = 3,417) and B) selected food categories (n = 1,574), United States, 2003–2012.

Seasonality

E. coli O157 outbreaks occurred year-round; however, nearly half occurred during July–September (175, 46%) (Figure 3). The seasonality of foodborne outbreaks varied by food category. Beef-associated outbreaks occurred most often in July (21 outbreaks, 27%), and leafy vegetable-associated outbreaks in September (8, 28%). Waterborne disease outbreaks occurred most often during June (6, 40%) and outbreaks associated with animal contact during August (13, 37%).

Geographic Distribution of Outbreaks and Multistate Outbreaks

Outbreaks were reported by 43 states (Figure 4). The median outbreak rate in northern states was >2 times the rate in southern states (0.015 vs. 0.006/100,000 population, p = 0.005), which was also true for foodborne disease outbreaks (0.007 vs. 0.002, p = 0.02). Most waterborne disease outbreaks were reported in states bordering the Mississippi River (13, 87%), including all 3 drinking water outbreaks and all 7 recreational water outbreaks associated with lakes or other natural, untreated bodies of water. Waterborne disease outbreaks reported by California (1) and Idaho (1) were associated with treated recreational water.

Of the 390 outbreaks reported, 44 (17%), all foodborne, were multistate. Multistate outbreaks were larger than single state outbreaks (median 22 cases vs. 5, p<0.0001). The food categories implicated in multistate outbreaks were beef (22 outbreaks, 17 attributed to ground beef), leafy vegetables (12), unpasteurized dairy products (3), sprouts (1), nuts (1), and other meats (bison, 1).

Settings

Among 222 foodborne outbreaks for which food preparation settings were reported, a single setting was reported for 183 (84%). Restaurants were the most common settings (73 outbreaks, 40%), followed by private homes (60, 33%) (Table 2). Beef was most frequently prepared in a home (34, 56%), followed by restaurants (15, 25%). Leafy vegetables were most often prepared in restaurants (13, 59%), followed by homes (4, 18%).

Most (17, 89%) outbreaks with person-to-person transmission and a reported setting occurred in institutions; all except 1 institutional outbreak occurred in a childcare center. Petting zoos and fairs were the most common setting for outbreaks attributed to animal contact (17, 71%). Waterborne outbreaks occurred most often in recreational settings (7, 47%), including lakes (4), a pool (1), a sports complex (1), and an interactive fountain (1).

Discussion

As in previous reports, our analysis found that food was the major mode of transmission for *E. coli* O157 outbreaks (*3*). Although beef, especially ground beef, remained the most common source (*3*), 14% of beef outbreaks were attributed to steak. Outbreaks attributed to foods generally consumed raw had higher hospitalization rates than other outbreaks, and leafy vegetables were the major source of outbreaks in the fall. Foodborne disease outbreak rates were highest in northern states, and most waterborne disease outbreaks occurred in states bordering the Mississippi River. New food vehicles were identified, including raw, prepackaged cookie dough (*6*), hazelnuts (*7*), strawberries (*8*), and salsa (*9*).

More outbreaks were reported during 2003–2012 than during the previous 20 years (*3*), although outbreaks were smaller (median 6 illnesses vs. 8). The hospitalization rate was higher than during the previous 20 years (27% vs. 17%), and the HUS rate was slightly higher (6% vs. 4%). Contaminated food was responsible for a larger percent of outbreaks during our study (65% vs. 52%), and the percent attributed to another or unknown mode decreased (10% vs. 21%). Among foodborne disease outbreaks, the percentage caused by *E. coli* O157 in beef decreased (31% vs. 47%), and among outbreaks associated with beef, the percentage linked to ground beef decreased (21% vs. 41%), whereas the percentage associated with other types of beef increased (9% vs. 6%). These differences might be partly attributable to continued improvements in surveillance, including the maturation of the national molecular subtyping network, PulseNet, which supported earlier detection of more outbreaks (*10*), as well as improved outbreak investigations

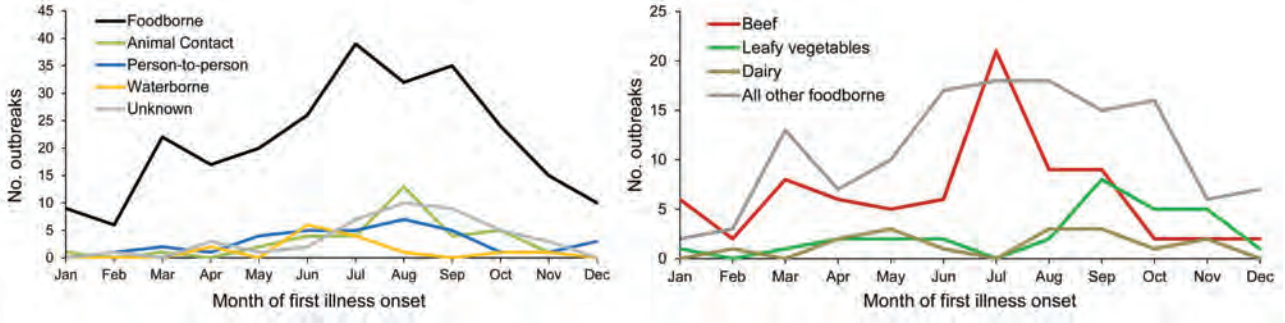

Figure 3. Number of *Escherichia coli* O157 outbreaks by month and by A) transmission mode (n = 390) and B) selected food categories (n = 255), United States, 2003–2012.

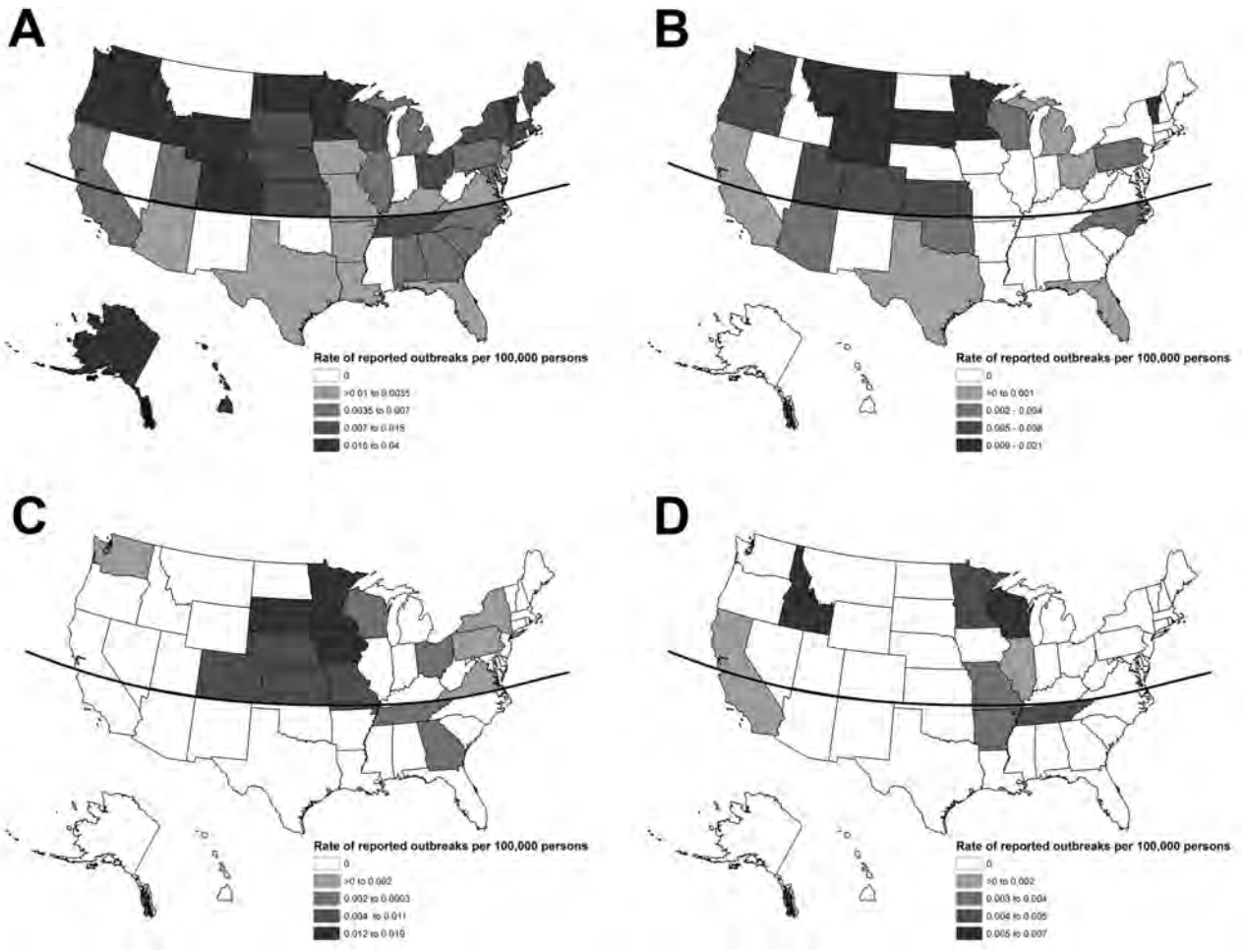

Figure 4. Single-state Escherichia coli O157 outbreaks (n = 346) by state and transmission mode, United States, 2003–2012. A) Foodborne transmission (n = 211); B) animal contact transmission (n = 39); C) person-to-person transmission (n = 39); D) waterborne transmission (n = 15). Curved line denotes 37°N latitude.

and systematic, electronic reporting of outbreaks of all transmission modes (*4*).

Beef, particularly ground beef, continues to be the major source of *E. coli* O157 outbreaks, likely because cattle are the main reservoir for *E. coli* O157. Contamination of raw beef usually occurs during slaughter and meat processing by contact with hides contaminated with feces (*11*,*12*). Grinding can spread contamination through vast amounts of ground beef (*11*). Mechanically tenderizing steaks internalizes surface contamination (*13*,*14*), so steaks not cooked thoroughly could harbor *E. coli* O157. The risk for contamination varies for different locations on the carcass (*15*). Cattle carcasses sampled for microbial contamination had higher bacteria counts on sites on the rear (*16*,*17*).

Our finding that outbreaks attributed to leafy vegetables, dairy products, fruits, and other meats were more severe than outbreaks attributed to beef could have several explanations, including strain virulence and patient age and sex. Outbreak reports did not provide information on strain virulence factors. Outbreaks attributed to foods generally consumed raw had higher hospitalization rates than those attributed to cooked foods. Outbreaks with high proportions of illnesses among young children, especially those attributed to animal contact, dairy products, other meats, and fruits, also had the highest rates of physician-diagnosed HUS, which might relate to the higher risk for HUS among children (*18*). However, this finding was not true for person-to-person outbreaks, in which the proportion of illnesses among young children was highest. The reasons for this finding are unknown, but intensive case finding efforts during suspected person-to-person outbreak investigations might identify more mild illnesses and asymptomatic patients, decreasing the overall hospitalization rate.

Higher hospitalization rates in outbreaks attributed to certain foods might be influenced by patient sex. For example, hospitalization rates were 35% for outbreaks associated with leafy vegetables, and 66% of illnesses were in female patients. Studies have found that women were at

Table 2. *Escherichia coli* O157 outbreaks by setting (n = 357), United States, 2003–2012

Transmission source	Setting/no. outbreaks							
	Restaurant/ banquet facility	Home	Institutional*	Fair/petting zoo	Recreational area/facility†	Other‡	Unknown	Total
Food	73	60	16	2	0	32	39	222
Beef	15	34	3	0	0	9	8	69
Poultry	0	0	1	0	0	0	0	1
Other meat	1	5	0	0	0	0	0	6
Dairy	0	2	0	0	0	11	0	13
Leafy vegetables§	13	4	3	0	0	2	4	26
Fruits	0	2	0	1	0	2	0	5
Sprouts	1	0	0	0	0	0	0	1
Nuts	0	1	0	0	0	0	0	1
Other foods	13	6	0	0	0	2	3	24
Food unknown	30	6	9	1	0	6	24	76
Animal contact	0	1	2	17	0	4	15	39
Person-to-person	0	2	17	0	0	0	20	39
Water	0	2	2	0	7	4	0	15
Other or unknown	0	0	0	0	0	0	42	42
All outbreaks	73	65	37	19	7	38	116	357

*Institutional setting comprises camp, daycare, hospital, nursing home, prison, school, church, and workplace cafeteria.
†Recreational area/facility comprises county and state park, beach, and a sports complex.
‡Other settings comprise a grocery store, commercial products, a 4H steer competition, communities, a farm, a National Western Stock Show, a public outdoor area, and a club.
§Setting was not available for 3 outbreaks involving leafy vegetables.

increased risk for HUS after *E. coli* O157 infection (*18*–*20*), although others have not found an association (*21*,*22*). Additionally, the proportion of illnesses in female patients might relate to gender-specific food preferences.

Most outbreaks occurred during the summer. Cattle shed the largest number of *E. coli* O157 organisms in their feces during summer months (*23*,*24*), coinciding with a higher prevalence of *E. coli* O157 on hides in processing plants (*25*). However, leafy vegetable-associated outbreaks exhibited distinct fall seasonality, which could be the result of summertime application to seedlings of irrigation water, soil amendments, or fertilizers that might contain more *E. coli* O157 organisms than other seasons. Therefore, leafy vegetables harvested during fall might be more likely to become contaminated than those grown at other times. Seasonality of leafy vegetable–associated outbreaks could also relate to harvest location. Nearly three quarters of leafy vegetable-associated outbreaks occurred during April–October; during this same time each year, most US-produced lettuce is harvested from the Salinas Valley (California) (*26*). For 4 of 5 recent outbreaks (2011–2012), detailed traceback information implicated lettuce harvested from farms in Salinas Valley (K.E. Heiman, unpub. data).

The cause of higher infection rates in northern states than southern states is unknown (*27*). Cattle density might play a role. In other countries, areas with the highest cattle density had some of the highest rates of human *E. coli* O157 infection (*28*). Cattle density does not appear to be higher in northern states compared with southern states (*25*), although it is very high in counties in California where most lettuce is produced (*29*). Other environmental factors might have a role. Edrington et al. found a stronger correlation between prevalence of *E. coli* O157 in cattle feces and longer day length than with higher ambient temperature, although both were statistically significant (*30*). However, several studies of prevalence of *E. coli* O157 in cattle feedlots have found either no variation by geography (*31*) or higher prevalence in herds in the south (*32*), where summertime daylight hours are shorter but ambient temperatures are higher. Geographic distribution might also be influenced by presence of cattle called "super shedders" that shed higher numbers of *E. coli* O157 bacteria (*33*). Differences in reporting by state could also affect geographic patterns (*4*).

Several factors might have contributed to our finding that waterborne outbreaks were reported primarily among states bordering the Mississippi River. Cattle density is high in the Midwest (*25*). *E. coli* O157-containing feces from cattle and other animals can be washed into nearby bodies of water, especially during heavy rainfall (*34*). Karst formations, which are rock formations that occur in several Midwest states, develop when acidic water begins to break down bedrock surfaces, allowing surface water to enter fractures in limestone and contaminate ground water; studies have shown that *E. coli* can survive in karst streams for prolonged periods (*35*). Karst formations exist in the counties that reported 2 of the 3 *E. coli* O157 outbreaks in which drinking water was the source; all were associated with well water. The remaining well water–associated outbreak occurred on a farm after heavy rain and flooding. However, karst formations are unlikely to explain outbreaks associated with untreated recreational water. Only 15 waterborne outbreaks were reported; therefore, interpretation of these findings is limited.

Our study had several limitations. Reported outbreaks likely underrepresent the number that occur (*36*).

Many go unrecognized or unreported because they are not detected, no common source is found, or resources are not available for investigation (*37*). Sources of outbreak-related illnesses may differ from those of sporadic illnesses, although our findings were similar to those of another US study that examined risk factors for sporadic *E. coli* O157 infections (*38*). We analyzed outbreaks by their primary mode of transmission; however, outbreak-related infections could be transmitted by a variety of modes (e.g., several illnesses were transmitted from person-to-person in an outbreak linked to spinach). State and local health departments investigated and reported outbreaks; we did not verify transmission mode. Our findings might be influenced by changes in surveillance: outbreaks transmitted person-to-person, by animal contact, and by an unknown mode were not systematically reported until 2009 (*4*). We used physician-diagnosed HUS, which might overestimate the rate of HUS (*39*); however, the rate of physician-diagnosed HUS for all outbreak-related illnesses was 6%, the same as was reported in a study that used defined laboratory criteria (*2*). We found one third of foodborne disease outbreak reports did not have an identified implicated food item; therefore, the foods in this analysis might not be representative of all foodborne *E. coli* O157 outbreaks.

Conclusions

More *E. coli* O157 outbreaks were reported during 2003–2012 than during the previous 20 years (*3*), probably because of continued improvements in surveillance. Outbreaks in the United States continue to be caused mostly by contaminated food, especially beef. People can protect themselves through proper handling of raw beef, by cooking nonintact beef products such as ground beef and mechanically tenderized steak to internal temperatures of at least 160°F, and by thorough handwashing after contact with animals or their environment. Clearly labeling mechanically tenderized beef might help consumers make safer choices. Pasteurizing dairy products and fruit juices is recommended. Further research is needed to identify interventions to reduce the risk of illness caused by consumption of raw produce. Ways must be found to encourage implementation of interventions such as decreasing contamination in the production of produce, vaccinating cattle, and irradiating foods (*40*). Consideration of the proximity of agriculture fields to cattle operations, as well as adherence to Good Agricultural Practices specified by the US Department of Agriculture (http://www.ams.usda.gov/AMSv1.0/gapghp) during production, will also help reduce risk. Continued surveillance is needed to monitor changes in food vehicles of contamination and to improve our understanding of the geographic distribution of outbreaks.

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Real-Time Microbiology Laboratory Surveillance System to Detect Abnormal Events and Emerging Infections, Marseille, France

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Infectious diseases are a major threat to humanity, and accurate surveillance is essential. We describe how to implement a laboratory data–based surveillance system in a clinical microbiology laboratory. Two historical Microsoft Excel databases were implemented. The data were then sorted and used to execute the following 2 surveillance systems in Excel: the Bacterial real-time Laboratory-based Surveillance System (BALYSES) for monitoring the number of patients infected with bacterial species isolated at least once in our laboratory during the study periodl and the Marseille Antibiotic Resistance Surveillance System (MARSS), which surveys the primary β-lactam resistance phenotypes for 15 selected bacterial species. The first historical database contained 174,853 identifications of bacteria, and the second contained 12,062 results of antibiotic susceptibility testing. From May 21, 2013, through June 4, 2014, BALYSES and MARSS enabled the detection of 52 abnormal events for 24 bacterial species, leading to 19 official reports. This system is currently being refined and improved.

Although infectious diseases were declared under control and considered to be a past public health problem during the second half of the 20th century (*1*), these diseases, including those that are well-known, emerging, and reemerging, remain a major threat to humanity. Indeed, infectious pathogens possess an amazing common capacity to emerge and spread in unpredictable ways before they are detected by public health institutions (*2*). Infectious diseases have a substantial effect on both global human demographics (they are the second leading cause of death in humans worldwide, accounting for ≈15 million deaths) (*3*) and the economy (*4*), which has led the public health community to reconsider them as a real threat. This alarming observation has led public health authorities to try to improve infectious disease surveillance.

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One of these strategies, known as traditional public health surveillance of infectious diseases, has been to use clinical case reports from sentinel laboratories or laboratory networks and direct reports of positive results from clinical laboratories to survey the presence of microbial agents known to be dangers to health in a precise population (*5*). Some examples of surveillance systems implemented by using this strategy are the National Tuberculosis Surveillance System in the United States (*6*), the surveillance system of the Netherlands Reference Laboratory for Bacterial Meningitis (*7*) and the European Gonococcal Antimicrobial Surveillance Programme (*8*).

Another strategy, known as syndromic surveillance, consists of developing real-time surveillance systems capable of detecting abnormal epidemiologic events, not on the basis of infectious disease diagnosis data, but rather on the basis of nonspecific health indicators, such as absenteeism, chief complaints, and prescription drug sales (*5*,*9*). Such surveillance systems can be implemented nationally, such as the Emergency Department Syndromic Surveillance System in England (*10*) or the National Retail Data Monitor in the United States (*11*), and regionally, such as the Emergency Department Syndromic Surveillance in Canada (*12*) or the European Antimicrobial Resistance Surveillance Network in Europe (*13*), or the systems can be administered by laboratories with large quantities of data and the financial and human resources to apply the information.

On the basis of our experience at the Assistance Publique–Hôpitaux de Marseille (AP-HM), we describe all the steps necessary for implementing a laboratory data–based syndromic surveillance system in a laboratory. Because of its simplicity, we believe that it can be rapidly applied and used as a first surveillance tool in well-established laboratories. We also show the advantages and limits of this surveillance system.

Materials and Methods

Study Setting

Marseille is the second-most populous French city (estimated population 850,726 persons in 2010). All data

analyzed in this article came from the 4 university hospitals of Marseille (North, South, Conception, and Timone hospitals). Cumulatively, these hospitals represent ≈3,700 beds, including ≈1,500 beds for the Timone Hospital, ≈600 beds for the North Hospital, ≈700 beds for the Conception Hospital, and ≈900 beds for the South Hospital. The AP-HM clinical microbiology laboratory is located at Timone Hospital; the laboratory performed ≈145,000 serologic tests and ≈200,000 PCRs and cultures of microorganisms from 220,000 samples in 2012 (*14*). This amount of data allowed us to implement our own laboratory-data–based syndromic surveillance system.

Organization of Surveillance Activity on Tools of AP-HM

The AP-HM laboratory–based surveillance consists of 3 following syndromic surveillance tools founded on Excel software (Mircosoft Corp., Redmond, WA, USA): 1 previously described system called EPIMIC (EPIdemiological biosurveillance and alert based on MICrobiologic data) (*15*,*16*), 1 surveillance system implemented for the surveillance of bacterial antibiotic resistance (MARSS, Marseille Antibiotic Resistance Surveillance System), and BALYSES (BActerial real-time LaboratorY-based SurveillancE System), which was developed for the surveillance of the number of patients infected by each bacteria species identified at least once in our laboratory. Our surveillance systems are defined as syndromic surveillance systems because no surveillance data are specifically collected for their use. The flow of information needed for each of the 3 surveillance systems is summarized in Figure 1. However, only BALYSES and MARSS are further described.

All of the data routinely used for the 2 surveillance systems are manually collected from the Timone Hospital laboratory information management systems and processed by using Microsoft Excel software (2007 version). Data are then entered in the 2 surveillance systems according to their nature. The 2 systems automatically compare the entered data with their specific thresholds. Alarms are emitted by the systems if the entered values exceed thresholds. The emitted alarms are analyzed weekly during a specific thematic epidemiology meeting with laboratory staff. If alarms are validated, further investigations are immediately conducted by biologists, clinicians, and medical residents. After the alarm is signaled, our institution's team in charge of nosocomial infections, called the Centre de Coordination de la Lutte contre les Infections Nosocomiales, initiates an investigation. Finally, if these investigations reveal that the alarm events were real epidemiologic events (thereafter called true alarms), official reports can be sent to an official regional public health institution, the Agence Régionale de la Santé (ARS).

Laboratory Data–Based Syndromic Surveillance System

BALYSES

The BALYSES surveillance system was implemented and has been routinely used since January 2013. The first version of BALYSES was implemented to automatically compare the weekly number of samples positive for each bacterial species identified at least once at our institution with the mean historical weekly values ± 2 SDs (Table 1, http://wwwnc.cdc.gov/EID/article/21/8/14-1419-T1.htm). In October 2013, BALYSES was improved to survey the weekly number of patients infected by each bacterial species (Figure 2; Table 1). Then, if alarms are emitted that indicate an abnormal increase in the number of isolations of a specific bacterial species, an additional Microsoft Excel interface is used to show more details, including the hospitals and units in which the patients received care, the types of samples from which the bacterial species were isolated, and the patients' identification numbers. BALYSES also automatically classifies the bacterial species from most to least abundant, according to the weekly number of infected patients, and calculates their weekly rank. It finally calculates the maximum number of patients infected by each of the bacterial species monitored, indicates the date of first isolation of the bacterial species at AP-HM, and identifies the historical rank (on the basis of the historical number of patients infected) among the other bacterial species.

MARSS

The MARSS surveillance program has been used since April 2013. Fifteen bacterial species are monitored by MARSS, including *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Proteus mirabilis*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Morganella morganii*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus*, and *S. epidermidis*. MARSS automatically compares the weekly number of isolates exhibiting a given β-lactam resistance phenotype to the mean value ± 2 SDs for the historical number of strains harboring this phenotype (Figure 3). Alarms are emitted when this threshold is exceeded. In parallel, MARSS emits alarms for key phenotypes to allow for their rapid identification and verification (Tables 2, 3).

Historical Databases

The detection of abnormal events necessitates the calculation of expected references, previously called historical thresholds. To define the expected references, 2 historical databases were built by using data extracted from the laboratory information management systems of the 4 university hospitals of Marseille. The first historical database consisted

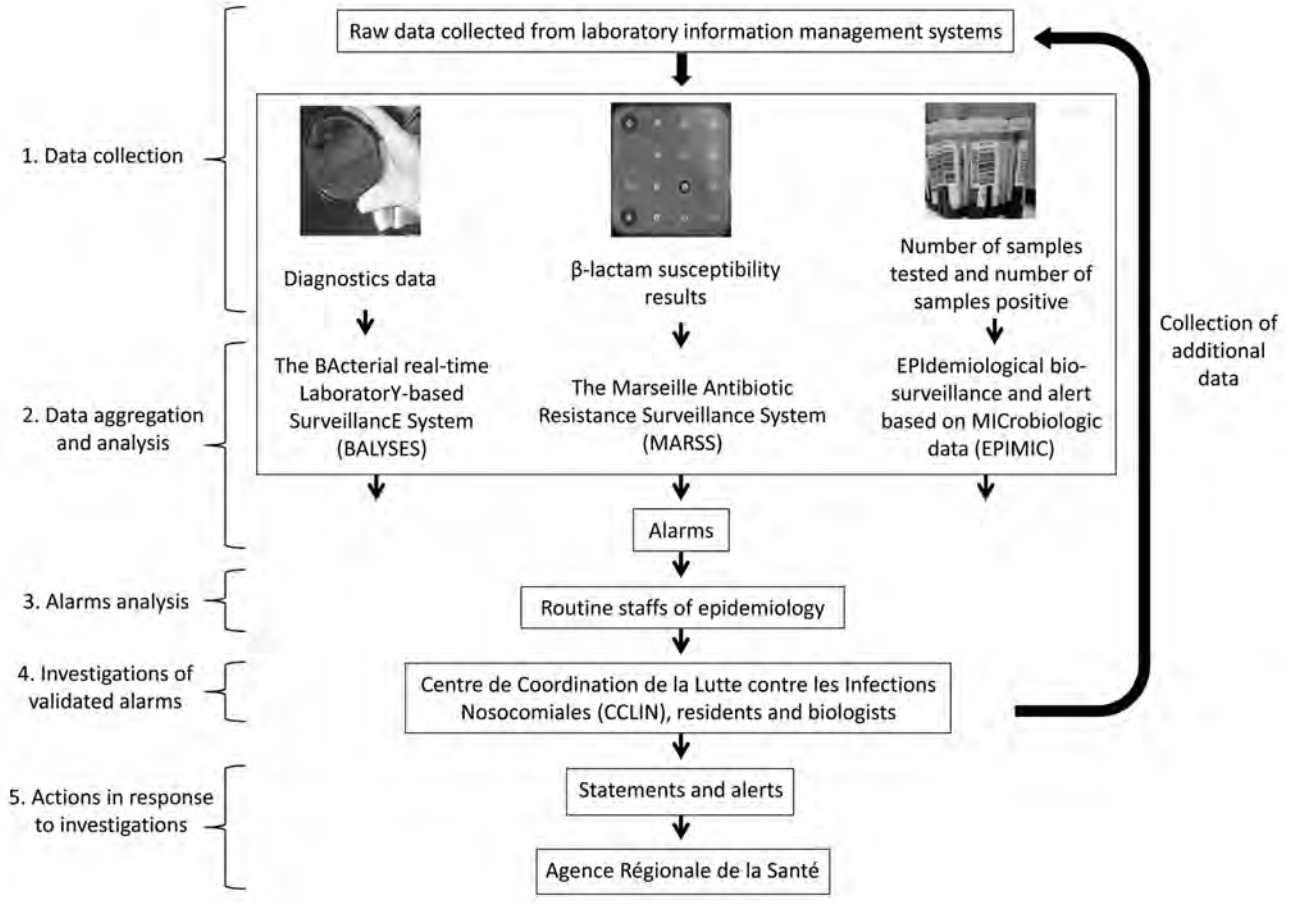

Figure 1. Workflow of real-time surveillance systems used by Institut Hospitalo–Universitaire Méditérranée Infection, Assistance Publique-Hôpitaux de Marseille, Marseille, France.

of all of the bacterial identifications obtained from January 2002 to December 2013 (excluding December 2002, data unavailable), including those described in a previous work (*17*), and a second database consisted of most antimicrobial resistance profiles obtained from October 2012 through March 2013. These data were then processed with Microsoft Excel software (2007 version) and sorted. The first database was then sorted, and only samples from which bacterial species were properly identified were conserved. Then, the duplicates for patient and bacterial species were removed. The second database was sorted into different Microsoft Excel spreadsheets for the most frequently isolated bacterial species. Duplicates occurring within the same week were then removed on the basis of the same methods.

Results

Databases and Surveillance Systems

The first version of the 11-year historical BALYSES database contained 161,374 bacterial identifications corresponding to 568 different bacterial species. The 10 most numerous bacterial species were *E. coli* (37,560 patients), *S. aureus* (23,562 patients), *S. epidermidis* (11,091 patients), *P. aeruginosa* (9,113 patients), *K. pneumoniae* (7,576 patients), *E. faecalis* (7,403 patients), *S. agalactiae* (4,473 patients), *E. cloacae* (4,453 patients), *P. mirabilis* (4,415 patients), and *Haemophilus influenzae* (2,424 patients). The 2013 updates increased the number of bacterial identifications to 174,853 and the number of monitored bacterial species to 611 (43 new bacterial species were added). Among them, 384 bacterial species, defined here as rare bacterial species, were identified <11 times in the 12-year period.

The historical MARSS database included 12,062 antibiograms from October 2012 to March 2013. Here, the 10 most frequently isolated bacterial species were *E. coli* (3,293 strains), *S. aureus* (1,613 strains), *Achromobacter xylosoxidans* (1,478 strains), *S. epidermidis* (822 strains), *E. faecalis* (749 strains), *K. pneumoniae* (729 strains), *P. mirabilis* (455 strains), *S. agalactiae* (322 strains), *E. cloacae* (278 strains), and *Staphylococcus hominis* (153 strains).

A

ID_bac_v2.0	Nb_patts_histori	Rank_historic	Date_1st_ID	Max	Mean	Mean - 2 SD	Mean + 2 SD	26/03/2014	02/04/2014	26/03/2014
Micrococcus luteus	379	38	12/01/2002	5	1	-1	4	4		16
Enterococcus avium	143	58	05/01/2002	3	1	-1	2	3		18
Moraxella catarrhalis	504	34	16/01/2002	3	0	-1	2	2		27
Haemophilus haemolyticus	1	545	10/09/2013	2	0	-1	1	1		35
Corynebacterium tuberculostearicum	64	100	04/08/2004	1	0	-1	1	1		35
Enterococcus casseliflavus	27	157	31/05/2002	1	0	-1	1	1		35
Parabacteroides distasonis	42	125	03/01/2002	1	0	0	0	1		35
Corynebacterium auris	20	178	14/10/2002	1	0	0	0	1		35
Fusobacterium gonidiaformans	3	354	20/10/2008	1	0	0	0	1		35
Comamonas kerstersii.	2	398	20/03/2010	1	0	0	0	1		35

B

Hospital	SYNERGIE_number	Kind of sample	Species found	Number of isolation at the hospital	Unit	Unit entire name	Number of isolation per
Conception	4032003305	Urines	Corynebacterium auris	1	2574	CENTER FOR KIDNEY TRANSPLANTATION	1
Conception	4032006110	Urines	Corynebacterium tuberculostearicum	1	2544	HEPATO-GASTROENTEROLOGY	1
Conception	4031997591	Hémo aerob	Micrococcus luteus	1	3926	EXTERNAL ORGANISMS	1
Conception	4032005916	Hémo ana	Parabacteroides Distasonis	1	2610	RECEPTION EMERGENCIES	1
Hôpital Nord	4032008019	L.ponction	Enterococcus avium	1	2091	HEAD AND NECK SURGERY	1
Hôpital Nord	4032001328	L.péritoné	Micrococcus luteus	1	1963	GENERAL AND DIGESTIVE SURGERY	1
Hôpital Nord	4031996497	Asp.bronch	Moraxella catarrhalis	1	2315	BRONCHI - ALLERGIES - SLEEP	1
Ste Marguerite	4032001082	Os	Enterococcus casseliflavus	1	5453	ORTHOPEDIC SURGERY	1
Timone	4032004580	Hémo aerob	Comamonas kerstersii.	1	771	NEUROLOGY	1
Timone	4042012135	Urines	Enterococcus avium	2	6110	RECEPTION SPECIALISED EMERGENCIES	1
Timone	4031997344	Os	Enterococcus avium	2	715	VASCULAR SURGERY	1
Timone	4032004373	Biopsie	Fusobacterium gonidiaformans	1	9916	EXTERNAL ORGANISMS	1
Timone	4032004048	CRA muco	Haemophilus haemolyticus	1	5476	SPECIAL PEDIATRY	1
Timone	4031995242	Anévrisme	Micrococcus luteus	1	3544	VASCULAR SURGERY	1
Timone	4031997103	Narine	Moraxella catarrhalis	1	3016	HEART HOSPITAL DEPARTMENT	1
Unknown	4031997163	Os	Micrococcus luteus	1	5595	0	1

Figure 2. Screen shots from the Bacterial Real-Time Laboratory-based Surveillance System. A) List of the 652 bacterial species followed by the Bacterial Real-time Surveillance System and all of the contained information. B) Interface summarizing information from the alarms. ID_bac_v2.0, all the bacterial species followed by the surveillance system; Nb_patts_histori, the historical number of patients infected by the bacterium; Rank_historic, the historical rank of a precise bacterium under surveillance; Date_1st_ID, the date of first identification of the bacterium.

Alarms Validated and Investigated, May 21, 2013– June 4, 2014

From May 21, 2013, through June 4, 2014 (55 weeks), BALYSES detected 21 alarms (6 confirmed events and 15 unconfirmed events), corresponding to ≈0.4 alarms per week. These alarms led to 5 official reports to the ARS of the Provence-Alpes-Côte d'Azur (PACA) region, France (Table 1; Figure 4). The positive predictive value for the study period was 0.28. Sixteen bacterial species triggered alarms in this surveillance system. The bacterial species that triggered alarms were *E. aerogenes* (3 alarms), *Aeromonas hydrophila* (2 alarms), *E. cloacae* (2 alarms), *K. oxytoca* (2 alarms), *M. morganii* (2 alarms), *E. coli* (1 alarm), *E. faecium* (1 alarm), *Gardnerella vaginalis* (1 alarm), *Haemophilus parahaemolyticus* (1 alarm), *Moraxella catarrhalis* (1 alarm), *Raoultella ornithinolytica* (1 alarm), *Staphylococcus capitis* (1 alarm), *Staphylococcus gallolyticus* (1 alarm), *Staphylococcus hominis* (1 alarm), and *Staphylococcus saprophyticus* (1 alarm). As an example of the system's usefulness, BALYSES allowed us to detect a real nosocomial transmission of *R. ornithinolytica* between 2 patients in the intensive care unit at the Timone Hospital on June 4, 2013 (Table 1).

In parallel, MARSS detected 31 alarms (16 confirmed events and 15 unconfirmed events, ≈0.6 alarms/week), which led to 15 official reports to the ARS of the PACA region, France (Table 4, http://wwwnc.cdc.gov/EID/article/21/8/14-1419-T4htm; Figure 4). The positive predictive value for the study period was 0.52. Thirteen bacterial species triggered alarms in MARSS. Here, the bacterial species, in order according to the number of alarms triggered, were *K. pneumoniae* (13 alarms), *E. cloacae* (3

A

Sum_week													130
Resistance phenotypes	AMX	TIC	AMC	TCC	CRO	FEP	IPM	Maximum for one specific week	Mean number of isolation	Standard deviation	Mean + 2 standard deviation	Mean – 2 standard deviation	03/10/2014
Wild	S	S	S	S	S	S	S	72	57	10	77	36	63
Low level penicillinase	I/R	I/R	S	S	S	S	S	33	17	6	30	5	23
Inhibitors resistant penicillinase	I/R	I/R	R	R	S	S	S	36	17	6	30	5	14
High level penicillinase	I/R	I/R	I/R	I/R	S	S	S	41	22	7	35	9	23
Extended-spectrum β-lactamase	R	R	S/I/R	S/I/R	I/R	I/R	S	17	9	3	16	3	5
High level cephalosporinase	R	R	R	R	R	S	S	5	1	1	4	–1	2

B

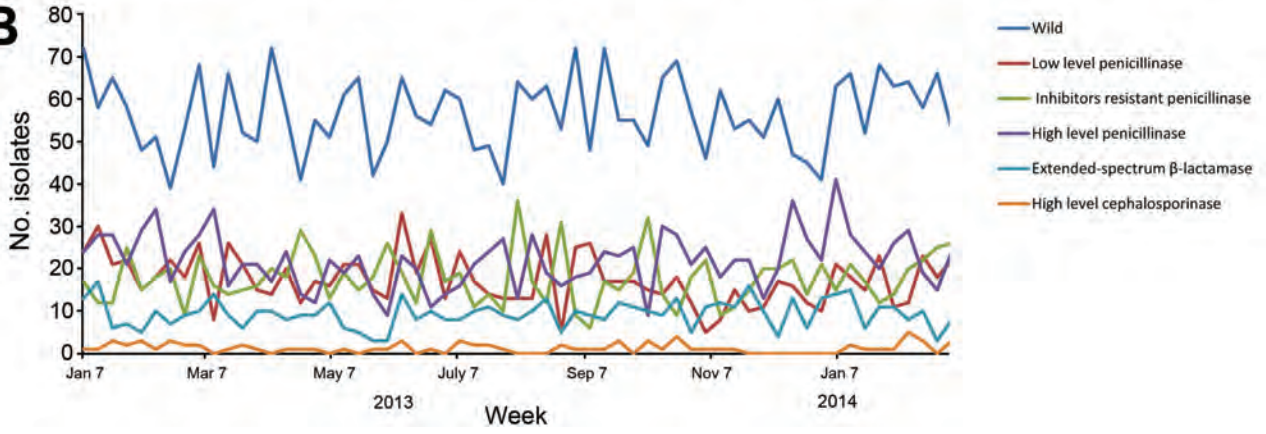

Figure 3. Marseille Antibiotic Resistance Surveillance System (MARSS) interface for *Escherichia coli*. A) Screen shot showing list of most of the β-lactam antibiotic resistance profiles coded for *E. coli* in MARSS. B) Example of graph created by using MARSS showing the evolution of the antibiotic resistance of *E. coli*.

alarms), *P. mirabilis* (3 alarms), *E. coli* (2 alarms), *E. aerogenes* (2 alarms), *Salmonella* spp. (2 alarms), *P. aeruginosa* (1 alarms), *Citrobacter koseri* (1 alarm), *M. morganii* (1 alarm), *S. marcescens* (1 alarm), *S. epidermidis* (1 alarm), and *S. agalactiae* (1 alarm). As an example of the system's usefulness, MARSS allowed us to detect a local outbreak of oxicillinase-48 carbapenemase–producing *K. pneumoniae* from July 2013 to October 2013 (11 patients infected) (unpub. data; Table 4, http://wwwnc.cdc.gov/EID/article/21/8/14-1419-T4.htm).

For clarification, not all of the true alarms led to official reports because we did not identify the reasons why these abnormal increases occurred (Tables 1, 4). Nevertheless, investigations are ongoing to try to elucidate these phenomena.

Discussion

Analysis of 2 Real-Time Laboratory-Based Surveillance Systems

Implementing surveillance systems on the basis of data that were not specifically collected for surveillance is one of the advantage of our systems. Indeed, these types of systems, syndromic surveillance systems, are well suited in places and situations in which surveillance tools are urgently needed (*18*). In our situation, this approach allowed us to rapidly implement the system and quickly detect abnormal events related to bacterial infections occurring in our institution (19 official reports) (Tables 1, 4; Figure 4).

The fact that all of the emitted alarms are systematically validated during epidemiologic meetings with microbiologists (Figure 1) is also a strength of this laboratory surveillance system. Thus, the system enables rapid verification and filtering of false alarms to ensure that the official reports sent to the regional health authorities (ARS) are correct. This facilitates a rapid public health response to counter possible epidemics. As an example, EPIMIC, our third surveillance system not described here (Figure 1) (*15*,*16*), allowed us to detect a nosocomial outbreak of the hypervirulent *Clostridium difficile* ribotype O27 that started in March 2013 (*19*). As we continue to fight this major public health problem, a list of recommended containment measures, such as systematic isolation of infected patients in special care units or systematic screening of patients at risk, is being published and transmitted to our institutional and regional health care providers.

Our 2 surveillance systems have been implemented by using Microsoft Excel software. This strategy makes the

Table 2. Summary of the normal phenotypes registered in MARSS*

Bacterial species	Resistance phenotypes	β-lactam antibiotics										
		AMX	TIC	AMC	TCC	TZP	FOX	OXA	CRO	FEP	CAZ	IPM
Escherichia coli	Wild-type	S	S	S	S				S	S		S
	Low-level penicillinase	I/R	I/R	S	S				S	S		S
	Inhibitor-resistant penicillinase	I/R	I/R	R	R				S	S		S
	High-level penicillinase	I/R	I/R	I/R	I/R				S	S		S
	ESBL	R	R	S/I/R	S/I/R				I/R	I/R		S
	High-level cephalosporinase	R	R	R	R				R	S		S
Klebsiella pneumoniae	Wild-type			S		S			S	S		S
	ESBL			I/R		I/R			I/R	I/R		S
	High-level cephalosporinase			I/R		I/R			I/R	S		S
	ESBL-TZP-sensible			I/R		S			I/R	I/R		S
Proteus mirabilis	Wild	S	S	S	S				S	S		S
	Low-level penicillinase	I/R	I/R	S	S				S	S		S
	Inhibitor-resistant penicillinase	R	R	R	R				S	S		S
	High-level penicillinase	R	R	I/R	I/R				S	S		S
	ESBL	R	R	I/R	I/R				I/R	I/R		S
	High-level cephalosporinase	R	R	R	R				R	S		S
Klebsiella oxytoca	Wild-type			S		S				S		S
	ESBL			I/R		I/R				I/R		S
	High-level penicillinase			I/R		S/I/R				S		S
	Low-level penicillinase			S		R				S		S
	ESBL–TZP-sensible			I/R		S				I/R		S
Enterobacter aerogenes	Wild-type				S	S			S	S		S
	Inhibitors-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Morganella morganii	Wild-type				S	S			S	S		S
	Inhibitor-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Serratia marcescens	Wild-type				S	S			S	S		S
	Inhibitor-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Enterobacter cloacae	Wild-type				S	S			S	S		S
	Inhibitor-resistant penicillinase				R	R			S	S		S
	ESBL				S/I/R	I/R			I/R	I/R		S
	High-level cephalosporinase				I/R	I/R			I/R	S		S
Pseudomonas aeruginosa	Wild-type		S		S	S				S	S	S
	Penicillinase		R		R/I/S	I/S				S	S	S
	High-level penicillinase		I/R		I/R	I/R				S	S	S
	ESBL		I/R		I/R	I/R				I/R	I/R	S
	Selective permeability to imipenem		S		S	S				S	S	R
	Penicillinase, loss of D2 porine		R		R	S				S	S	R
Acinetobacter baumannii	Wild-type		S		S						S	S
	Penicillinase		R		R/I/S						S	S
	ESBL		I/R		I/R						I/R	S
Streptococcus agalactiae	Wild							S	S			
	Oxacillin-resistant							I/R	S			
Enterococcus faecalis	Wild-type	S										
E. faecium	Wild-type	I/R										
Staphylococcus aureus	Wild-type						S					
	Methicillin-resistant						I/R					
S. epidermidis	Wild-type						S					
	Methicillin-resistant						I/R					

*MARSS, Marseille Antibiotic Resistance Surveillance System; AMC, amoxicillin; TIC, ticarcillin; AMC, amoxicillin/clavulanic acid; TIC, ticarcillin/clavulanic acid; TZP, piperacillin/tazobactam; FOX, cefoxitin; OXA, oxacillin; CRO, ceftriaxone; FEP, cefepime; CAZ, ceftazidime; IMP, imipenem; S, susceptible; I, intermediate; R, resistant; ESBL, extended-spectrum β-lactamase.

systems easy to handle and allows rapid modifications and improvements without the need for in-depth computer skills. These advantages may not be the case for fully designed website surveillance systems such as the Swiss Antibiotic Resistance Surveillance database (*20*) or the Real-Time Outbreak and Disease Surveillance (RODS) (*21*). These aspects

Table 3. Summary of the alarm phenotypes defined in MARSS*

Bacteria species	Alarm triggering key phenotypes
Escherichia coli, Proteus mirabilis	Carbapenem resistance
Klebsiella pneumoniae	Carbapenem resistance
Klebsiella oxytoca	Carbapenem resistance
Enterobacter aerogenes, *Morganella morganii*, *Serratia marcescens, Enterobacter cloacae*	Carbapenem resistance
Pseudomonas aeruginosa	Carbapenem resistance
Acinetobacter spp.	Carbapenem and colistin resistance
Streptococcus agalactiae	Ceftriaxone resistance
Enterococcus faecalis	Amoxicillin resistance
Enterococcus faecium	Amoxicillin susceptible
Staphylococcus aureus	Vancomycin resistance

*MARSS, Marseille Antibiotic Resistance Surveillance System.

are key factors for the optimal long-term use at the hospital level because surveillance systems can be considered complex socio-technical systems with the objective of assisting users during abnormal epidemic events (*22*).

The implementation of our 2 surveillance systems required 1 full-time PhD student for 4 months and a computer with standard configuration equipped with Microsoft Office version 2003 or 2007. In France, the national research agency requires that the minimum salary of a PhD student is 33,000€ per year. Considering that the average price for a basic computer equipped with Microsoft Office is ≈500€ and that the PhD student's salary for the 4 months was 11,000€, plus the administrative and management costs, the total consolidated cost of these surveillance systems was ≈13,800€ (US $17,000).

The use of our own microbiology laboratory data ensures the availability and the completeness of the data. These problems are frequently mentioned when surveillance systems collect data from various health care institutions. For example, the designers of the German Surveillance System of Antibiotic Use and Bacterial Resistance encountered problems comparing antibiogram data between participating intensive care units. Indeed, in Germany, laboratories did not apply 1 standard to determine antibiotic-resistance profiles of the bacterial species (*23*). Moreover, the increasing number of intensive care units joining the surveillance system may effect the comparability of collected data because recently added intensive care

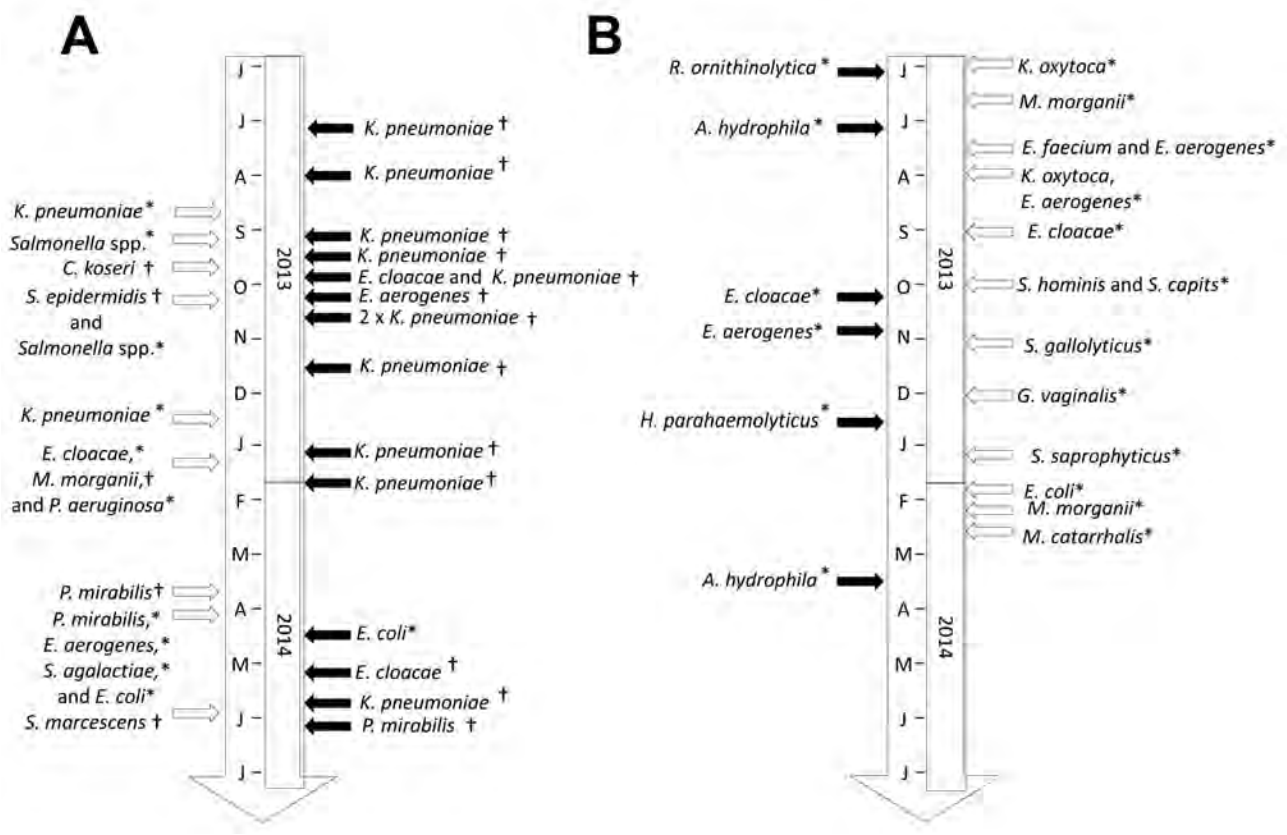

Figure 4. Time chart of the confirmed and unconfirmed events identified by the Marseille Antibiotic Resistance Surveillance System (MARSS) and the Bacterial real-time Laboratory-based Surveillance System (BALYSES). A) List of all the abnormal events (confirmed or not) detected by MARSS. B) List of all the abnormal events (confirmed or not) detected by BALYSES. Open arrows, unconfirmed events; solid arrows, confirmed events; asterisk (*), alarm due to abnormal increases or abnormal isolations; dagger (†), alarm due to strain with abnormal antibiotic susceptibility results.

units may use different antibiotic drugs, thus leading to different antimicrobial resistance profiles (*24*). Poor quality data were also observed in the emergency department syndromic surveillance system in New York, primarily because of the lack of human resources (*25*).

However, our surveillance systems have 2 main limitations. The first limitation is the statistical analysis used for the detection of abnormal events. As described before, our surveillance systems compared entered data with the historical means ± 2 SDs. For our purposes, this tool was simple to develop and was used effectively to detect abnormal events. However, these statistics do not consider seasonal variations in pathogen isolation, especially for rare bacterial species. To address this problem, Enki et al. improved the detection algorithms according to the frequency of isolation of the 3,303 pathogens included in the 20-year LabBase surveillance database recovered from the UK Health Protection Agency (*26*). They discovered that although all of these organisms varied greatly in their isolation frequency, most of them could be surveyed by using quasi-Poisson or negative binomial models for which the variance is proportional to the mean. In MARSS, the use of moving averages in our kinetic graphs or of cumulative sum control charts, as has been done in RODS (http://openrods.sourceforge.net/), could also be effective improvements for the detection of abnormal events.

The second limitation was that all of the data in our system were manually collected and entered into the surveillance system. This aspect can introduce bias into our data analysis. For example, we have already observed false alarms after shifts in data collection because of national holidays or because of the lack of human resources, which is a problem also observed in other surveillance systems, such as the emergency department syndromic surveillance system in New York (*25*). To address these issues, simple solutions can be developed, such as implementing and using informatic tools for automatic collection and processing of the collected data. This solution was implemented by the designers of ASTER, the French military decision-supported surveillance system (*22*).

With knowledge of the previously mentioned weaknesses, we are currently working to improve our 2 surveillance systems. Thus, a surveillance platform that will merge all of the surveillance activities and will contain stronger statistical tools for the surveillance of abnormal events is under development. This platform will help us survey abnormal events by using all of the clinical microbiology data available in the laboratory. Moreover, our monitoring activity is expanding to other laboratories in the PACA region. We are implementing a regional laboratory surveillance system that will allow us, on the basis of the clinical microbiology data that are collected every week, to gain a better understanding of the local dissemination of pathogens at the regional level and to survey weekly isolation frequencies. Finally, another surveillance system based on matrix-assisted laser desorption/ionization–time of flight spectra of bacteria is currently under development in our laboratory. A prototype is used weekly in our laboratory to try to detect epidemics, including the possible nosocomial transmission of bacterial clones.

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etymologia

Escherichia coli [esh"ə-rik'e-ə co'lī]

A gram-negative, facultatively anaerobic rod, *Escherichia coli* was named for Theodor Escherich, a German-Austrian pediatrician. Escherich isolated a variety of bacteria from infant fecal samples by using his own anaerobic culture methods and Hans Christian Gram's new staining technique. Escherich originally named the common colon bacillus *Bacterium coli commune*. Castellani and Chalmers proposed the name *E. coli* in 1919, but it was not officially recognized until 1958.

"Escherich, Theodor" by Unknown, retouched by Lichtspiel. Public domain image via Wikimedia Commons (https://commons.wikimedia.org/wiki/File:Escherich,_Theodor.jpg#/media/File:Escherich,_Theodor.jpg)

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Underrecognition of Dengue during 2013 Epidemic in Luanda, Angola

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During the 2013 dengue epidemic in Luanda, Angola, 811 dengue rapid diagnostic test–positive cases were reported to the Ministry of Health. To better understand the magnitude of the epidemic and identify risk factors for dengue virus (DENV) infection, we conducted cluster surveys around households of case-patients and randomly selected households 6 weeks after the peak of the epidemic. Of 173 case cluster participants, 16 (9%) exhibited evidence of recent DENV infection. Of 247 random cluster participants, 25 (10%) had evidence of recent DENV infection. Of 13 recently infected participants who had a recent febrile illness, 7 (54%) had sought medical care, and 1 (14%) was hospitalized with symptoms consistent with severe dengue; however, none received a diagnosis of dengue. Behavior associated with protection from DENV infection included recent use of mosquito repellent or a bed net. These findings suggest that the 2013 dengue epidemic was larger than indicated by passive surveillance data.

Dengue is a potentially fatal acute febrile illness caused by any of 4 mosquito-transmitted dengue viruses (DENV-1–4). The disease is endemic throughout the tropics (*1*), but is underrecognized in sub-Saharan Africa (*2*,*3*), where an estimated 64 million DENV infections occurred in 2010 (*4*). Although dengue was identified in travelers returning from Angola in the 1980s (*5*), locally acquired cases had not been reported until an outbreak in 2013 that was initially thought to have resulted from importation of DENV by immigrant workers from Asia. However, the only virus detected during the outbreak was a strain of DENV-1 that molecular epidemiologic analysis indicated had been circulating in western and west-central Africa for roughly 4 decades (*6*–*9*), demonstrating regional endemicity of dengue.

During the 2013 epidemic, the Angola Ministry of Health was notified of a total of 1,214 dengue case-patients, nearly all (98%) of whom resided in the capital, Luanda, which has an estimated population of 3–14 million (Angola Ministry of Health and World Health Organization, unpub. data). Serum specimens from suspected cases were tested with a dengue rapid diagnostic test (RDT; SD BIOLINE Dengue Duo, Standard Diagnostics, Haryana, India), and positive cases were defined by detection of nonstructural protein 1 antigen, anti-DENV IgM, or both. In total, specimens from 811 (67%) persons with suspected dengue tested RDT-positive, including those from 246 (30%) hospitalized patients and from 11 (1.4%) patients who died. The highest weekly incidence occurred during May 17–23, 2013, when 125 cases were reported, of which 101 (81%) were RDT-positive.

Dengue is a focal disease, and cases frequently cluster around the households of infected persons (*10*,*11*). Previous household-based cluster investigations in Indonesia (*12*), Nicaragua (*13*), Thailand (*14*), and Vietnam (*15*) demonstrated DENV infection rates of 2.2%–12.4% among persons residing within 10–100 m of index case-patients. These studies enabled detection of unrecognized dengue cases and identification of household risk factors for DENV infection, such as the presence of uncovered water storage containers (*12*) and lack of piped household water supply (*14*). Household-based cluster investigations are therefore a useful tool to estimate the extent of dengue in regions where case reporting may be suboptimal and can also facilitate identification of local risk factors for DENV infection.

Methods

We conducted household-based cluster investigations in Luanda to detect unreported cases and identify demographic characteristics and household and behavioral risk factors for infection. Clusters consisted of households located within a 25-m radius of the following: 1) residences of dengue case-patients who sought medical care, were reported as having a suspected dengue case, and tested positive by RDT (case clusters); or 2) randomly selected households from throughout Luanda in which no known dengue case-patient resided (random clusters).

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Table 1. Demographic characteristics and medical history among participants in household cluster investigations of DENV infection, Luanda, Angola, 2013*

Characteristic	Random clusters, n = 26	Case clusters, n = 21
Households per cluster, median (range)	3 (1–7)	4 (1–12)
Participants per household, median (range)	3 (1–13)	4 (1–12)
All participants	247	173
Male, no. (%)	98 (42)	78 (45)
Age, y, median (range)	22 (0–94)	25 (0–79)
Participants with evidence of recent DENV infection	25 (10)	16 (9)
Male, no. (%)	11 (48)	8 (50)
Age, y, median (range)	23 (7–65)	22 (4–42)
Fever within past 30 d, no. (%)	10 (43)	3 (23)
Fever in household member within past 30 d, no. (%)	9 (47)	10 (67)

*DENV, dengue virus. N = 420; 35 participants with equivocal anti-DENV IgM ELISA results were excluded from analysis.

Case clusters were identified by contacting RDT-positive dengue case-patients or their parents and querying whether they were available for a household visit, which was made within 30 days of the index case-patient's reported date of illness onset. Case clusters were studied even if the index case-patient did not participate in the investigation.

The protocol for selection of random clusters was as follows: 1) randomly selecting and traveling to 1 of 8 regions of Luanda; 2) spinning a 1-sided object (e.g., pen, bottle); 3) traveling in the indicated direction for ≈30 min by automobile without accounting for the degree of traffic congestion; 4) parking the automobile and spinning the 1-sided object again; 5) traveling by foot in the indicated direction for ≈5 min; 6) again spinning the 1-sided object; and 7) offering participation in the investigation to the nearest household in the direction indicated by the object. If the selected household was unoccupied or declined participation, the team returned to the automobile and repeated the process.

The head of the household in case and random clusters was informed of the purpose of the investigation. Households were not revisited if the head of household was unavailable. If heads of household agreed to participate in the investigation, all available household members were offered the opportunity to participate, which included the following: 1) completing a questionnaire that collected information on demographics, medical history, and mosquito avoidance strategies; and 2) providing a serum specimen for dengue diagnostic testing. Heads of household completed an additional questionnaire regarding household characteristics. All communications and questionnaires were in Portuguese. The cluster study was conducted during June 28–July 2, 2013. Specimens were processed on the day of collection, stored at -20°C, and shipped on dry ice to the Centers for Disease Control and Prevention (CDC) Dengue Branch (San Juan, Puerto Rico), for dengue diagnostic testing by real-time reverse transcription PCR (rRT-PCR)

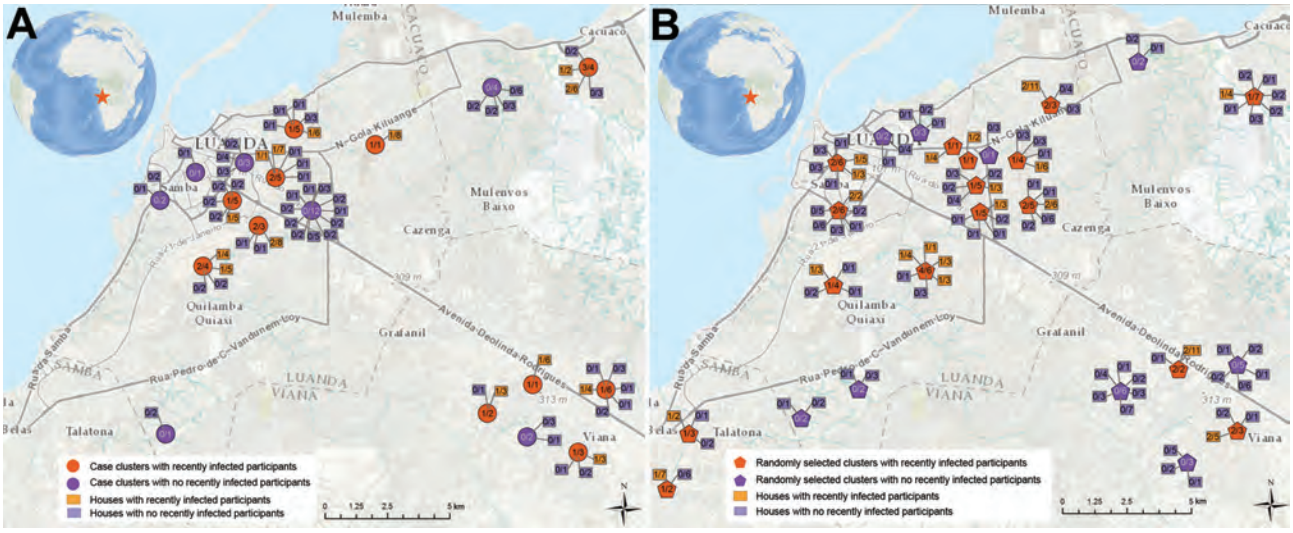

Figure. Locations of case and random cluster investigations conducted in Luanda, Angola, 2013. Clusters were conducted within a 25-m radius of A) the residences of known dengue case-patients (case clusters) or B) households in which no known dengue-case-patients resided (random clusters). The numbers in each cluster foci indicate the number of households with ≥1 recently infected household member per number of households included in each cluster. The numbers in each household indicate the number of recently infected participants in each household per the number of participants in each household. Coordinates were not available for 2 case clusters and 1 random cluster. Maps created by using the Geospatial Research, Analysis and Services Program (US Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA).

(*16*) and anti-DENV IgM capture ELISA (InBios International, Inc., Seattle, WA, USA). Data were compiled in a Microsoft Access (Microsoft Corp., Redmond, WA, USA) database.

Participants were household members who completed a questionnaire and provided a serum specimen. Current DENV infection was defined as detection of DENV nucleic acid by rRT-PCR. Recent DENV infection was defined as detection of anti-DENV IgM by ELISA. Dengue, dengue with warning signs, and severe dengue were defined by 2009 World Health Organization guidelines (*1*).

To identify differences in characteristics between recently infected participants and uninfected participants, we fitted generalized linear models with each of the variables of interest as the predictor. Random effects were included for households nested within clusters to account for correlation. Because it was unclear whether inference could be made to the greater population of Luanda, p values were computed through a permutation test, in which DENV infection statuses were permuted within households. Thus, results are only applicable to the surveyed population.

The investigation protocol underwent institutional review at CDC and was determined to be public health response and not research. As such, institutional review board approval was not required.

Results

Serum specimens and questionnaires were collected from 455 cluster participants (Table 1). Similar numbers of households per cluster and participants per household were included in case and random clusters. Age and sex of participants had a similar distribution in case and random clusters. No participants had evidence of current DENV infection by rRT-PCR, 41 (9%) had evidence of recent DENV infection by IgM ELISA, and 35 (8%) had equivocal IgM ELISA results and were excluded from further analysis. Age and sex distributions were similar for recently-infected case and random cluster participants. Recently-infected participants from random clusters more frequently reported having fever in the past month and less frequently reported a febrile household member in the past month.

Of 173 participants from 67 households in 21 case clusters, 16 (9%) had evidence of recent DENV infection (Figure). Of 247 participants from 90 households in 26 random clusters, 25 (10%) had evidence of recent DENV infection. Most case (55%) and random (77%) clusters contained at least 1 recently infected participant. Approximately one fifth of case and random cluster households had at least 1 recently infected participant.

Recently infected participants were significantly younger and had spent significantly less time in Luanda than uninfected participants (Table 2). Roughly one third of recently infected participants and also uninfected participants reported having fever in the past 30 days. Of 13 recently infected and recently febrile participants, 5 (38%) reported symptoms consistent with dengue with warning signs (severe abdominal pain) and 1 (8%) reported symptoms consistent with severe dengue (hematemesis). Seven (54%) recently infected and recently febrile participants sought medical care; 1 (14%) was hospitalized, and none reported receiving a diagnosis of dengue. Recently infected and febrile participants who sought care frequently (71%) received a diagnosis of malaria, as were uninfected, recently febrile participants who sought care (58%).

Having used a bed net or mosquito repellent in the past 30 days were significantly associated with protection from recent DENV infection ($p = 0.05$ and $p = 0.03$, respectively; Table 2). Although most participants' homes had piped water, delivery of household water by public water truck was also significantly associated with protection from DENV infection ($p = 0.04$).

Discussion

In this investigation, ≈10% of case and random cluster participants had evidence of recent DENV infection. A possible explanation for why no participants had current DENV infection is that the cluster investigations were conducted ≈6 weeks after the apparent peak of the epidemic. Therefore, although DENV circulation may have been declining when surveys were conducted, anti-DENV IgM, which may persist for months after infection (*17*), was still detectable. This low rate of current DENV infection among cluster participants is in contrast to findings of an investigation recently conducted near the peak of a dengue outbreak in Mombasa, Kenya, in which nearly 7% of participants had evidence of current DENV infection and another 7% had recent infection (*18*).

Persons with evidence of recent DENV infection were most frequently 10–19 years of age, and this finding likely led to confounding in the observation that persons with recent DENV infection spent less time in Luanda than uninfected persons. Unfortunately, our sampling method did not allow for statistically valid age-matched comparisons. In addition, the expected natural history of DENV transmission in a disease-endemic area would support the idea that persons 10–19 years of age were more likely to have higher rates of infection than adults who had lived in Luanda for many years, had been previously infected with DENV-1, and were thus protected from infection in 2013. Taken together with molecular evidence of dengue endemicity in Angola (*7*,*8*), these observations indicate a level of dengue endemicity equivalent to that observed in the Americas (*4*), where adolescents are routinely one of the most affected age groups (*19*–*21*).

Although multiple pieces of evidence indicate that dengue is endemic in Luanda, none of the recently infected persons who sought medical care had received a diagnosis

Table 2. Demographic, illness, behavioral, and household characteristics of participants with or without evidence of recent DENV infection that were identified though household cluster investigations, Luanda, Angola, 2013*

Characteristic	Participants with evidence of recent DENV infection, N = 41	Participants without evidence of recent DENV infection, N = 379	p value
Demographic			
Age, median (range)	14 (4–65)	24 (0–94)	**0.002**
Male sex, no (%)	19 (49)	157 (43)	0.65
Time in Luanda, y (range)	13 (3–58)	20 (0–80)	**0.01**
Medical history, no. (%)			
Fever in past 30 d	13 (33)	109 (29)	0.89
Sought medical care	7 (54)	62 (57)	0.42
Hospitalized	1 (14)	6 (10)	0.21
Diagnosis of dengue	0 (0)	4 (6)	–
Diagnosis of malaria	5 (71)	36 (58)	0.09
Diagnosis of typhoid fever	1 (14)	4 (6)	–
Other†/unknown diagnosis	1 (14)	18 (29)	–
Minor bleeding‡	2 (15)	6 (10)	0.13
Dengue with warning signs	6 (46)	23 (37)	0.57
Severe dengue	1 (8)	5 (8)	0.86
Behavioral, no. (%)			
Traveled outside of Luanda in past 30 d	0 (0)	24 (6)	0.07
Used bed net in past 30 d	2 (5)	69 (19)	**0.05**
Used repellent in past 30 d	2 (5)	46 (12)	**0.03**
Household, no. (%)			
Water supply			
Piped water supply	28 (76)	186 (67)	0.77
Public water truck	4 (11)	84 (30)	**0.04**
Other§/unknown	5 (14)	59 (21)	0.81
Had febrile household member in past 30 d	19 (56)	176 (56)	0.98
Has screened windows	1 (3)	46 (15)	0.14
Usually leave windows open	30 (79)	258 (78)	0.39
Has air conditioning	15 (39)	142 (43)	0.72
Use mosquito coils in house or yard	23 (62)	176 (53)	0.87

*Thirty-five participants with equivocal anti-DENV IgM ELISA results were excluded from analysis. DENV, dengue virus; –, insufficient numbers for permutation test. Boldface type indicates significance.
†Influenza, arterial hypertension, diabetes, abortion.
‡Petechiae, epistaxis, gingival bleed.
§Well, rain water.

of dengue. Instead, most received a diagnosis of malaria, including 1 person who was hospitalized with an illness consistent with severe dengue. Thus, as has been observed in other regions of sub-Saharan Africa (*2*,*18*,*22*–*24*), dengue and other acute febrile illnesses may be frequently overlooked in Luanda, where malaria is in fact rare (*25*). Observations from this investigation therefore suggest that the number of RDT-positive dengue cases reported to the Ministry of Health was likely a large underestimation of the true magnitude of the 2013 epidemic.

An unexpected finding of this investigation was that the rate of detection of recently infected participants was equivalent in both case and random clusters. This finding is in contrast to those of most prior household cluster surveys, in which few persons or none were found to be recently infected in randomly selected clusters (*13*,*14*,*26*,*27*). One possible explanation for these differences is that the Luanda investigation was conducted in an urban environment soon after the peak of a large epidemic, whereas previous studies were conducted during periods of nonepidemic levels of transmission. Similar to results of this investigation, a recent 3-year cluster study conducted in urban Vietnam found that the incidence of having detectable anti-DENV IgM was twice as high in participants from case clusters than from control clusters (*15*). Alternatively, because some random clusters were surveyed on the same day and by the same teams investigating case clusters, teams may not have traveled sufficiently far from case clusters to obtain independent findings. Because dengue is a focal illness that travels in "waves" outward from urban environments (*11*,*28*), the timing and distance required to be independent from a site of known or suspected DENV transmission are unclear.

Behavior associated with protection from DENV infection included having recently used mosquito avoidance strategies (such as applying mosquito repellent or sleeping under a bed net) and delivery of household water supply by public water truck. Although the latter finding should be further investigated to both validate and explore the reasons behind its association with protection from DENV infection, use of mosquito repellent is a well-documented approach to mosquito avoidance that has been repeatedly associated with protection from DENV infection (*1*). Bed net use was previously associated with protection from DENV infection among soldiers in Somalia (*29*) but is not typically thought to be associated with protection from DENV infection because *Aedes aegypti* mosquitoes, which

were the dominant vector detected during the Luanda epidemic (*8*), are most active at dusk and dawn (*1*). Bed net use may therefore be associated with protection from early morning biting. In line with this observation, a serosurvey in Mombasa, Kenya, conducted in 2013, found that leaving windows open at night was associated with DENV infection (*18*), possibly because *Aedes* mosquitoes enter the home in the evening and feed on the host in the early morning (*30*,*31*).

Strengths of this investigation include using community-level surveys to demonstrate the extent of dengue in a region where clinical awareness and reporting infrastructure were suboptimal. Moreover, the recent availability of an RDT enabled early detection of dengue cases, without which the epidemic may not have been recognized. Also, by using well-validated diagnostic tests to detect evidence of current or recent DENV infection in cluster participants, the likelihood that additional infections were missed is minimal. Conversely, infection with flaviviruses can result in cross-reactive antibody (*32*), creating the possibility of false-positive anti-DENV IgM diagnostic test results. Although the IgM ELISA used in this assay has been previously demonstrated to be highly specific for anti-DENV IgM (InBios DENV Detect IgM Capture ELISA, product insert; CDC Dengue Branch, unpub. data), it is nonetheless possible that some proportion of participants with recent DENV infection were misclassified due to false-positive diagnostic test results.

The circulation of DENV-4 was recently detected in Luanda (*33*,*34*) and may be associated with future epidemics in the region. Because early identification and proper management of dengue patients can reduce case-fatality rates among hospitalized patients from ≈10% to <0.5% (*35*), clinical awareness of dengue should be improved in Luanda and throughout sub-Saharan Africa through clinical dengue patient management trainings (e.g., http://www.cdc.gov/dengue/training/cme.html). Case reporting should also be improved by instituting routine laboratory-based surveillance for acute febrile illnesses in Africa, which will assist in better defining the epidemiology of dengue and other emerging infectious diseases (*33*,36).

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Health Care–Associated Infection Outbreak Investigations in Outpatient Settings, Los Angeles County, California, USA, 2000–2012

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Health care services are increasingly delivered in outpatient settings. However, infection control oversight in outpatient settings to ensure patient safety has not improved and literature quantifying reported health care–associated infection outbreaks in outpatient settings is scarce. The objective of this analysis was to characterize investigations of suspected and confirmed outbreaks in outpatient settings in Los Angeles County, California, USA, reported during 2000–2012, by using internal logs; publications; records; and correspondence of outbreak investigations by characteristics of the setting, number, and type of infection control breaches found during investigations, outcomes of cases, and public health responses. Twenty-eight investigations met the inclusion criteria. Investigations occurred frequently, in diverse settings, and required substantial public health resources. Most outpatient settings investigated had ≥1 infection control breach. Lapses in infection control were suspected to be the outbreak source for 16 of the reviewed investigations.

Health care services are increasingly delivered in outpatient settings rather than inpatient, acute-care settings. Nationwide, nearly 1.2 billion outpatient visits occur per year (*1*). Outpatient facilities encompass a broad array of facilities, such as primary care clinics, ambulatory surgery centers, pain clinics, oncology clinics, imaging facilities, dialysis centers, urgent care centers, and other specialized facilities. The types of procedures performed in outpatient settings are also diverse and include myriad procedures, from podiatry and nail clipping to advanced surgeries (e.g., joint replacements).

Procedures performed in outpatient settings are often invasive and carry risks of infection. Many of these procedures were previously performed in hospitals in which infection control practices are subject to regular oversight and regulation (*2*). Despite the increase in ambulatory care, there has not been a corresponding increase in infection control oversight in outpatient settings, and data are insufficient on the rates of infections resulting from procedures performed in outpatient settings (*3*).

At the same time, the amount of literature reporting a need for infection control oversight in outpatient settings is increasing. For example, during 2001–2011, there were ≥18 outbreaks of viral hepatitis associated with unsafe injection practices in outpatient settings, such as physician offices or ambulatory surgery centers (*4*). In addition, in an infection control audit conducted by the Centers for Medicare and Medicaid Services (CMS) in 2008, a total of 46 (68%) of 68 ambulatory surgery centers surveyed had ≥1 lapse in infection control; 12 (18%) had lapses identified in ≥3 of 5 infection control categories (*5*). CMS now requires adherence to its infection control surveyor worksheet for participation in CMS by ambulatory surgery centers (*6*). However, many outpatient settings opt out of participation in CMS or are not licensed by state health departments and are thus not held to the standardized infection control standards.

We recognized the infection control concerns associated with outpatient settings. Therefore, the Los Angeles County Department of Public Health (LACDPH) conducted an analysis to characterize health care–associated infection (HAI) outbreak investigations in Los Angeles County outpatient settings.

Databases and Study Population

An outpatient setting is defined as a distinct health care entity, either hospital-based or nonhospital-based, that operates exclusively on an outpatient basis for patients who do not require hospitalization and who are expected to stay <24 hours.

In California, all outbreaks, confirmed or suspected, are mandated to be reported to the respective local health department. We defined an outbreak as a suspected occurrence of cases of a disease above the expected or baseline level, over a given period of time, in a geographic area or facility, or in a specific population group (*7*). This review analyzed all HAI outbreak investigations that occurred in an outpatient setting, which included some investigations that found

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no cases but instead were enacted as an inquiry into deficient medical practices. The LACDPH is responsible for investigating communicable disease outbreaks in this county, as reported by general acute care hospitals, outpatient settings, schools, residential facilities, and other sources. Reported outbreaks are documented in the LACDPH disease control outbreak log (outbreak log) with details of the investigation, suspected source, and number of cases.

To identify suspected and confirmed HAI outbreaks that occurred in outpatient settings and which were investigated by the LACDPH during January 2000–December 2012, we reviewed the outbreak log database and other internal publications, records, and correspondence. Outbreaks were classified as confirmed if the implicated infectious disease agent was verified by laboratory findings. Outbreaks were classified as suspected when such findings could not be verified. We classified outbreak investigations by oversight (licensure and accreditation status), hospital affiliation, and type of setting. Settings that were licensed by the California Department of Public Health Licensing and Certification Division at the time of the outbreak investigation were considered to be licensed. Settings with documented accreditation at the time of the investigation by an accrediting organization recognized by the California Medical Board were considered to be accredited. Outpatient settings affiliated with a hospital were under the common ownership, licensure, or control of the hospital (*8*). Ophthalmology offices, hospital clinics, urology offices, radiology offices, pain clinics, orthopedist offices, oncology offices, obstetrics/gynecology clinics, and medical spas were grouped together into offices/clinics.

Suspected and confirmed HAI outbreak investigations were classified by number and type of infection control breaches, time until reporting, duration of investigation, and number and outcome of cases. The time until reporting of an outbreak was defined as time from onset of the first case or the time from first known exposure to the breach until notification of the Los Angeles County Department of Public Health. Duration of investigation was defined as time from notification of the LACDPH to closure in the outbreak log.

Public health response was separated into several categories, including site visit(s), medical record review, patient notification, active surveillance, recommendations to facility, sample collection, laboratory analysis, and environmental investigation. Patient notification refers to the process of informing patients about potential exposures through mailed notification letters or postage of a letter in the facility. Active surveillance is surveillance in which the LACDPH proactively solicited infection reporting (e.g., analyzed current patient medical records from facilities for case finding or surveying patients to identify additional cases).

Sample collection involved the ascertainment of biologic specimens from patients (e.g., from blood, wound, urine), environmental samples (e.g., water, air), medication samples, and samples from equipment (swab specimens from inside or outside equipment). Laboratory analyses included genetic typing, pulsed-field gel electrophoresis for DNA fingerprinting, and genomic sequencing. Laboratory analysis was either conducted by the LACDPH Laboratory or sent to the Centers for Disease Control and Prevention (CDC) laboratory or the California Department of Public Health laboratory for testing. Environmental investigations were conducted in conjunction with the LACDPH Environmental Health Division and involved evaluating facility layouts and environmental infection control procedures.

Infection control characteristics were classified into 10 categories. These categories included breaches in hand hygiene, use of personal protective equipment, injection safety, medication documentation, equipment processing and sterilization, written infection control policies and procedures, and staff credentials. Because all investigations were undertaken conducted as outbreak investigations under public health authority and considered routine public health activities, this analysis was exempt from institutional review board review.

Measures

We examined outbreak investigations by setting type, oversight, infection control findings, duration, and outcome of cases. The criterion for statistical significance was a 2-sided χ^2 test p value <0.05. Analyses were conducted by using SAS statistical software version 9.3 (SAS Institute Inc., Cary, NC, USA).

Characterization of Outbreak Investigations

Twenty-eight investigations of HAI outbreaks in outpatient settings in Los Angeles County met the inclusion criteria; 22 were classified as confirmed outbreaks and 6 were classified as suspected outbreaks. The summary characteristics for the 28 investigations are shown in Table 1 (http://wwwnc.cdc.gov/EID/article/21/8/14-1251-T1.htm).

Most identified outbreak investigations were in facilities not affiliated with a hospital (20, 71.4%). The most common settings for outbreak investigations were ambulatory surgery centers (6, 21.4%) and dialysis centers (6, 21.4%). Almost half (13, 46.4%) of the outbreak investigations occurred in settings that were licensed by the California Department of Public Health Licensing and Certification Program. We identified only 2 (7.1%) outpatient settings that were accredited at the time of the investigation, but accreditation history was infrequently documented and was difficult to ascertain. The distribution of outbreak investigations by setting type is shown in Table 2.

Outbreaks were reported 0–1,160 days after onset of the first case or exposure of the first case (median 69 days).

Table 2. Distribution of selected health care–associated infection outbreaks in outpatient settings, by hospital affiliation and setting type, Los Angeles County, California, USA, 2000–2012

Setting	No. outbreak investigations (%)	No. cases (%)
Licensed by state		
Yes	13 (46.4)	111 (66.1)
No	15 (53.6)	57 (33.9)
Hospital affiliation		
Yes	8 (28.6)	42 (25.0)
No	20 (71.4)	126 (75.0)
Type		
Office/clinic	11 (39.3)	53 (31.5)
Ambulatory surgery center	6 (21.4)	26 (15.5)
Dialysis center	6 (21.4)	70 (41.7)
Contracted home health agency	5 (17.9)	19 (11.3)

The longest delay in reporting came from an investigation into a cosmetic surgeon who had been collecting and transplanting cartilage without proper consent, storage, or donor testing. The total case-patient count was 168 (mean 6/outbreak, range 0-36); 59 case-patients (35.1%) were hospitalized, and 5 case-patients (3%) died. Bacterial agents were implicated in 50% (14) of identified outbreak investigations. Viral and fungal agents were the next most common agents implicated in these investigations.

Public Health Response

Investigations lasted a median of 36 days (range 7–94 days). The most common actions taken by the LACDPH were conducting one or more site visits (78.6% of investigations); providing written recommendations to the facility (78.6%); medical record reviews of cases and other patients (75%); formal interviews of facility staff (64.3%); and laboratory analysis (60.7%). Acute Comunicable Disease Control also often consulted CDC (50.0%) and the California Department of Public Health (35.7%) during investigations. Other outside partners consulted included the Food and Drug Administration, the Medical Board of California, and the California Board of Pharmacy. Patients who did not have cases were notified of possible risk in 7.1% of investigations. In 1 investigation, nearly 2,300 patients were notified of possible exposure. Public health response by the LACDPH is summarized in Table 3.

Infection Control Breaches

Of the 28 outbreak investigations, 22 (78.6%) had ≥1 infection control breach identified (Table 4). The mean number of infection control breaches identified by the LACDPH during the outbreak investigations was 2.4 (range 0–8). The most common breaches recorded were associated with injection safety (10, 35.7%), equipment processing and sterilization (10, 35.7%), medication documentation (7, 25.0%), and environmental cleaning (6, 21.4%). Injection safety violations included reuse of single-dose medications and not using aseptic technique to enter multidose vials. In 2010, the LACDPH investigated a hepatitis C infection that occurred at an unlicensed pain clinic in which the patient had received epidural injections from a multidose saline vial accessed with a needle that had been used on a previous patient who had hepatitis C. Breaches in equipment processing and sterilization included incomplete disinfection of reusable dialyzers after dialysis and use of incorrect cleanser and disinfection method for endoscopes. In an investigation at a urology office, it was discovered that the facility had been improperly cleaning and disinfecting cystoscopes for >10 years.

Suspected Sources of Outbreaks

Lapses in infection control were suspected as the source for 16 (57.1%) of the outbreak investigations reviewed. Suspected causes included single-use medication used on multiple patients, reuse of finger stick blood glucose meters on multiple patients, deficiencies in dialyzer reprocessing, and improper equipment cleaning and disinfection. Two (7.1%) outbreak investigations identified externally contaminated medication as the suspected source; these medications were manufactured at compounding pharmacies. Nine (32.1%) investigations did not identify a source of the outbreak. One suspected outbreak investigation found no cases and thus identified no source.

Table 3. Public health response during outbreak investigations, Los Angeles County, California, USA, 2000–2012

Public health response activity	No. (%) outbreak investigations
Site visit	22 (78.6)
Medical record review	21 (75.0)
Formal staff interviews	18 (64.3)
Sample collection	13 (46.4)
Environmental sample*	9 (32.1)
Biologic specimen (patient)	6 (21.4)
Medication sample	4 (14.3)
Laboratory analysis	17 (60.7)
Los Angeles County Public Health Laboratory	14 (50.0)
Centers for Disease Control and Prevention	9 (32.1)
Environmental health investigation	7 (25.0)
Patient interviews	6 (21.4)
Patient notification	2 (7.1)
Active surveillance	8 (28.6)
Review of facility policies and procedures	15 (53.6)
Written recommendations to facility	22 (78.6)

*Includes air, water, and equipment isolates.

Table 4. Infection control breaches noted in outbreak investigations, Los Angeles County, California, USA, 2000–2012

Infection control breach	No. (%) outbreak investigations
Injection safety	10 (35.7)
Injection preparation technique and environment	7 (25.0)
Single-use medication policies	2 (7.1)
Logging exposure events	2 (7.1)
Equipment processing and sterilization	10 (35.7)
Log of equipment maintenance	2 (7.1)
Documentation or manuals for equipment	2 (7.1)
Medication documentation	7 (25.0)
Dosage or lot number	3 (10.7)
Open date or expiration date	5 (17.9)
Environmental cleaning	6 (21.4)
Hand hygiene	5 (17.9)
Personal protective equipment	3 (10.7)
Proper glove use	2 (7.1)
Documentation of infection control policies and procedures	5 (17.9)
Credentials of staff	5 (17.9)
Single-use equipment (e.g., blood glucose meters)	4 (14.3)
Knowledge and adherence to policies and procedures	4 (14.3)

Oversight of Settings and Infection Control

The number of infection control breaches in unlicensed and licensed settings was determined. We found that mean number of infection control breaches was significantly higher when identified in unlicensed versus licensed settings. (3.3 vs. 1.3 breaches; $p<0.001$).

Conclusions

The LACDPH documented considerable illness and death associated with the 28 suspected and confirmed HAI outbreak investigations in outpatient settings included in this study. Cumulatively, more than one third of case-patients associated with these investigations were hospitalized; the mortality rate for these case-patients was 3%. Deaths and illnesses attributed to HAIs are largely considered preventable, which makes these high rates a critical concern (*14*).

Analysis showed diversity in types of outpatient and outbreak settings in Los Angeles County. A dozen different types of outbreak settings were identified, including surgery centers with multiple operating rooms; small medical spas and pain clinics; and home-like settings in assisted living centers with visiting nurses. All of these settings performing a variety of services. In addition, we demonstrated that outbreak investigations require substantial public health resources. The 28 investigations required considerable public health response, including site visits, laboratory analysis, and patient notification; the investigations lasted, on average, >1 month.

We found that infection control breaches served as the suspected source of most outbreaks. The average investigation identified several infection control lapses. The most common infection control lapses identified are consistent with those found by a national audit of ambulatory surgery centers (*5*). In a 1991 analysis of outbreaks in outpatient settings and emergency departments during 1961–1990, most identified sources were related to contaminated medical equipment and multidose medication use (*15*). Our analysis found injection safety violations and equipment cleaning issues were the most frequent sources of outbreaks. Contamination of multidose vials and reuse of syringes were common infection control breaches in Los Angeles County during 2000–2012, similar to findings of Goodman and Solomon in 1991 (*15*). The lack of change in 5 decades related to outbreak source and infections resulting from preventable unsafe behaviors is alarming. Most outbreaks documented could have been prevented by using standard precautions and practicing basic infection control (*14*,*16*). These findings highlight a need for more infection control oversight of outpatient settings, as well as better reporting from outpatient settings.

There were limitations to this analysis. This retrospective review relied on availability and completeness of investigation documents. It is possible that some investigations were not documented in the outbreak log or recalled by LACDPH personnel and consequently not included in this review. Accreditation history was difficult to obtain and was incomplete because accreditation status was not collected upon initial investigation and records from the Medical Board of California do not include settings that are now closed. Passive HAI surveillance in outpatient settings in Los Angeles County partly contributed to delayed report to the LACDPH because it heavily relies on facilities to identity and report outbreaks and conditions. The median time between onset of the first case or exposure of the first case and report to the LACDPH was 69 days; some reported years following the first exposure. It is not unusual for detection and investigation of an outbreak to occur several months or even years after the event (*17*). Delayed reporting might be a result of difficulty in tracking infections in outpatient populations (*18*). As a result of reporting issues, the findings of this study might be an underestimation of illness and death associated with HAIs in outpatient settings in Los Angeles County.

Many outpatient settings have limited patient follow-up; therefore, an outbreak in a particular outpatient facility may not be properly recognized and reported to public health authorities. Once an outbreak is identified, reporting to the LACDPH often does not follow protocol partially because some facilities are unaware of the protocol or fear repercussions of an investigation. Delayed reporting can have serious consequences for public health intervention and patient safety because it hampers the ability of public health officials to perform timely investigations and halt an outbreak. To improve reporting of postprocedure infections, outpatient settings should be encouraged to use standardized, validated reporting tools when applicable.

The National Healthcare Safety Network is a useful system for active and passive surveillance of HAIs and can

be applied to outpatient settings. Public health officials are encouraged to support the expansion of HAI surveillance and be vigilant in reaching out to and educating outpatient facilities on reporting requirements.

Finally, CDC and the Healthcare Infection Control Practices Advisory Committee created the Guide to Infection Prevention in Outpatient Settings: Minimum Expectations for Safe Care, which is intended to provide infection control and prevention recommendations to outpatient settings. Recommendations include development of an infection prevention program in the facility, specific infection prevention education and training of health care personnel, surveillance of HAIs, and adherence to standard precautions (*19*). This document might serve as a guide to outpatient settings in Los Angeles County and elsewhere for infection prevention practices.

This analysis of outbreak investigations in Los Angeles County demonstrated that HAI outbreaks in outpatient settings occur in diverse settings and can have severe impacts on affected patients and public health. Infection control standards and appropriate event reporting that are promoted, enhanced, and enforced in outpatient settings might help ensure patient safety.

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Response Strategies against Meningitis Epidemics after Elimination of Serogroup A Meningococci, Niger

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To inform epidemic response strategies for the African meningitis belt after a meningococcal serogroup A conjugate vaccine was introduced in 2010, we compared the effectiveness and efficiency of meningitis surveillance and vaccine response strategies at district and health area levels using various thresholds of weekly incidence rates. We analyzed reports of suspected cases from 3 regions in Niger during 2002–2012 (154,392 health area weeks), simulating elimination of serogroup A meningitis by excluding health area years with identification of such cases. Effectiveness was highest for health area surveillance and district vaccination (58–366 cases; thresholds 7–20 cases/100,000 doses), whereas efficiency was optimized with health area vaccination (5.6–7.7 cases/100,000 doses). District-level intervention prevented ≤6 cases (0.2 cases/100,000 doses). Reducing the delay between epidemic signal and vaccine protection by 2 weeks doubled efficiency. Subdistrict surveillance and response might be most appropriate for meningitis epidemic response after elimination of serogroup A meningitis.

For several decades, epidemic meningitis has been a major health problem in the African meningitis belt. *Neisseria meningitidis* serogroup A (NmA) has been responsible for most localized epidemics or epidemic waves, and other meningococcal serogroups occasionally caused epidemics (*1*,*2*). After the introduction of an NmA conjugate vaccine (PsA-TT, MenAfrivac; Serum Institute of India Ltd., Hadapsar, Pune, India), implemented since 2010 in mass campaigns focused on persons 1–29 years of age, no epidemics caused by NmA have occurred in countries where the vaccine is administered (i.e., vaccinated countries). Seasonal hyperendemicity continues to occur during the dry season because of meningococci and pneumococci in similar proportions (*3*), and NmA has been identified only exceptionally (*4*,*5*). So far, epidemic control measures have consisted of reactive vaccination campaigns in epidemic districts by using serogroup A/C or A/C/W polysaccharide vaccines combined with adapted treatment protocols. To detect epidemics, national routine surveillance of suspected cases of acute bacterial meningitis has been conducted in all meningitis belt countries according to World Health Organization (WHO) guidelines (*1*), although the recommendation of splitting large districts (>100,000 inhabitants) into subdistricts is not always followed. Districts notifying weekly incidences of 5 cases/100,000 persons were considered in alert, and those notifying weekly incidences of 15 cases/100,000 persons were considered in epidemic (*6*); however, specific conditions enabled declaration of an epidemic at a threshold of 10 cases/100,000 persons.

Since PsA-TT was introduced in 2010, NmA incidence has been substantially lower than historical levels, and no replacement by other serogroups has been observed (*7*). Consequently, the overall incidence of suspected meningitis cases has declined in all vaccinated countries, and established surveillance and vaccination strategies might no longer be appropriate. Epidemic detection and response remains important because serogroups W and X have epidemic potential (*1*,*2*,*4*,*8*,*9*). A polysaccharide vaccine is available against *N. meningitidis* serogroup W (NmW); vaccines for serogroup X (NmX) are under development. NmA epidemics might continue to occur, requiring mass vaccination campaigns with PsA-TT.

One approach to adapting epidemic response strategies to the changing epidemiology is to lower the weekly incidence thresholds to <10 cases/100,000 persons, which would increase the risk for an increased number of false alerts in small districts. Another approach is to analyze surveillance data at a finer spatial scale than district level. As in Burkina Faso (*2*,*10*) and Niger (*11*), epidemics of any serogroup usually are highly localized in few neighboring health centers, whereas most health centers in the district in question remain (hyper-)endemic. District-level incidences are therefore diluted and may hide epidemic activity. Consequently, surveillance at the health center

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level could detect epidemics earlier and enable targeting of reactive vaccination, making the overall strategy more effective and efficient. Early epidemic detection through surveillance at the health center level could increase the effectiveness and efficiency of response strategies, particularly in the anticipated situation of eliminated NmA meningitis and overall reduced meningitis incidence.

Therefore, during 2002–2012, we evaluated the effectiveness and efficiency of surveillance and vaccine response strategies in Niger using various epidemic thresholds and comparing health area and district intervention. Our analysis was based on surveillance data of suspected and confirmed cases and considered 2 scenarios: the historical situation before PsA-TT introduction (PsA-TT was introduced in Niger in 3 phases: the first in August 2010, the second in November 2010, and the third in October 2011) and a simulation of NmA elimination.

Methods

Databases

The surveillance data used for this analysis were collected during 2002–2012 in Niger. For national routine surveillance, all health centers in Niger report suspected meningitis case counts each week to health district administration, where data are aggregated to a district case count and forwarded to the national level for reporting (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/21/8/14-1316-Techapp1.pdf) (*12*,*13*). To analyze response strategies at the health area level, we retrieved the original health center counts of suspected meningitis cases from health district administrations and compiled them in a new database. A health area is a geographic area that encompasses all villages served by the same health center, which is an exclusive association. We assessed the completeness of this health area database of suspected cases by comparing the resulting weekly district case counts with those in the national surveillance reports. On the basis of the completeness of this health area–level database (online Technical Appendix), we selected 3 regions—Tahoua, Tillabery, and Dosso—for further analysis (Figure 1). Together, these regions had 7,648,150 inhabitants during 2012 (48% of Niger's population), 19 health districts (47%), and 373 health areas (51%). Niger used *Haemophilus influenzae* type b vaccine since 2006 but uses no pneumococcal vaccine in the widened vaccine program.

Data on confirmed cases came from countrywide surveillance based on PCR (*11*). For this surveillance, we requested that all cerebrospinal fluid samples from persons with suspected meningitis in the health centers be sent to the Centre de Recherche Médicale et Sanitaire (Niamey, Niger) for testing by multiplex PCR (*14*). After merging with the study database of suspected cases, we obtained the combined information about suspected and confirmed meningococcal case counts for every health area and week.

Using these data, we prepared a database simulating the situation after NmA elimination by excluding all health area years with at least 1 NmA case or without any laboratory information. A health area year corresponded to a health area that appears in the annual reporting file, so that in our analysis health area years represented all the health areas that appear in annual reporting files during the study

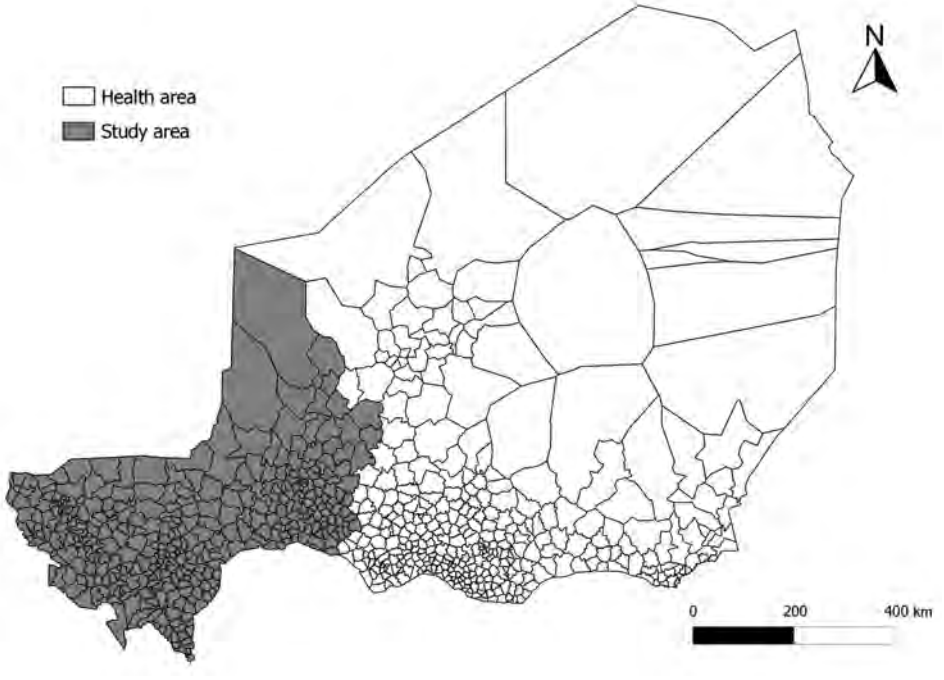

Figure 1. Location of the study area, Niger. This area comprises the 379 health areas in 3 regions (Tahoua, Tillabery, and Dosso).

period. Only 1 NmA case was identified in the study region after PsA-TT introduction. To validate the representativeness of this database for meningitis epidemiology after NmA elimination, we compared the distribution of annual incidences between health areas before and after PsA-TT introduction (Figure 2). To prepare the database simulating the situation after NmA elimination, we took into account the exact week of district vaccination, which was conducted in 3 phases: September 2010, December 2010, and November 2011. The Niger national ethics committee approved this research (no. 014/2012/CCNE).

Statistical Analysis

The Institut National de la Statistique provided the number of inhabitants in each village according to the 2001 national census. We aggregated the villages' populations at the health area level and applied a mean annual growth rate of 3.3% (*15*). We calculated weekly incidence rates (WIR) as the weekly number of cases per 100,000 inhabitants and annual incidence (AI) as the number of cases per 100,000 inhabitants during an epidemiologic year. To compile cases belonging to the same meningitis season (approximately December–May), we defined an epidemiologic year as July 1 of calendar year *n*–1 through June 30 of calendar year *n*.

We evaluated the effectiveness and efficiency of surveillance and vaccine response strategies by calculating the number of potentially vaccine-preventable cases and the number of vaccine doses needed per epidemic. These calculations were repeated with the database including NmA to determine whether the situation differed from that simulating NmA elimination.

Using receiver–operator curves as previously described (*10*), we chose candidate epidemic thresholds to optimize the detection of annual incidences that are above the 95th or 97.5th percentiles of health area annual incidences in the databases (online Technical Appendix Figure 3). An epidemic was defined as weekly incidence rate in a health area that exceeded the corresponding threshold for at least 1 week.

We evaluated 3 strategies: 1) surveillance (including data analysis) and vaccination at health area level; 2) surveillance at health area level and vaccination of the entire district; and 3) surveillance and vaccination at district level. We approximated the number of potentially vaccine-preventable cases (Nvp) as Nvp = N*3w* × P*Nm* × VC × VE, where N*3w* is the number of suspected cases in the surveyed health area or district from 3 weeks after the threshold is exceeded (assuming that campaign implementation and effective protection from vaccine antibody would take at least 3 weeks after signal detection); P*Nm* is the percentage of suspected cases confirmed as Nm, estimated as 50% in both epidemic and endemic periods, on the basis of previous surveillance reports (*16*); VC is the expected vaccine coverage during a mass campaign in response to an outbreak, estimated at 80% (*5*); and VE is the expected vaccine effectiveness, estimated at 80%. Because no data for NmW and future NmX vaccines (polysaccharide or conjugate) were available to inform this assumption, we used the available vaccine effectiveness data of NmA polysaccharide vaccine (*17*) and NmW and NmA polysaccharide vaccine combined (*18*). This assumption might be conservative, in particular because conjugate vaccines will be used for epidemic response against non-A serogroups (*19*). We calculated the number of vaccine doses needed per epidemic as the age group 1–29 years of the total population, which was 74% for the study regions according to census data (*20*); and the total number of preventable cases

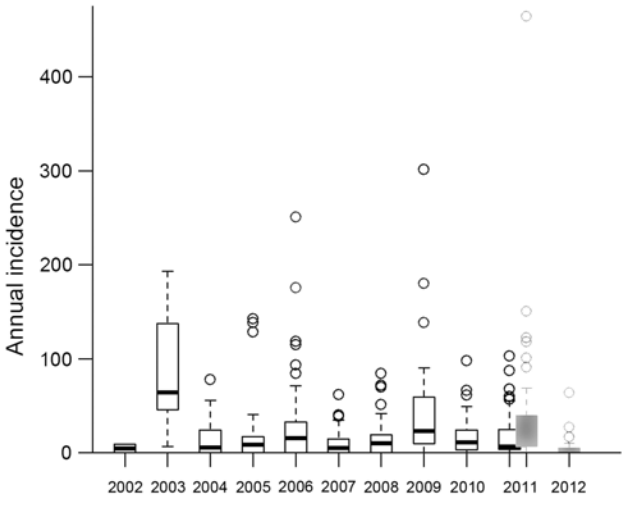

Figure 2. Annual incidences of suspected meningitis per 100,000 inhabitants in health areas before and after introduction of PsA-TT (in 2010) in a database simulating elimination of serogroup A meningococci. Tahoua, Tillabery, and Dosso regions, Niger, 2002–2012. The period before PsA-TT (2002–October 2011, last phase of vaccination during November 2011) (white bars) comprises 433 health area years. The period after PsA-TT (October 2010–December 2012, first phase of vaccination during September 2010) (gray bars) comprises 98 health area years. Excluded were health area years during which at least 1 serogroup A case was detected or for which no serogroup information was available. The number of health areas in this database varied by year from 2 (2002) and 10 (2003) to 126 (2011). Each circle corresponds to annual incidence in a health area. Dark lines are parts of the boxplot, as follows: for 2003 (complete boxplot), the first line corresponds to the minimal annual incidence of health area, the second line corresponds to the limit of the first quartile, and the third (darkest) corresponds to the median. The space between the second and third lines corresponds to the second quartile. The fourth line is the limit of the third quartile and the last line is the limit of the fourth quartile of annual health area incidence. PsA-TT, serogroup A meningococcal conjugate vaccine (MenAfrivac [Serum Institute of India Ltd., Hadapsar, Pune, India]).

in the population of interest for 100,000 vaccine doses. To evaluate the sensitivity of our estimates to a longer delay from signal detection to effective vaccine protection, we varied this delay from 3 weeks to 6 weeks. We used the rate ratio test to compare annual incidences assuming that they follow a Poisson distribution.

All analyses were performed in R software version 2.15.2 (http://www.R-project.org). Maps were created with QGIS software version 1.8.0 (http://qgis.osgeo.org).

Results

Descriptive Epidemiology

Our study comprised 154,392 weekly health area reports. Among the reports were 14,921 suspected cases, of which 13,620 (91.3%) occurred during calendar weeks 1–20, corresponding to the meningitis season (online Technical Appendix Figure 2). At district level, median AI was 10.5 cases per 100,000 inhabitants; WIR peaked at 10 cases per 100,000 during most years, and maximum incidences were 50 in Tillabery region during 2006 and 30 in Tahoua region during 2008. At health area level, median AI was 0 cases per 100,000 inhabitants, with a maximum of 1,384 for health areas in Tillabery, 959 in Tahoua, and 777 in Dosso. WIR peaked at 613 cases per 100,000 inhabitants.

In the database simulating NmA elimination, median district-level AI was 10.5 cases (range 0–166) per 100,000 inhabitants; WIR peak was a median of 2 (maximum 50) per 100,000. Median health area–level AI was 10 cases (maximum 464) per 100,000 inhabitants; WIR peaked at 172 per 100,000.

In the database simulating NmA elimination, median district-level AI was 13 cases (range 0–166) per 100,000 inhabitants before and 4 (range 0–41) per 100,000 after PsA-TT introduction. Median health area–level AI was 10 cases (range 0–302) per 100,000 inhabitants before and 9 (range 0–464) per 100,000 after PsA-TT introduction (Figure 2). Mean health area–level AI was 21 cases per 100,000 inhabitants before and 26 per 100,000 after PsA-TT introduction ($p = 0.43$). The similarity of these numbers suggests that the simulation of NmA elimination based on data before PsA-TT introduction is representative of the situation after PsA-TT introduction.

Evaluation of Strategies

In the database simulating NmA elimination, the thresholds providing best sensitivity and specificity to detect AI beyond the 95th and 97.5th percentiles were, at health area level, WIR of 20 and 15 cases per 100,000 inhabitants, respectively, and at district level, WIR of 4 per 100,000, for both percentiles (online Technical Appendix Figures 3, 4).

The total number of epidemics requiring a response during the 11-year period in the study area varied from 3 to 15 for district surveillance and from 49 to 233 for health area surveillance. The number of total vaccine-preventable cases varied from 0 to 6 (thresholds 2–7/100,000) with district surveillance and vaccination, from 27 to 213 cases (thresholds 7–20/100,000) with health area surveillance and vaccination, and from 58 to 366 cases with health area surveillance combined with district-level vaccination (thresholds 7–20/100,000) (Table 1). Although the latter strategy was most effective, it required the largest number of vaccine doses (8.7–25.7 million, depending on the threshold), whereas the 2 other strategies consumed a similar number of doses (≈0.5–3 million). Efficiency was lowest for district surveillance (≤0.24 vaccine-preventable cases/100,000 doses) and highest for health area surveillance and vaccination (7.7 cases/100,000 doses). Overall, the difference in effectiveness or efficiency for different geographic levels of intervention was greater than for any

Table 1. Comparison of estimated vaccine-preventable meningitis cases using different strategies of surveillance and meningococcal vaccine response in a situation simulating elimination of meningococcal serogroup A, Tahoua, Tillabery and Dosso regions, Niger, 2002–2012*

Strategy, surveillance–vaccination	Threshold	Total no. epidemic signals	Population affected by signal	Vaccine doses in persons 1–29 y†		Vaccine-preventable cases‡				
						No.		Per 100,000 cases		
				Total	Median	Total	Median	Total	Median	Range
Health area–health area	7	233	3,741,116	2,768,426	9,721	213	0.32	7.70	2.31	0–178.35
	10	165	2,453,831	1,815,835	9,346	119	0.32	6.54	2.38	0–178.35
	15	80	1,142,888	845,737	8,817	53	0.00	6.32	0.00	0–178.35
	20	49	641,378	474,620	8,272	27	0.00	5.60	0.00	0–41.02
Health area–district	7	233	35,297,443	25,866,453	258,625	366	0.96	1.42	0.44	0–14.38
	10	165	31,304,108	23,165,040	259,647	246	2.80	1.06	0.27	0–13.23
	15	80	17,062,861	12,626,517	284,407	126	0.96	1.00	0.30	0–7.87
	20	49	11,757,953	8,700,885	287,661	58	0.80	0.66	0.23	0–3.36
District–district	2	15	4,053,961	2,999,931	216,403	6	0.00	0.20	0.00	0–1.56
	4	8	2,710,118	2,005,487	269,749	5	0.00	0.24	0.00	0–1.56
	7	3	936,406	692,940	250,957	0	0.00	0.00	0.00	0

*Weekly incidence rate thresholds (cases/100,000 inhabitants were selected on the basis of best performance (sensitivity and specificity) to identify years of high annual incidence (531 health area years). The 3 regions had 7.5 million inhabitants.
†Case-patients <2 y of age were not excluded.
‡Median, across all health areas or districts with epidemic signal; total: for entire study area of 3 regions (population 7,648,128); mean: mean per signal (health area or district level).

change of threshold. Among individual health areas, the effect of any vaccine response followed a skewed distribution: the median number of preventable cases per 100,000 doses was close to 0 in most scenarios, and a maximum of 178 cases were preventable.

When the assumed delay between epidemic signal and effective protection from vaccine antibody increased from 3 to 5 weeks, efficiency was halved, and the difference among the strategies was halved. At 6 weeks' delay, health area surveillance and vaccination strategy remained the most efficient (2–5 cases/100,000 doses). District surveillance and vaccination at any threshold failed to prevent cases in a delay of ≥4 weeks (Figure 3).

When we evaluated data within the entire database for 2002–2012, including health areas with NmA identification in a year (Table 2), the total number of epidemics requiring response varied from 18 to 92 for district activity and from 232 to 844 for health area activity. The number of total vaccine-preventable cases varied less with geographic level than with threshold (overall range 652–3,739 cases). By contrast, efficiency varied more strongly with geographic level than with threshold; health area surveillance and vaccination prevented 21–26 cases per 100,000 doses, compared with 14–16 cases for district intervention.

Discussion

Using meningitis surveillance data from Niger during an 11-year period, we estimated that after elimination of NmA from the meningitis belt surveillance and epidemic vaccine response would be more effective and efficient if conducted at the health area level rather than at the district level, as is currently done in most meningitis belt countries. In Niger, health areas typically have a median population of 14,440 inhabitants, ≈20-fold smaller than districts (median 295,200 inhabitants). Although the WHO epidemic response guidelines recommend splitting large districts (>100,000 inhabitants) into subdistricts for data analysis (*12*), surveillance data during the last decade have been analyzed at no higher resolution than district level in most instances. Our analysis confirms that before NmA elimination and before PsA-TT introduction, the district-level strategy yielded good effectiveness and efficiency comparable with health area–level analysis of surveillance data, while it minimized the number of individual events requiring a response. In light of these data, we recommend that more countries explore the expected impact and feasibility of subdistrict-level surveillance and vaccination after NmA elimination.

Although meningococcal polysaccharide vaccines have been used in the meningitis belt for several decades, including occasionally for prevention strategies, no evidence has been found of a major effect on meningococcal epidemiology beyond outbreak control. By contrast, PsA-TT, a protein-conjugate vaccine, appears to have eliminated NmA disease in the region, probably because conjugate vaccines induce much higher antibody levels than do polysaccharide vaccines (*21*), leading to mucosal immunity and persisting longer. Mucosal immunity leads to reduced transmission in vaccinees and to indirect protection against infection and disease for the entire population. PsA-TT mass campaigns focusing on persons in a large range of ages have achieved high coverage in Niger (*22*), such that circulation of NmA has been drastically reduced and NmA disease eliminated. This interpretation is supported by evidence from Chad (*23*) and Burkina Faso (*24*). Further surveillance is needed to show the duration of this elimination, but if a long-term vaccination strategy that includes protection of new birth cohorts were established, this situation could be sustainable. Consequently, surveillance and epidemic response for meningitis outbreaks of other etiologies such as other meningococcal serogroups or pneumococcus will need to be adapted for the long term.

In our analysis, in the event of a health area–level epidemic signal, vaccinating the entire district achieved higher effectiveness than did focusing on the epidemic health area, whereas the latter appeared more efficient relative to the number of vaccine doses required. An intermediate strategy might involve ring vaccination of health areas neighboring the epidemic area. In any case, because vaccine campaigns would be launched in small health areas after as few as 2 or 3 cases, the choice of exact strategy will need to account for country-specific factors, such as logistic and economic constraints and preferences. The availability of laboratory confirmation will provide further arguments.

Given the short duration of individual meningococcal meningitis epidemics, rapid decision making and implementation of mass campaigns is essential to maximizing the impact of the epidemic response. In our analysis, decreasing the delay from signal detection to effective vaccine protection by 2 weeks doubled efficiency (and number of cases prevented). The updated version of the WHO guidelines further emphasizes this aspect. However, several relatively fixed factors make it challenging to reduce the currently observed delay, such as weekly rhythm of data analysis, storage of vaccines at a central location in a country or even at international level, with corresponding administrative and transport delay, and time required to mobilize vaccination teams. More reactive signal detection in health areas, possibly combined with decentralized vaccine management at the regional level, is therefore an interesting option for improving the impact of reactive vaccination.

Our analysis indicated that several hundred cases were preventable during the 11-year period; however, among individual epidemics, a median of near 0 cases were prevented per 100,000 doses, and a maximum of 180 cases were prevented. This finding indicates that in many epidemic health areas, no cases would be prevented by reactive vaccination,

Table 2. Comparison of estimated vaccine-preventable meningitis cases using different strategies of surveillance and meningococcal vaccine response before introduction of PsA-TT, Tahoua, Tillabery, and Dosso regions, Niger, 2002–September 2011

Strategy, surveillance–vaccination	Threshold*	Total no. epidemic signals	Population affected by signal	Vaccine doses in persons 1–29 y		Vaccine preventable cases				
						No.		Per 100,000 doses		
				Total	Median	Total	Median	Total	Median	Range
Health area–health area	7	844	14,742,136	10,909,180	10,051	2,282	0.64	20.92	5.18	0–595.34
	10	679	11,567,626	8,560,043	10,081	1,924	0.64	22.48	5.41	0–595.34
	15	469	7,849,057	5,808,302	9,587	1,332	0.64	22.93	5.59	0–482.10
	20	358	5,791,671	5,791,671	9,307	1,087	0.64	25.36	6.14	0–482.10
	30	235	3,719,950	3,719,950	8,919	717	0.64	26.05	8.25	0–482.10
Health area–district	7	844	47,090,036	34,846,626	250,877	3,739	4.64	10.73	3.42	0–70.20
	10	679	44,361,151	32,827,251	251,352	3,353	5.76	10.21	2.55	0–70.20
	15	469	36,118,246	26,727,502	251,574	2,689	4.80	10.06	2.32	0–69.16
	20	358	29,902,138	22,127,582	250,956	2,276	4.48	10.29	1.75	0–58.53
	30	235	23,115,711	17,105,626	244,380	1,612	2.88	9.43	1.27	0–58.53
District–district	2	92	29,251,073	21,645,794	244,595	3,103	12.00	14.33	6.05	0–61.19
	4	68	21,069,851	15,591,689	244,595	2,343	13.60	15.03	5.35	0–61.19
	7	44	14,075,282	10,415,708	252,666	1,483	17.44	14.24	7.93	0–46.79
	10	18	6,021,142	4,455,645	252,049	652	18.40	14.64	9.74	0–35.28
	15	18	6,021,142	4,455,645	252,049	652	18.40	15.64	9.74	0–35.28

*Weekly incidence rate thresholds were selected on the basis of best performance (sensitivity and specificity) to identify years of high annual incidence (2,534 health area years). The 3 regions had 7.5 million inhabitants.

but a large benefit would appear in a few situations, probably where the outbreak is longer. Our analysis overestimates current potential vaccine impact because a proportion of the outbreaks resulted from NmX, for which no vaccine exists. Other assumptions may lead to overestimation or underestimation of vaccine-preventable cases, but regardless, the overall expected impact from reactive vaccination after NmA elimination may be limited. An exception might be a strong increase in the percentage of Nm among suspected cases that result from emergence of non-A serogroups that might increase the effectiveness of any strategy.

Bringing data resolution for meningitis surveillance to the health area level would require relatively few additional means: throughout the meningitis belt, suspected case data are collected in health centers and transmitted in aggregate form by districts to the national level so that a lower degree of aggregation would accelerate data availability. Routine procedures for data management and analysis would need to be slightly modified, but analyses would consist mostly in automated signal detection. Estimates of the referring population usually are available from national census data of villages and health intervention planning. However, several countries may encounter difficulties related to the availability of reliable and up-to-date demographic estimates at health center level. In this case, countries could consider and test other subdistrict resolutions of data analysis. In contrast, substantial efforts would be needed to bring vaccine response to the health area level because bringing response to this level would require decentralization of vaccine management, including prior distribution of vaccine to regions or districts. As a positive side effect, bringing vaccine response to the health area level would further speed up reactive vaccination and thus increase the effectiveness of outbreak response. More focused vaccination would require fewer human resources than do districtwide campaigns and therefore reduce health care disruption, a major shortcoming of mass campaigns. In any instance, countries will need to explore feasibility of subdistrict surveillance and vaccination, identify optimal resolutions, and base decisions on their means and priorities.

Our analysis has several limitations, related mainly to the nature of the available data. We selected only 3 regions in Niger, for which health area counts of suspected case corresponded best with district counts documented nationally. Our analysis therefore did not include densely populated regions at the border with Nigeria, which have regularly reported epidemics, including epidemics caused by NmW or NmX. Furthermore, reporting and sample submission might have been more exhaustive and precise during epidemic situations, leading to overestimation of incidence and of the contribution of epidemic agents. When preparing the database simulating NmA elimination, we excluded health areas without any laboratory confirmation in a year, which includes health areas without any suspected cases. That most health areas report at least 1 case per year probably has led to an overestimation of the median annual incidence after NmA elimination. However, this fact does not affect our estimation of effectiveness and efficiency because these health areas would not have contributed to any epidemic signal. Finally, we calculated incidence rates using the 2001 population census data, to which we applied a constant population growth rate, instead of accounting for potential spatio-temporal variations in migration, ethnic factors, and fecundity rate. We believe that the differences we found among surveillance strategies are sufficiently robust to justify revisiting the current surveillance strategies. By combining routine case reporting and confirmed case incidence at health area level, our database provides a rare opportunity for investigating alternative surveillance strategies around the change in serogroup. Other countries

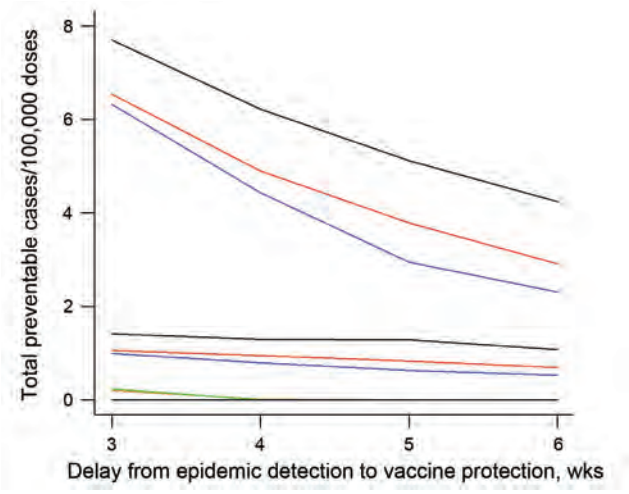

Figure 3. Comparison of preventable meningitis cases per 100,000 vaccine doses, given different surveillance and meningococcal vaccine response strategies, in a situation simulating elimination of *Neisseria meningitides* serogroup A, Tahoua, Tillabery, and Dosso regions, Niger, 2002–2012. Three, 4, 5, and 6 weeks delay were considered between epidemic detection and effective vaccine protection. The strategies were surveillance and vaccination at health area level (health area–health area, top 3 lines), surveillance at health area level combined with vaccination of the district (health area–district, middle 3 lines), and surveillance and vaccination at district level (district–district, bottom 3 lines). For the health area–health area and health area–district strategies, the black line indicates preventable cases/100,000 vaccine doses at an incidence threshold of 7 cases/100,000 inhabitants; red line, threshold of 10 cases/100,000 inhabitants; and blue line, threshold of 15 cases/100,000 inhabitants. For the district–district strategy, the green line indicates preventable cases/100,000 vaccine doses at an incidence threshold of 4 cases/100,000 inhabitants; orange line, threshold of 2 cases/100,000 inhabitants; and black line, threshold of 7 cases/100,000 inhabitants.

should analyze suspected case incidence after PsA-TT introduction at health center level over the next few years to validate our findings and to inform optimal epidemic surveillance and response strategies.

Our evaluation highlights the value of continuous meningitis surveillance over longer periods and at subdistrict resolution and has the potential to guide future recommendations for reactive vaccination. Also, given a possibly limited absolute impact of reactive vaccination, the results recall that improved prevention strategies should be developed to reduce the effects of acute bacterial meningitis in the African meningitis belt, for example using multivalent meningococcal and pneumococcal vaccines in extended target age groups.

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H.B.M. and J.E.M. contributed substantially to the conception of the work; H.B.M., I.I., J-P.M.P., O.O.M.O.-B., and A.F. acquired the data; H.B.M., J.P., and J.E.M. analyzed and interpreted the data; H.B.M. drafted the manuscript; J.P., I.I., J.M., O.O., A.F., and J.E.M. conducted the critical revision of the work for important intellectual content; H.B.M., J.P., I.I., J-P.M.P., O.O.M.O.-B., A.F., and J.E.M. gave final approval of the version submitted and agree to be accountable for all aspects of the work.

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Phylogeography of Influenza A(H3N2) Virus in Peru, 2010–2012

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It remains unclear whether lineages of influenza A(H3N2) virus can persist in the tropics and seed temperate areas. We used viral gene sequence data sampled from Peru to test this source–sink model for a Latin American country. Viruses were obtained during 2010–2012 from influenza surveillance cohorts in Cusco, Tumbes, Puerto Maldonado, and Lima. Specimens positive for influenza A(H3N2) virus were randomly selected and underwent hemagglutinin sequencing and phylogeographic analyses. Analysis of 389 hemagglutinin sequences from Peru and 2,192 global sequences demonstrated interseasonal extinction of Peruvian lineages. Extensive mixing occurred with global clades, but some spatial structure was observed at all sites; this structure was weakest in Lima and Puerto Maldonado, indicating that these locations may experience greater viral traffic. The broad diversity and co-circulation of many simultaneous lineages of H3N2 virus in Peru suggests that this country should not be overlooked as a potential source for novel pandemic strains.

Worldwide, influenza virus causes substantial illness and death and considerable public health costs (*1*). Like other countries, Peru experiences a significant number of influenza cases (*2*,*3*). The epidemiology of influenza virus in tropical and low- to middle-income countries and the role they play in global influenza ecology remains unclear (*4*). One outstanding question is whether a global source–sink dynamic exists. In the source–sink model, countries have putative tropical sources of influenza characterized by year-round (or multiannual) transmission, local persistence of influenza lineages, and relatively high genetic diversity. Then, it is postulated, that influenza lineages migrate and seed seasonal epidemics in cooler temperate regions, where they experience interseasonal extinction (*5*). Determining if and where this source–sink dynamic exists is of major importance because the results could guide targeted influenza surveillance for vaccine recommendations, pandemic planning, and prediction of novel strains (*4*,*6*).

Most analyses of whether a global source population exists have focused on East and Southeast Asia, in part because several pandemic and seasonal epidemics appear to have originated in those areas (*7*–*11*). Because of the lower availability of local influenza sequence data from tropical Latin America, relatively little is known about the possible role that region plays in global influenza dynamics (*12*). Nonmolecular epidemiologic studies have hinted at climate-driven patterns of influenza virus spread in South America; for example, diffusion of influenza activity from tropical to temperate areas has been noted in Brazil (*13*). Peru's diverse climates make it an ideal location to test aspects of the source–sink model in Latin America, particularly because some tropical areas in Peru are known to experience year-round influenza activity (*14*). In recent years, prospective community-based influenza-like illness (ILI) surveillance cohorts were established in multiple regions of Peru, providing a unique opportunity to examine the epidemiology of human influenza virus (*15*).

Our study objectives were to determine whether 1) a source–sink influenza dynamic exists within Peru, including the existence of genetically diverse hubs and virus lineage persistence between seasons; 2) Peru could act as a global source for influenza virus lineages that could seed temperate regions; and 3) influenza virus is circulating within Peru in a closed system. We also sought to compare the spatial dynamics of influenza A(H3N2) virus across the 4 climatically and demographically diverse Peruvian sites.

We based our analysis on human influenza A(H3N2) virus because, over a long-term scale, it is the best represented lineage in sequence databases, and it has caused regular seasonal influenza epidemics in both hemispheres, including in Latin America (*16*,*17*). Although much attention has been paid to the study of pandemic influenza A(H1N1) pdm09 virus (*18*), H3N2 virus remains a significant cause of influenza in Peru, is a dominant seasonal influenza A

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virus subtype in other regions of the world, and causes substantial illness and death in Peru and beyond. A key aspect of this study is that we obtained samples from diverse ecologies and populations, including viruses from large urban and semirural locations and diverse altitudes and climates, and the distance between study sites was sufficient to allow spatial analysis. In addition, the prospective cohort studies involved continuous, active, year-round surveillance that enabled capture of any interseasonal strains.

Materials and Methods

Study Setting, Enrolment Criteria, and Field Procedures

In 2009, the United States Navy Medical Research Unit No. 6 (NAMRU-6), the Centers for Disease Control and Prevention (CDC), and the Peruvian Ministry of Health established a community-based prospective ILI cohort (Proyecto Influenza) in 4 ecologically distinct regions of Peru. Sites were chosen to represent the diverse ecologies, climates, and population structure in Peru. Lima, on the central desert coast, is Peru's capital and largest city and a transport hub for the rest of the nation. Lima has a population of 8,348,400 persons and a temperate climate with little rain (*19*). Puerto Maldonado, in the southern Amazon Basin, has a population of 89,500 persons. The city has high annual rainfall and a warm, humid climate year-round (*19*). Cusco is a high-altitude (3,200 meters) city in the southern Andes Mountains. This southern highlands city has a population of 420,030 persons (*19*). Tumbes is a northern equatorial coastal city of 157,760 persons (*19*).

Enrollment criteria and field procedures were as described elsewhere (*15*). In brief, during 2010–2012, households were selected from each study site by using a computer-based randomization process. An adult head and all residents of the household were eligible for enrollment. Participants were assessed 3 times per week for the development of ILI. For children <5 years of age, ILI was defined as sudden onset of fever (≥38°C) and cough, sore throat, or coryza. For persons ≥5 years of age, ILI was defined as sudden onset of fever (≥38°C) with cough, sore throat, or both. We administered a household enrolment form in which sociodemographic and risk factor data were collected. Nasal and throat swab samples for virus identification were obtained from persons with signs meeting the ILI case definition; a rapid influenza test was performed so that immediate medical referral could be made if necessary.

Ethical Approval

The NAMRU-6 Institutional Review Board approved the study. Informed written consent was obtained at the time of enrolment from each adult participant and from a parent or guardian of children. NAMRU-6 participation was under protocol NMRCD.2009.005, which is in compliance with all applicable US federal regulations governing the protection of human subjects.

Detection of Influenza Virus in Nasal or Throat Swab Specimens

Nucleic acid was extracted from nasal and throat swab specimens in universal transport media by using the QIAamp Viral RNA Isolation Kit (QIAGEN, Valencia, CA, USA). Reverse transcription PCR (RT-PCR) for influenza detection, including subtype, was performed by using primers and probes from the CDC Human Influenza Virus Real-Time RT-PCR Diagnostic Panel (Influenza Reagent Resource, CDC, Atlanta, GA, USA). Original respiratory samples were then stored at −80°C at NAMRU-6 Peru.

Identification of Sequences for Phylogenomic Analyses and Generation of Sequence Data

Over the study period, we randomly selected 100 H3N2 virus–positive (RT-PCR cycle threshold <29) specimens from each study site (400 total). Original respiratory specimens were sent (at −80°C) from NAMRU-6 Peru to the J. Craig Venter Institute (Rockville, MD, USA) for extraction and hemagglutinin (HA) gene sequencing. GenBank accession numbers for the consensus sequences are available in online Technical Appendix Table 1 (http://wwwnc.cdc.gov/EID/article/21/8/15-0084-Techapp1.pdf). Viral RNA was isolated by using the ZR 96 Viral RNA Kit (Zymo Research Corporation, Irvine, CA, USA). The influenza A virus genomic RNA segments were simultaneously amplified from purified RNA (3 mL) by using a multisegment RT-PCR strategy (*20*,*21*). Amplicons were sequenced by using the Nextera DNA Sample Preparation Kit Library construction and the Illumina MiSeq version 2 platform (both from Illumina, Inc., San Diego, CA, USA) or the Ion Xpress Plus Fragment Library Kit and the Ion Torrent PGM platform (both from Thermo Fisher Scientific, Waltham, MA, USA). The sequence reads were sorted by barcode and trimmed, and chimeric influenza virus sequences and noninfluenza sequences were removed. The next-generation sequencing reads were then mapped to the best matching reference virus by using the CLC Bio Assembly Cell 3.0 program clc_ref_assemble_long (http://www.clcbio.com/products/clc-assembly-cell/) (*22*). At loci where next-generation sequencing platforms agreed on a variation (as compared with the reference sequence), the reference sequence was updated to reflect the difference. A final mapping of all next-generation sequences to the updated reference sequences was then performed.

Collation of Background Sequence Data, Alignment, and Evolutionary Model Selection

Global background H3N2 HA sequences were obtained from the National Institute of Allergy and Infectious

Disease Influenza Research Database (IRD; http://www.fludb.org/brc/home.spg?decorator=influenza) (*23*) and the Global Initiative on Sharing Avian Influenza Data EpiFlu Database (http://platform.gisaid.org/epi3/frontend#f989c). Sequences for viruses obtained during January 2004–August 2013 from the following regions were sampled (nos. in parentheses indicate no. of sequences): South America, excluding Peru (193); Australia, New Zealand, and Oceania, excluding Hawaii (259); East and Southeast Asia (374); Middle East/Central Asia, including Russia (110); Europe (235); Central America and the Caribbean (116); Mexico (27); Canada (234); the United States, including Hawaii (549); and Africa (79). In addition, 16 sequences for strains collected in Peru during 2006–2013 were obtained through IRD or the EpiFlu Database. A total of 2,192 background sequences were selected (online Technical Appendix Tables 2–4).

To improve phylogenetic resolution, only complete or near-complete HA sequences (containing at least the entire HA1 region) were included. For geographic regions with an abundance of full HA1 sequences in GenBank (e.g., Asia, United States), intermittent sequences were manually selected from a list sorted by country in the IRD. For underrepresented geographic regions (e.g., Africa, South America), all available full HA1 sequences were included to overcome ascertainment bias. Accession numbers (GenBank and EpiFlu Database) for these comparator sequences are shown in online Technical Appendix Tables 2–4.

Untranslated regions were trimmed, and duplicate sequences were removed, resulting in a final dataset of 2,581 sequences 1,639–1,700 nt in length; 1 partial sequence was 1,324 nt long. A second dataset of 389 sequences (1,700 nt long) was constructed for viruses from Peru. All sequences were aligned before inspection by using the MUSCLE algorithm in MEGA5.2 and hand-edited for final correction (*24*) A best-fit model of nucleotide substitution (general time-reversible with a gamma-distributed rate variation among sites and a proportion of invariant sites) was selected by using jModelTest2 software (*25*).

Global Phylogenetic Analysis

A maximum-likelihood tree of all 2,581 H3 sequences was inferred by using RAxML software version 7.26 (*26*). Statistical robustness was tested by nonparametric bootstrap resampling analysis (500 replicates). Inferred maximum-likelihood trees were viewed and annotated by using FigTree software (http://tree.bio.ed.ac.uk/software/figtree/).

Bayesian Analyses of Peruvian Sequences

We analyzed 389 HA time-stamped sequences (i.e., labeled with the time of sampling to the nearest day) for viruses from Peru by using the Bayesian Markov chain Monte Carlo method in BEAST (*27*); the results enabled inference of the time-scale of the viruses' epidemiologic histories. For this analysis, we selected a Bayesian skyline demographic model was selected and, assuming a strict molecular clock rate (under a uniform prior), we selected the Hasegawa-Kishino-Yano nucleotide substitution model with a discrete-gamma distribution in place of other, more complex models that likely overparameterized the data. The analysis was run by using a 500,000,000-step Markov chain, sampling every 50,000 states. A 10% burn-in was removed, and statistical convergence was determined by parameter values with effective sample size values >200. The posterior distribution of trees was summarized as the maximum clade credibility tree, as generated by using TreeAnnotator version 1.75 (http://beast.bio.edu.ac.uk/TreeAnnotator/) and visualized by using FigTree.

For viruses from Peru, the posterior distribution of HA trees from BEAST was also used to assess the strength of geographic clustering in the data by using the phylogeny-trait association test available in the Bayesian Tip-association Significance testing package (*28*). For this analysis, each sequence was given a geographic code reflecting its place of origin. The overall statistical significance of geographic clustering of all Peruvian sequences by location was determined by calculating observed and expected association index and parsimony score statistics for the entire Peruvian sequence dataset, where the null hypothesis is that clustering by geographic location is not more than that expected by chance. In addition, the maximum clade statistic was used to compare the strength of clustering at each location by calculating the expected and observed mean clade size from each of the 4 study locations. A significance level of $p<0.05$ was used in all cases.

Results

Of the 400 H3N2 PCR–positive specimens selected from the NAMRU-6 repository, 389 HA segments were successfully sequenced (online Technical Appendix Table 1). The distribution of successfully sequenced H3N2 HA genes by year and location relative to other co-circulating influenza virus subtypes in the study period is presented in Table 1. Well-distributed sampling in all sites for all years was impossible because of differences in specimen quality and because overall H3N2 virus activity in the cohorts was considerably less overall during 2011–2012 than in 2010, partly due to the dominance of influenza B virus in 2012. Thus, the sampling was skewed toward 2010 and toward fewer sequences for Cusco and Puerto Maldonado in 2012 and Tumbes in 2011.

Phylogenetic analysis of the 389 study sequences for viruses from Peru and 2,192 global HA sequences revealed extensive geographic mixing (Figures 1, 2; fully labeled

Table 1. Distribution of sequenced influenza A(H3N2) virus strains, compared with all confirmed cases of influenza and influenza-like illness, Peru, 2010–2012

Year, location	No. sequenced influenza A(H3N2) strains*	No. other strains or illnesses				
		All H3N2	Influenza A(H1N1) pdm09	Influenza B	Influenza illness	Influenza-like illness
2010						
All	227	414	138	306	858	1,716
Lima	41	95	38	96	229	458
Cusco	31	42	63	74	179	358
Tumbes	92	155	25	83	263	526
Puerto Maldonado	63	122	12	53	187	374
2011						
All	105	219	36	16	271	542
Lima	13	35	6	1	42	84
Cusco	65	101	2	0	103	206
Tumbes	2	17	11	14	42	84
Puerto Maldonado	25	66	17	1	84	168
2012						
All	57	87	57	233	377	754
Lima	27	45	29	48	122	244
Cusco	0	7	7	74	88	176
Tumbes	28	42	18	18	78	156
Puerto Maldonado	2	38	3	93	134	268
2010–2012						
All	389	1,485	462	1110	3057	6,114

*Strains sequenced during this phylogeographic study of influenza A(H3N2) virus in Peru.

tree in the online Technical Appendix Figure). Perhaps the most notable observation from this analysis was the interseasonal extinction of virus clades from Peru in all regions of the country, even in a tropical region where molecularly confirmed year-round influenza transmission has been noted (*14*). In addition, the phylogeny showed extensive global mixing of H3N2 viruses, with co-circulation of clades from Peru with those from all Northern and Southern Hemisphere regions, including in countries in Latin and North America, Africa, Europe, Central Asia, and East

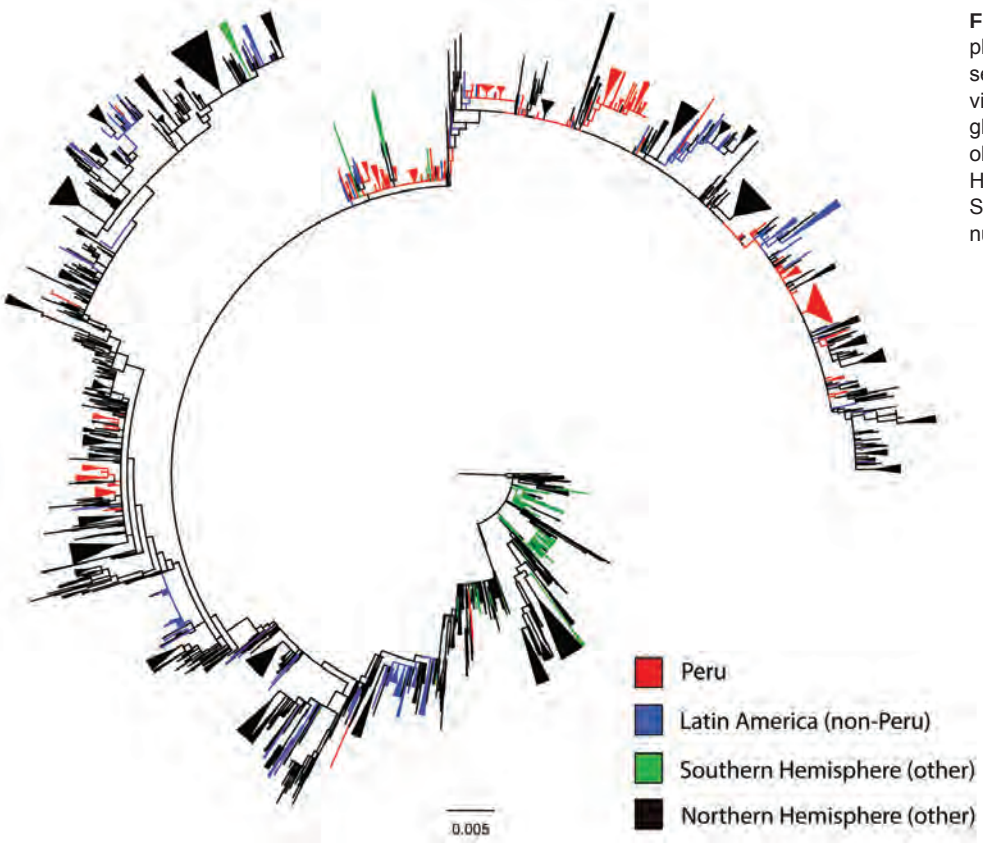

Figure 1. Maximum-likelihood phylogeny of hemagglutinin sequences of influenza A(H3N2) viruses from Peru and other global locations, rooted with the oldest available sequence (A/Hong Kong/CUHK52390/2004). Scale bar indicates number of nucleotide substitutions per site.

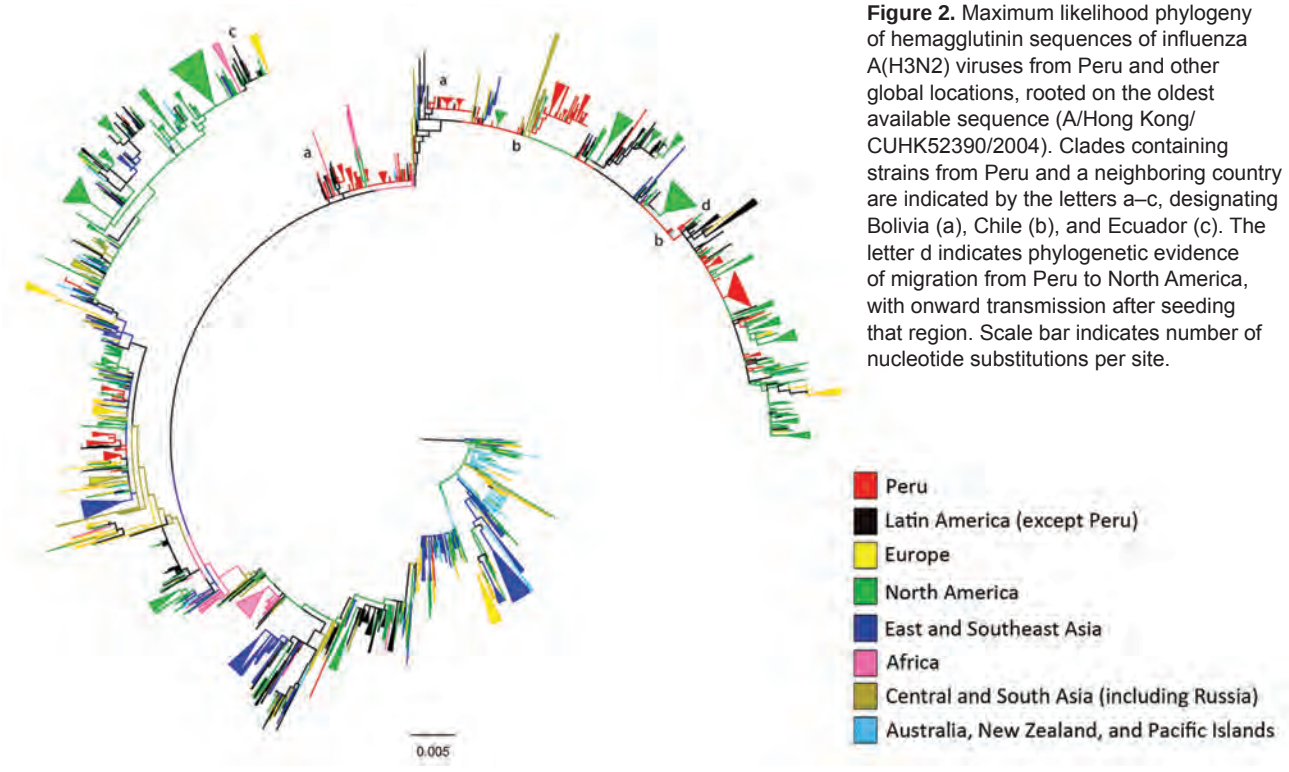

Figure 2. Maximum likelihood phylogeny of hemagglutinin sequences of influenza A(H3N2) viruses from Peru and other global locations, rooted on the oldest available sequence (A/Hong Kong/CUHK52390/2004). Clades containing strains from Peru and a neighboring country are indicated by the letters a–c, designating Bolivia (a), Chile (b), and Ecuador (c). The letter d indicates phylogenetic evidence of migration from Peru to North America, with onward transmission after seeding that region. Scale bar indicates number of nucleotide substitutions per site.

and Southeast Asia. In one instance, onward transmission of virus was noted after migration from Peru to the United States (Figure 2, section d).

Viruses from each study location in Peru formed weak to moderately supported clades with sequences for viruses from other localities (bootstrap values were usually <70% but occasionally >80%), reflecting a relative lack of phylogenetic resolution in the data at this scale (online Technical Appendix Figure). In contrast, smaller but often better supported clades (frequently with bootstrap values >70%) containing H3 virus sequences from multiple locations in Peru were observed (online Technical Appendix Figure).

Closer examination of the phylogenetic analysis of sequences for viruses from Latin America showed evidence for the presence of weakly supported sublineages consisting predominantly of strains from Peru but also containing strains from Chile and Bolivia (Figure 2); this finding is indicative of viral traffic between these border-sharing countries. Analysis of clustering with strains from Ecuador was limited by a paucity of sequences, but evidence of strongly supported clustering with strains from Peru was found (Figure 2). In addition, strains from Peru fell into some weakly supported multinational sublineages containing strains from Brazil, Venezuela, Paraguay, Nicaragua, Colombia, Argentina, and Mexico, which suggests H3N2 viral traffic throughout the Americas (online Technical Appendix Figure).

Analyzed separately, the maximum clade credibility tree (Figure 3) for strains from Peru showed substantial HA diversity each year; many clades co-circulated at each location. The smaller-sized locations of Tumbes, Puerto Maldonado, and Cusco had a wide range of co-circulating clades, similar to those of larger travel hubs, such as Lima (Table 2). This analysis also showed a short time to most common recent ancestor (mean 3.8 y, 95% highest posterior density 3.1–4.6 y), as has been shown for most other studied localities (*5*,*29*). A similarly short mean time to most recent common ancestor (1.6 y, 95% highest posterior density 1.1–2.1 y) was obtained for 2010, the most sampled year, providing the most precise single-season estimate.

To determine the phylogeographic structure in the data, we performed phylogeny-trait association tests (Table 3). For strains from Peru, the results confirmed a stronger spatial clustering of sequences at all sites than would be expected by chance alone ($p<0.01$), but the results also showed clear evidence of some viral traffic among sampling locations, as noted in the phylogenetic analysis. Furthermore, the maximum clade statistic was significant ($p = 0.009$) in all 4 study sites, reflecting predominantly local evolution in these localities. Differences in the observed and expected maximum clade values tentatively suggested that Lima exhibited the least structure (i.e., most mixing; difference of 5.50) and Tumbes the strongest spatial structure (difference of 10.33) (Table 3).

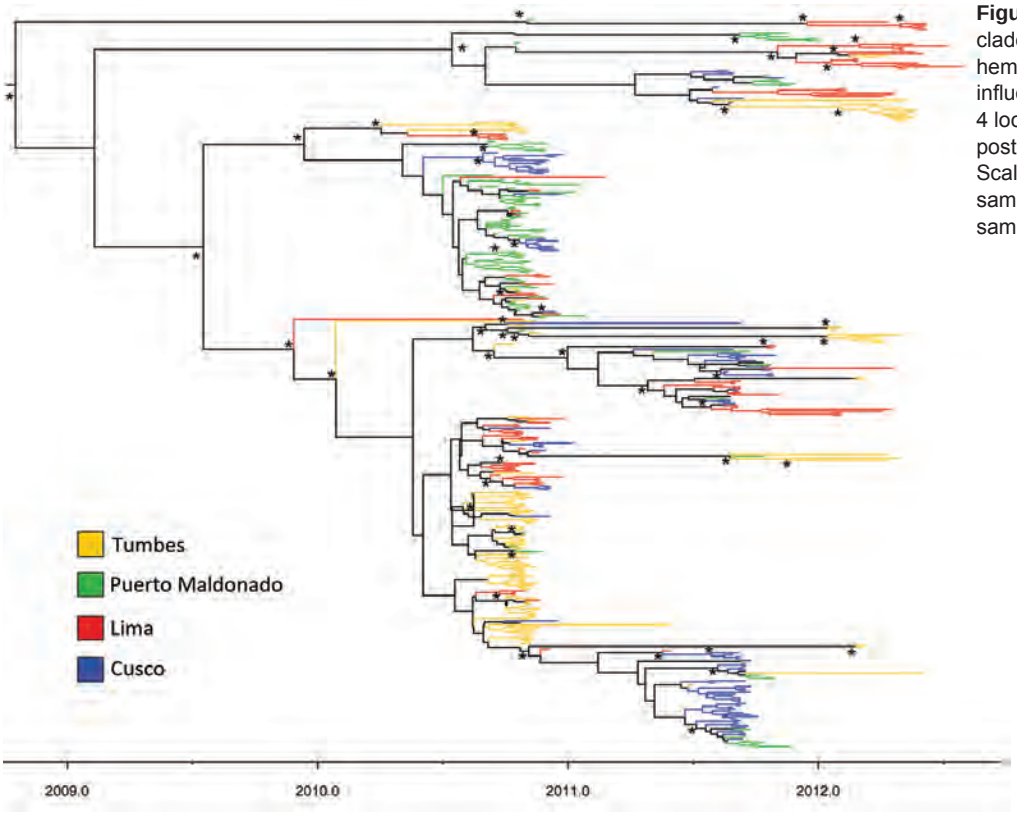

Figure 3. Time-scaled maximum clade credibility phylogeny of hemagglutinin sequences for influenza A(H3N2) viruses from 4 locations in Peru. *Indicates posterior probabilities >0.9. Scale bar refers to year of sampling to indicate time of sampling for each virus.

Discussion

Our phylogenetic analysis showed that the level of international H3N2 viral traffic was high and that mixing of Peruvian HA sequences with those from multiple regions of the world was rapid and widespread (Figures 1, 2). These findings support a continuous H3N2 gene flow in and out of Peru rather than a local closed system in which viruses evolve entirely within the country. Mixing of viruses between all study sites in Peru and other countries may also suggest gene flow in and out of Peruvian locations outside the main air-transport hub of Lima. However, such a conclusion comes with a strong caveat because we may not have sampled all Lima source lineages that seed peripheral locations in the country. Of note, we found evidence of H3N2 virus migration between Peru and its neighbors, although this conclusion was limited by a relative paucity of sequences from these other Latin American countries.

At each study site in Peru, we found multiple co-circulating clades of influenza virus that regularly underwent extinction (Figures 1, 2), suggesting that much of the genetic diversity of viruses in Peru results from global lineages that pass through the country, rather than from local evolution associated with long-term local persistence. In particular, all sampled strains, even those from tropical Peruvian sites like Tumbes and Puerto Maldonado, underwent extinction rather than persisted over time, thus regularly halting local evolution of imported influenza viruses. That the time to most common recent ancestor of the whole sample (mean 3.8 y) was much shorter than the known history of H3N2 virus in Peru is also consistent with the idea that the influenza virus gene pool in Peru is being frequently replenished from other regions.

Our findings are consistent with those of studies in countries with temperate regions, such as Australia, New Zealand, and countries in North America, which showed regular introduction of new H3N2 virus lineages and seeding of local seasonal epidemics rather than the interseasonal persistence of lineages (*29–31*). Such studies have similarly revealed that the genetic diversity of seasonal influenza in temperate locales primarily results from the ongoing introduction of genetically divergent lineages during seasonal epidemics (*5*,*30–32*).

In contrast, interseasonal persistence of H3N2 influenza virus has been documented in subtropical and tropical

Table 2. Number of circulating influenza A(H3N2) virus clades, Peru, 2010–2012*

Location	No. clades circulating, by year		
	2010	2011	2012
Lima	8	6	5
Puerto Maldonado	6	4	0
Cusco	4	9	0
Tumbes	13	1	5

*Data are derived from the phylogenetic tree in Figure 3.

Table 3. Results of phylogeny-trait association testing for influenza A(H3N2) viruses in Peru, 2010–2012*

	Association index (95% CI)†			Parsimony scores (95% CI)†			Mean maximum clade size (95% CI)‡			
Location	Observed	Expected	p value§	Observed	Expected	p value§	Observed	Expected	p value¶	Difference#
All	8.53 (7.25–9.81)	33.02 (31.52–34.56)	<0.001	73.72 (70.00–77.00)	211.00 (205.65–217.36)	<0.001	–	–		–
Lima	–	–		–	–		8.04 (6.0–10.0)	2.6 (2.18–3.16)	0.009	5.44
Cusco	–	–		–	–		12.4 (12.0–15.0)	2.82 (2.36–3.44)	0.009	9.58
Puerto Maldonado	–	–		–	–		8.2 (6.0–14.0)	2.7 (2.28–3.45)	0.009	5.50
Tumbes	–	–		–	–		13.68 (10.0–22.0)	3.35 (2.76–4.99)	0.009	10.33

*Results were determined by a Bayesian analysis of phylogeographic structure. p values correspond to the proportion of trees from the null distribution equal to, or more extreme than, the median posterior of the statistic.
†Association index and parsimony scores only determined for all locations combined.
‡Maximum clade size statistics only determined for each specific location.
§p<0.001 confirms a stronger observed spatial clustering of sequences from Peru at all sites than would be expected by chance alone.
¶p = 0.009 reflects predominantly local evolution in the 4 locations.
#Difference between observed and expected clade size.

locations like Hong Kong and Southeast Asia (*7*,*8*,*10*). A more recent study has shown evidence for multiyear pandemic influenza A(H1N1)pdm09 strain persistence in tropical areas of western Africa that are relatively isolated (*33*). In contrast, an analysis of H3N2 virus persistence over a 15-year period in subtropical China did not demonstrate interseasonal persistence, and the sample size in that study was much larger than that in our study (*9*).

Our findings did not offer support to a source–sink dynamic within Peru, and they also indicate that Peru is an unlikely common tropical source of persistent lineages that seed other countries in Latin America or the rest of the world. Instead, our findings are more consistent with a shifting metapopulation model of H3N2 virus, such that the virus may pass through any region for a variable amount of time rather than perpetually circulating in fixed locations in the tropics and consistently seeding temperate regions each year (*11*,*34*). Such a shifting metapopulation model may also explain why some studies show apparent persistence in some tropical and subtropical locations over certain years and others do not (*7*–*9*,*33*). This model is also compatible with the existence of temporary source populations in locations throughout the world. Indeed, we provide some phylogenetic evidence that Peru may occasionally, but not consistently, act as a temporary source, spreading virus from Peru to another country, from which onward transmission continues (Figure 2, section d).

H3 virus sequences for viruses from Peru also exhibited some clustering by sampling location, a finding consistent with semilocalized seasonal H3N2 virus epidemics in each region of Peru (Figure 3), although with migration between localities. Such semilocalized epidemics have been observed in other areas (*29*). These data also provided some evidence for weaker spatial clustering in Lima compared with other localities. This evidence is not surprising because Lima has the largest population and, thus, movement of humans around, in, or out of the city would generally be expected to be greater than in other areas. In this context it is perhaps surprising that Puerto Maldonado, the least populous site, had a similar strength of spatial clustering. This locality has been characterized by rapid population growth, likely due to widespread mining and associated activities (*35*). Hence, it is possible that frequent human movement in and out of this location is creating more diffusion of influenza virus. In addition, the true population of this area may be considerably higher than suggested by official statistics.

These findings have implications for public health practice in Peru and Latin America. For example, they suggest that future novel strains of influenza virus may enter Peru at multiple locations rather than just through its major air-transport hub (Lima) (*36*). Moreover, the rapid diffusion of influenza virus throughout Peru, even in the more remote regions, also serves as a potent reminder of how quickly influenza virus can disseminate. We identified Lima and Puerto Maldonado as possible diffusion hubs for influenza virus; perhaps both cities could be prioritized for heightened influenza surveillance if a novel influenza subtype is introduced into Peru.

Although Peru does not appear to be a global source population for influenza viruses, the diversity and cocirculation of many simultaneous lineages of H3N2 virus in the country means that it should not be overlooked as a potential source for novel pandemic strains, particularly given that there is some evidence of high-risk animal farming practices and low biosecurity in this country (*37*). Similarly, the rapid, widespread, and unpredictable migration of global strains into Peru and widespread global mixing shown in this study emphasize that vaccine recommendations in either hemisphere

need to be based on well distributed, widespread global H3N2 virus sampling from as many sentinel laboratories as possible (*6*).

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Influenza A Viruses of Human Origin in Swine, Brazil

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The evolutionary origins of the influenza A(H1N1)pdm09 virus that caused the first outbreak of the 2009 pandemic in Mexico remain unclear, highlighting the lack of swine surveillance in Latin American countries. Although Brazil has one of the largest swine populations in the world, influenza was not thought to be endemic in Brazil's swine until the major outbreaks of influenza A(H1N1)pdm09 in 2009. Through phylogenetic analysis of whole-genome sequences of influenza viruses of the H1N1, H1N2, and H3N2 subtypes collected in swine in Brazil during 2009–2012, we identified multiple previously uncharacterized influenza viruses of human seasonal H1N2 and H3N2 virus origin that have circulated undetected in swine for more than a decade. Viral diversity has further increased in Brazil through reassortment between co-circulating viruses, including A(H1N1)pdm09. The circulation of multiple divergent hemagglutinin lineages challenges the design of effective cross-protective vaccines and highlights the need for additional surveillance.

Influenza A viruses circulating in swine (swIAVs) are of major economic concern for the swine industry and a pandemic threat for humans. The H1N1 influenza pandemic of 2009 was associated with a virus of swine origins (*1*) that caused its first outbreak in humans in Mexico in early 2009 (*2*). However, the evolutionary origins of influenza A(H1N1)pdm09 (pH1N1) virus in swine are poorly understood, and no potential progenitor viruses have been detected in swine in any part of the world. The relatively small number of swIAVs that have been characterized in Latin America make it particularly difficult to confirm or negate the possibility of pH1N1 evolving in swine in Mexico or another Latin American country before emergence in humans.

Since 2009, transmission of pH1N1 virus from humans to pigs has been documented in numerous countries spanning 6 continents (*3*–*13*), including many countries where influenza viruses previously had not been detected in swine, such as Australia (*14*), Finland (*15*), and Cameroon (*16*). pH1N1 virus has been identified in swine in several Latin American countries, including Argentina (*17*), Brazil (*18*,*19*), Colombia (*20*), and Mexico (*21*), because of human-to-swine transmission events that have occurred since 2009. In Argentina, multiple subtypes of viruses of human seasonal virus origin also have been identified in swine (*22*). Although Brazil hosts one of the largest swine populations in the world (≈41 million hogs), little evidence existed of swIAV circulation in swine herds in Brazil before 2009 (*23*–*25*). Influenza virus in pigs was first detected in Brazil in 1974, and the isolated virus was closely related to the classical North American swine virus A/swine/Illinois/1/63/H1N1 (*24*). However, relatively little clinical illness was observed in pigs in Brazil until 2009. Since 2009, Brazil's swine population has experienced outbreaks of pH1N1 that are associated with respiratory illness. These outbreaks have been located primarily in the country's major swine production regions in southern, midwestern, and southeastern Brazil (Figure 1). As a result, surveillance efforts for IAVs in swine populations in Brazil have increased, revealing additional influenza virus diversity. Serum collected from swine in southeastern Brazil during January–March 2009 indicated the widespread presence of antibodies cross-reactive to multiple antigenically distinct subtypes in swine: North American classical swine H1N1 (44.4%), North American triple-reassortant swine H3N2 (23.5%), and human-like H1N1 (38.3%) (*25*). In southern Brazil, the seroprevalence of the H3N2 subtype was recently found to be ≈20% (*23*). A human-like H1N2 virus was isolated from captive wild boars (*26*) and from swine (*27*) in Brazil.

Here, a phylogenetic analysis of newly sequenced influenza viruses from Brazil's swine herds provides evidence that multiple IAVs of human seasonal virus origin have been circulating in swine for more than a decade. These particular H3N2 and H1N2 swIAV clades appear to be specific to Brazil. The co-circulation of multiple genetically diverse swIAV lineages of the H1N1, H1N2, and H3N2 subtypes introduces new challenges for the control of influenza in Brazil's swine herds, including development of cross-protective vaccines.

Methods

Data Collection and Sequencing

A total of 1,881 nasal swab and 89 lung tissue samples were collected from swine from 131 pig farms in southern,

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Figure 1. Areas of swine production in 7 states in southern, midwestern, and southeastern Brazil: Rio Grande do Sul (RS) State (total swine population ≈7.0 million), Santa Catarina (SC) State (≈9.0 million swine), Paraná (PR) State (≈6.0 million swine), Mato Grosso (MT) State (≈2.4 million swine), Mato Grosso do Sul (MS) State (≈1.3 million swine), Minas Gerais (MG) State (≈5.4 million swine), and São Paulo (SP) State (≈1.8 million swine). Red dots indicate pig farms sampled where at least 1 sample was positive for influenza by PCR; blue dots indicate farms where all samples were negative for influenza.

midwestern, and southeastern Brazil during 2009–2012 (Figure 1). Nursery and growing pigs were selected for nasal swab sampling during 2 cross-sectional studies in 2010 and 2011, where pigs showing typical clinical signs of influenza infection (e.g., fever, labored abdominal breathing, and dyspnea) were preferentially chosen for sampling. Lung samples were collected from pigs of different production phases (suckling, nursery, and fattening) and submitted to a private diagnostic laboratory for necropsy and/or histopathologic examination, and positive lung samples for IAV by immunohistochemical analysis were sent to the virology laboratory at EMBRAPA (Brazilian Agricultural Research Corporation, Concordia, Brazil) for virus isolation, reverse transcription quantitative PCR (RT-qPCR), and sequencing.

IAV was detected from nasal swab samples from pigs on 24 of 62 farms. Fifty-nine (3.13%) of 1,881 nasal swab samples analyzed were positive by RT-qPCR. Moreover, 58 (65.16%) of 89 lung samples with pneumonic lesions, from 44 pig farms, were positive for IAV by RT-qPCR. Viral RNA was extracted from the original samples and tested for the matrix gene of IAV and pH1N1 IAV by 2 separate RT-qPCRs (*28*,*29*). Virus was isolated by inoculating lung tissue supernatant or nasal swab samples into MDCK cells or into specific pathogen–free embryonated chicken eggs (*30*). To confirm virus isolation, we tested the supernatants or allantoic fluids for hemagglutinin (HA) activity and by RT-qPCR. Nasal swab and lung samples were considered negative for IAV if a second passage in specific pathogen–free chicken eggs or in MDCK cells was negative by HA test and RT-PCR. Of the 117 PCR-positive samples, 41 virus isolates were obtained: 14 from nasal swab samples and 27 from lung samples.

We conducted whole-genome sequencing using an ABI 3130xl analyzer (Applied Biosystems, Foster City, CA, USA) and Illumina MiSeq (Illumina, San Diego, CA, USA). PCR was conducted by using a set of primers specific for the amplification of the 8 IAV gene segments (*31*,*32*) and a primer set for the amplification of pH1N1 gene segments. For sequencing on an Illumina MiSeq, RT reaction and amplification of the 8 IAV gene segments were conducted by using primers as previously described (*32*,*33*). In total, at least partial sequence data were obtained for 35 IAVs. Of these, 16 swIAVs provided whole-genome sequence data of sufficient quality to be included in the final phylogenetic analysis.

Phylogenetic Analysis

Nucleotide alignments were generated for 11 discrete datasets: H1 (human seasonal virus–like, 209 viruses), H1 (pandemic virus–like, 451 viruses), H3 (463 viruses), N1 (pandemic

virus–like, 311 viruses), N2 (683 viruses), and the 6 internal gene segments (polymerase basic [PB] 2, PB1, polymerase acidic [PA], nucleoprotein [NP], matrix [MP], and nonstructural [NS]). Each dataset comprised 1) Brazilian swIAVs sequenced for this study; 2) Brazilian swIAVs that we sequenced and published previously (*26*,*27*); and 3) related human and swine viruses, collected globally, that were downloaded from the Influenza Virus Resource (*34*) available in GenBank, which were studied previously (*35*) (Table 1; online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/8/14-1891-Techapp1.pdf). To accommodate relatively long but not fully complete sequences, the H3 alignment was trimmed to 1158 nt and the N2 alignment was trimmed to 1161 nt, and sequences less than this length were excluded.

For each of the 5 sets of sequence data, an alignment was generated by using MUSCLE v3.8.31 (*36*), with manual correction using the program Se-Al (http://tree.bio.ed.ac.uk/software/seal/). The phylogenetic relationships of each of the 5 datasets were inferred by using the maximum-likelihood (ML) method available in RAxML v7.2.6 (*37*), incorporating a general time-reversible model of nucleotide substitution with a g-distributed rate variation among sites. To assess the robustness of each node, we conducted a bootstrap resampling process (500 replicates), again using the ML method available in RAxML v7.2.6. Because of the computational complexity of these processes, we used the high-performance computational capabilities of the Biowulf Linux cluster at the National Institutes of Health (http://biowulf.nih.gov).

Divergence Times

A time-scaled Bayesian approach was used to estimate the timing of the human-to-swine transmission events associated with the H1, H3, and N2 segments, due to the long branch lengths separating the Brazilian swine viruses from the most closely related human seasonal viruses. We used a relaxed uncorrelated lognormal molecular clock, a flexible Bayesian skyline plot (BSP) demographic model (10 piece-wise constant groups), and a general-time reversible model of nucleotide substitution with a γ-distributed rate variation among sites. Markov chain Monte Carlo was run separately 3 times for each dataset for at least 100 million iterations, with subsampling every 10,000 iterations. The analysis used BEAST v1.8.0 (*38*) and the BEAGLE library (*39*) to improve computational performance. All parameters reached convergence, as assessed visually by using Tracer v1.6 (http://tree.bio.ed.ac.uk/software/tracer/), with statistical uncertainty reflected by values of the 95% highest posterior density (HPD). The initial 10% of the chain was removed as burn-in, runs were combined by using LogCombiner v1.8.0 (http://beast.bio.ed.ac.uk/logcombiner), and maximum clade credibility (MCC) trees were summarized by using TreeAnnotator v1.8.0 (http://beast.bio.ed.ac.uk/treeannotator).

Results

Among the newly sequenced swIAVs from Brazil, we identified multiple previously uncharacterized clades of viruses that are most closely related to human seasonal H3N2 and H1N2 viruses that circulated in humans during the 1990s and early 2000s, respectively (Figures 2–4). All of these viruses had matrix segments of pH1N1 origin, and all other internal genes that could be sequenced were also of pH1N1 origin (Table 1).

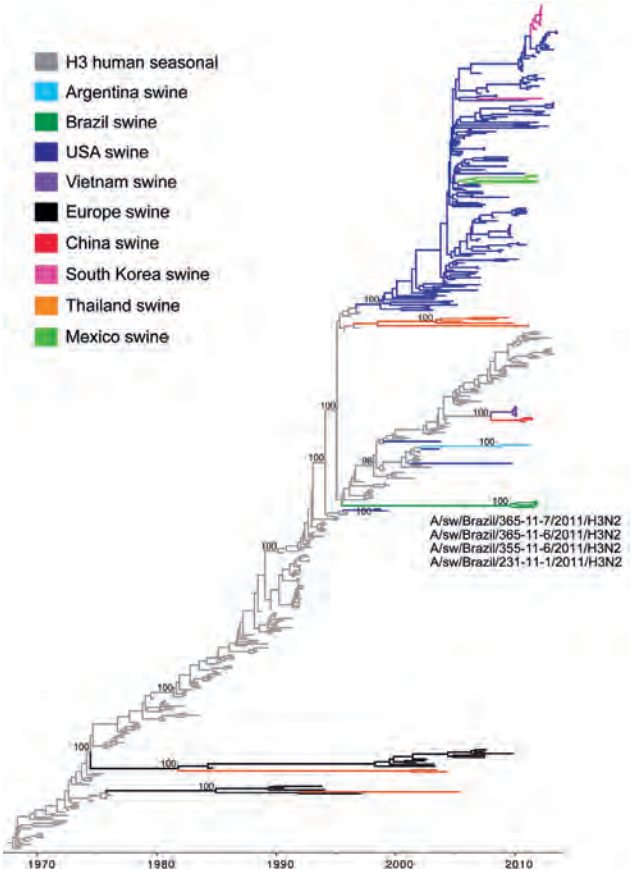

Figure 2. Phylogenetic relationships between human and swine influenza H3 segments. Time-scaled Bayesian maximum clade credibility (MCC) tree inferred for the hemagglutinin (H3) sequences of 463 viruses, including 4 viruses sequenced for this study from swine in Brazil, A/swine/Brazil/365-11-7/2011(H3N2), A/swine/Brazil/231-11-1/2011(H3N2), A/swine/Brazil/355-11-6/2011(H3N2), and A/swine/Brazil/365-11-6/2011(H3N2); 251 human seasonal H3 viruses collected globally during 1968–2013; and 208 closely related swine viruses collected globally that have been studied previously (*35*). Gray indicates branches of human seasonal influenza A(H3N2) virus origin. Branches associated with viruses from swine are shaded by country/area of origin: light blue, Argentina; dark green, Brazil; dark blue, United States; purple, Vietnam; black, Europe; red, China; pink, Korea; orange, Thailand; light green, Mexico. Posterior probabilities >0.9 are included for key nodes.

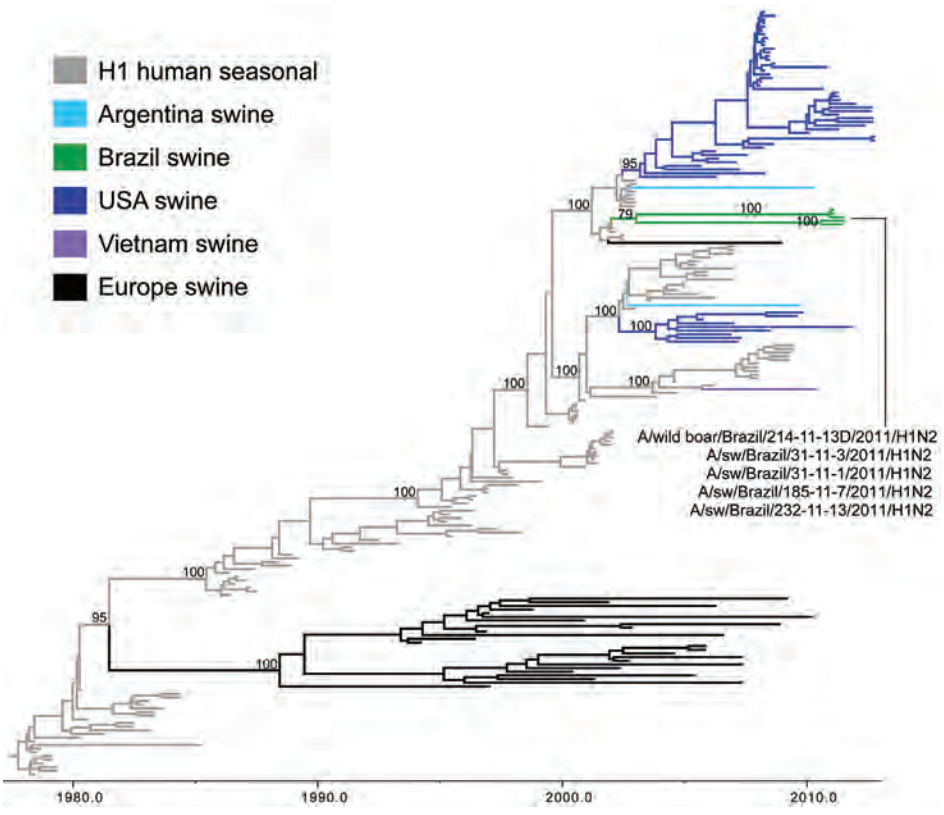

Figure 3. Phylogenetic relationships between human and swine influenza H1 segments. Time-scaled Bayesian maximum clade credibility (MCC) tree inferred for the hemagglutinin (H1) sequences of 209 viruses, including 2 swine viruses from Brazil sequenced for this study, A/swine/Brazil/185-11-7/2011(H1N2) and A/swine/Brazil/232-11-13/2011(H1N2); 3 swine influenza A viruses from Brazil sequenced previously, A/wild boar/Brazil/214-11-13D/2011(H1N2) (*26*), A/swine/Brazil/31-11-1/2011(H1N2), and A/swine/Brazil/31-11-3/2011(H1N2) (*27*); 120 human seasonal H1 viruses collected globally during 1978–2008; and 84 closely related swine viruses collected globally that have been studied previously (*35*). Gray indicates branches of human seasonal influenza A(H3N2) virus origin. Branches associated with viruses from swine are shaded by country/area of origin: light blue, Argentina; dark green, Brazil; dark blue, United States; purple, Vietnam; black, Europe; red, China; pink, Korea; orange, Thailand; light green, Mexico.

Identification of New Human-Origin H3N2 IAVs in Swine

On the H3 phylogeny, the 4 H3N2 viruses from swine in Brazil in 2011 are monophyletic, forming a single clade supported by 100% posterior probability (Figure 2) and 100% bootstrap support (online Technical Appendix Figure 1): A/swine/Brazil/365-11-7/2011(H3N2), A/swine/Brazil/231-11-1/2011(H3N2), A/swine/Brazil/355-11-6/2011(H3N2), and A/swine/Brazil/365-11-6/2011(H3N2) (Table 1; online Technical Appendix Table). This clade is phylogenetically distinct from other swIAVs found in Argentina and North America that also are of human seasonal H3N2 virus origin and appears to be a previously uncharacterized introduction of a human seasonal H3N2 virus into swine (Figure 2; online Technical Appendix Figure 1). The human H3N2 viruses that are most closely related to the Brazilian swIAVs were isolated during the late 1990s (e.g., A/Malaysia/13241/1997[H3N2]) and have a long branch length separating the clade of swIAVs from Brazil from the older human virus diversity.

The time-scaled MCC tree (Figure 2) indicates that human-to-swine transmission could have occurred at any time along this long branch between the node representing the estimated time to the most recent common ancestor of the clade of swIAVs from Brazil (2009.6; 95% HPD 2008.5–2010.4) and the node representing the time to the most recent common ancestor for the swine clade and most closely related human virus (1995.5; 95% HPD 1995.1–1995.9). The much higher intensity of sampling of human viruses than swine viruses means that the long branch length is more likely to represent unsampled swine viruses than unsampled human viruses and the actual time of human-to-swine introduction is likely to be closer to 1995.5. Detection of H3N2 swIAVs in 3 Brazilian states (Santa Catarina, Rio Grande do Sul, and Mato Grosso do Sul; Figures 1, 2) is consistent with extensive circulation of this swIAV within swine populations in southern and midwestern Brazil.

Identification of New Human-Origin H1N2 IAVs in Swine

On the H1 phylogeny, the 5 H1N2 viruses collected from swine in Brazil during 2011 also are monophyletic, although the clade is supported by just below the 70% bootstrap threshold on the ML tree (64% bootstrap support, online Technical Appendix Figure 2) and 80% posterior probability threshold on the MCC tree (79%, Figure 3). The lower support for this clade appears to be related to the early divergence of these swIAVs into 2 well-supported subclades (100% posterior probabilities). One subclade comprises 3 swIAVs collected in Paraná and Rio Grande do Sul States: A/swine/Brazil/31-11-1/2011(H1N2), A/swine/Brazil/31-11-3/2011(H1N2), and A/wild boar/Brazil/

214-11-13D/2011(H1N2) (Table 1; Figures 1, 3). The second subclade comprises 2 swIAVs collected in Santa Catarina State: A/swine/Brazil/185-11-7/2011(H1N2) and A/swine/Brazil/232-11-13/2011(H1N2). The presence of H1N2 viruses from domestic swine and a wild boar within the first subclade is consistent with viral transmission between wild and domestic swine in Brazil.

The estimated time of transmission of the human H1N2 viruses into swine is 2002.1–2003.1 (95% HPD 2001.8–2004.7), which is consistent with the period of global circulation of the unusual reassortant H1N2 virus in humans

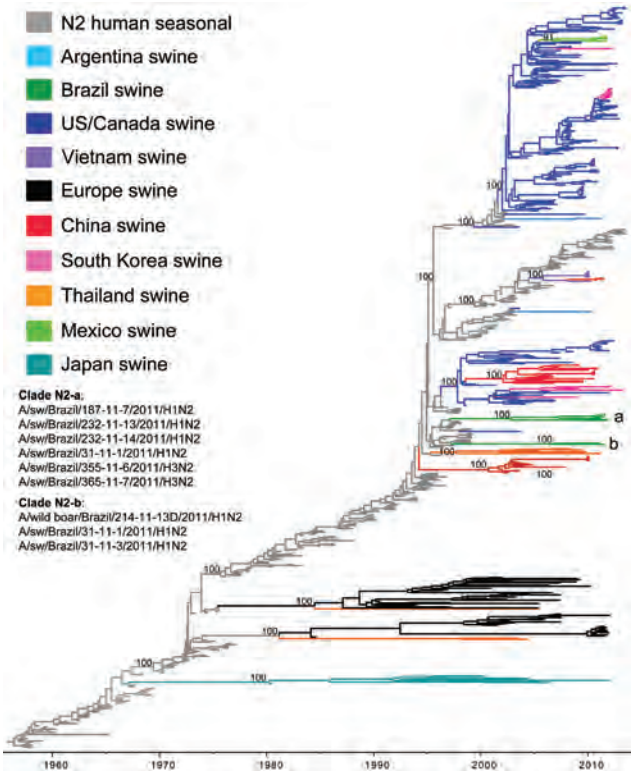

Figure 4. Phylogenetic relationships between human and swine influenza N2 segments. Time-scaled Bayesian maximum clade credibility (MCC) tree inferred for the neuraminidase (N2) sequences of 682 viruses, including 6 swine viruses from Brazil sequenced for this study, A/swine/Brazil/185-11-7/2011(H1N2), A/swine/Brazil/232-11-13/2011(H1N2), A/swine/Brazil/232-11-14/2011(H1N2), A/swine/Brazil/31-11-1/2011(H1N2), A/swine/Brazil/355-11-6/2011(H3N2), and A/swine/Brazil/365-11-7/2011(H3N2); 2 swine influenza A viruses from Brazil sequenced previously, A/wild boar/Brazil/214-11-13D/2011(H1N2) (*26*) and A/swine/Brazil/31-11-3/2011(H1N2) (*27*); 325 human seasonal N2 viruses collected globally during 1957–2013; and 350 closely related swine viruses collected globally that have been studied previously (*35*). Gray shading indicates branches of human seasonal influenza A(H3N2) virus origin. Branches associated with viruses from swine are shaded by country/area of origin: light blue, Argentina; dark green, Brazil; dark blue, United States; purple, Vietnam; black, Europe; red, China; pink, Korea; orange, Thailand; light green, Mexico; turquoise, Japanese swine viruses. Posterior probabilities >0.9 are included for key nodes, and the 2 clades of Brazilian swine viruses are labeled a and b (Table 1).

(2001–2003). The most closely related human virus was isolated in 2002: A/New York/417/2002/H1N2. Because of the low availability of human H1N2 sequences from this period and the low posterior probability (MCC tree) and bootstrap support (ML tree) for the clade of Brazilian H1N2 viruses, we cannot conclude with certainty that the H1N2 swIAVs identified in Brazil represent a single viral introduction from humans into pigs instead of 2 contemporaneous introductions involving similar human H1N2 viruses. The most parsimonious explanation, however, is that a single introduction of a human seasonal H1N2 virus into swine occurred, and shortly thereafter the virus diversified into 2 subclades that co-circulated in swine until at least 2011, when these H1N2 viruses were identified in swine in Brazil.

Two Introductions of Human N2 Segments in Swine

In contrast to the H3 and H1 phylogenies (Figures 2, 3), the Brazilian swIAVs are not monophyletic on the N2 phylogeny, representing 2 different introductions of the N2 segment from human seasonal H3N2 viruses into swine in Brazil (Figure 4). One N2 clade (clade N2-a, Figure 4) is defined by 100% posterior probability support and 90% bootstrap support (online Technical Appendix Figure 3) and comprises 6 Brazilian swIAVs: A/swine/Brazil/185-11-7/2011(H1N2), A/swine/Brazil/31-11-1/2011(H1N2), A/swine/Brazil/232-11-13/2011(H1N2), A/swine/Brazil/232-11-14/2011(H1N2), A/swine/Brazil/355-11-6/2011(H3N2), and A/swine/Brazil/365-11-7/2011(H3N2) (Table 1). Within the N2-a clade, the 4 H1N2 viruses cluster separately from the 2 H3N2 viruses as 2 genetically distinct subclades (90% and 100% bootstrap support, respectively; online Technical Appendix Figure 3). The 6 swIAVs in N2-a were collected from 4 Brazilian states: Santa Catarina, Rio Grande do Sul, Paraná, and Mato Grosso do Sul (Figure 1). The other N2 clade (clade N2-b, Figure 4) consists of 2 swIAVs from Brazil of the H1N2 subtype, defined by 100% posterior probability and 90% bootstrap support (online Technical Appendix Figure 3): A/wild boar/Brazil/214-11-13D/2011(H1N2) and A/swine/Brazil/31-11-3/2011(H1N2). These swIAVs were collected in Paraná and Rio Grande do Sul States, consistent with dispersal within southern Brazil despite the low number of samples (Figure 1; Table 1).

Both the Brazilian N2-a and N2-b clades are closely related to human seasonal H3N2 viruses that circulated during the late 1990s (e.g., A/New York/521/1998[H3N2] for clade N2-a and A/New York/251/1998[H3N2] for clade N2-b) and therefore have similar estimated times of human-to-swine transmission on the MCC tree: 1997.1–2002.8 (95% HPD 1996.3–2005.6) for clade N2-a and 1997.2–2006.9 (95% HPD 1996.1–2009.0) for N2-b (Figure 4). Again, the higher intensity of influenza surveillance in humans than in swine suggests that the 2 human-to-swine

Table 1. Characteristics and phylogenetic position of 16 influenza A viruses from swine sequenced at EMBRAPA (Brazilian Agricultural Research Corporation)*

Virus	State†	Date collected	Specimen type	Isolation method	Segments			
					HA‡	NA§	Matrix	Other internal¶
A/swine/Brazil/185-11-7/2011/H1N2	SC	2011 Jul 6	NS	ECE/MDCK	H1s	N2-a	pdm	pdm
A/swine/Brazil/232-11-13/2011/H1N2	SC	2011 Aug 17	NS	ECE	H1s	N2-a	pdm	pdm
A/swine/Brazil/232-11-14/2011/H1N2	SC	2011 Aug 17	NS	ECE	–	N2-a	pdm	–
A/swine/Brazil/31-11-1/2011/H1N2	PR	2011 Feb 28	Lung	ECE	H1s	N2-a	pdm	pdm
A/swine/Brazil/31-11-3/2011/H1N2	PR	2011 Feb 28	Lung	ECE	H1s	N2-b	pdm	pdm
A/wild boar/Brazil/214-11-13D/2011/H1N2	RS	2011 Jul 25	Lung	#	H1s	N2-b	pdm	pdm
A/swine/Brazil/231-11-1/2011/H3N2	SC	2011 Aug 17	NS	ECE/MDCK	H3	–	pdm	–
A/swine/Brazil/355-11-6/2011/H3N2	RS	2011 Oct 27	NS	ECE	H3	N2-a	pdm	–
A/swine/Brazil/365-11-6/2011/H3N2	MS	2011 Nov 10	NS	ECE/MDCK	H3	–	pdm	–
A/swine/Brazil/365-11-7/2011/H3N2	MS	2011 Nov 10	NS	MDCK	H3	N2-a	pdm	pdm
A/swine/Brazil/12A/2010/H1N1	SC	2010 Jan 30	Lung	ECE	H1p	N1p	pdm	–
A/swine/Brazil/18/2012/H1N1	RS	2012 Feb 10	Lung	ECE	H1p	N1p	pdm	pdm
A/swine/Brazil/66/2011/H1N1	SC	2011 Apr 13	Lung	ECE	H1p	N1p	pdm	pdm
A/swine/Brazil/107/2010/H1N1	SC	2010 Jul 15	NS	ECE	H1p	N1p	pdm	pdm
A/swine/Brazil/132/2009/H1N1	SC	2009 Sep 9	Lung	ECE	H1p	–	pdm	–
A/swine/Brazil/263/2012/H1N1	SC	2012 Nov 27	Lung	ECE	H1p	N1p	pdm	pdm

*ECE, embryonated chicken egg; HA, hemagglutinin; MS, Mato Grosso do Sul State; NA, neuraminidase; NS, nasal swab; pdm, pandemic; PR, Parana State; RS, Rio Grande do Sul State; SC, Santa Catarina State; –, insufficient sequence data available for analysis.
†Location of virus collection among 4 states in southern and midwestern regions Brazil provided for each sample (Figure 1).
‡Viruses are classified as related to human seasonal H1 (H1s, Figure 3), human seasonal H3 (Figure 2), or pandemic H1 (H1p) (online Technical Appendix Figure 4, http://wwwnc.cdc.gov/EID/article/21/8/14-1891-Techapp1.pdf).
§Viruses are classified as pandemic (N1p; online Technical Appendix Figure 5) or 1 of the 2 clades of human seasonal N2 (a and b; Figure 4).
¶Polymerase basic 2, polymerase basic 1, polymerase acidic, nucleoprotein, and nonstructural.
#Sequencing was performed directly from the original lung sample for A/wild boar/Brazil/214-11-13D/2011/H1N2.

transmission events associated with clades N2-a and N2-b are more likely to have occurred during late 1990s. These estimates overlap with the timing of the introduction of the H3 segment into swine in Brazil (Figure 2), and a parsimonious explanation is that the H3 clade and clade N2-a both represent the same introduction of human H3N2 viruses during the late 1990s, although additional sequencing is needed. In contrast, no evidence exists that the N2 of human H1N2 origin has continued to circulate in swine in Brazil beyond its initial introduction during the early 2000s. Rather, during the decade since the introduction of H1N2 viruses into the swine population, the H1N2 viruses have acquired through reassortment 2 different N2 segments of human H3N2 origin that circulated in swine in Brazil (N2-a and N2-b), representing 2 independent reassortment events between H1N2 and H3N2 viruses.

Introductions of Human Pandemic H1N1 IAVs into Swine in Brazil

The 6 viruses of human pH1N1 origin that we sequenced from swine in Brazil were not monophyletic on the H1 or N1 tree, indicating that these viruses represent multiple separate human-to-swine introductions rather than clonal expansion of a single human pH1N1 introduction into pigs (Table 1; online Technical Appendix Figures 4, 5). Additional pH1N1 HA and neuraminidase (NA) sequences were available in GenBank from swine in Brazil; these sequences also were not monophyletic or closely related to the pH1N1 viruses that were sequenced in our study, suggesting additional introductions of human pH1N1 viruses into swine herds in Brazil. The lower phylogenetic resolution of the recently emerged pH1N1 virus and large number of singleton viruses make precise estimation of the number of human-to-swine introductions difficult. As a lower-bound estimate based on monophyletic clusters supported by high bootstrap values (>70) on the pandemic H1, N1, and PB2 trees (online Technical Appendix Figures 4–6), we conservatively estimate 8 introductions of pH1N1 from humans into swine in Brazil, including additional sequence data from GenBank (Table 2). Including singleton viruses and poorly supported clusters, as many as 15 putative human-to-swine introductions in Brazil were evident on the HA phylogeny (online Technical Appendix Figure 4). Some of the long branch lengths separating singleton swine viruses from human viruses might represent onward transmission of a pH1N1 introduction in the swine population in Brazil, but additional sequencing is needed to confirm sustained circulation. Although the resolution of the phylogeny inferred for the matrix segment is even lower, because of the short sequence length, the swIAVs from Brazil also are not monophyletic on the matrix tree, consistent with multiple introductions of pH1N1 into swine in Brazil (online Technical Appendix Figure 7). Phylogenies also suggest that human pH1N1 viruses have been transmitted multiple times to swine in other Latin America countries, including Argentina, Colombia, and Mexico (Table 2; online Technical Appendix Figures 4–6). Only in Costa Rica was the pH1N1 population monophyletic with high bootstrap support that suggests a single human-to-swine introduction.

Table 2. Introductions of influenza A(H1N1)pdm09 virus from humans to swine in Latin America

Introduction	Viruses	Collection date	Bootstrap*
Brazil			
1	A/swine/Brazil/1/2009/H1N1; A/swine/Brazil/2/2009/H1N1; A/swine/Brazil/3/2009/H1N1	2009 Aug 11; 2009 Aug 11; 2009 Aug 11	75 (HA), 72 (NA)
2	A/swine/Brazil/5/2009/H1N1; A/swine/Brazil/6/2009/H1N1	2009 Aug 28; 2009 Aug 28	97 (HA)
3	A/swine/Brazil/8/2009/H1N1; A/swine/Brazil/9/2009/H1N1; A/swine/Brazil/10/2009/H1N1	2009 Oct 5; 2009 Oct 5; 2009 Oct 5	85 (HA), 85 (NA)
4	A/swine/Brazil/12/2009/H1N1; A/swine/Brazil/13/2009/H1N1	2009 Oct 25; 2009 Nov 10	97 (HA)
5	A/swine/Brazil/19/2010/H1N1; A/swine/Brazil/20/2010/H1N1	2010 Sep 9; 2010 Sep 9	100 (HA), 100 (NA)
6	A/swine/Brazil/107/2010/H1N1 (SC); A/wild boar/Brazil/214-11-13D/2011/H1N2 (RS)	2010 Jul 15; 2011 Jul 25	84 (PB2), 83 (PB1)
7	A/swine/Brazil/31-11-1/2011/H1N2 (PR); A/swine/Brazil/31-11-3/2011/H1N2 (PR)	2011 Feb 28; 2011 Feb 28	100 (PB2), 100 (PB1)
8	A/swine/Brazil/185-11-7/2011/H1N2 (SC); A/swine/Brazil/232-11-13/2011/H1N2 (SC)	2011 Jul 6; 2011 Aug 17	99 (PB2)
Argentina			
1	A/swine/Argentina/CIP051-A204/2012/H1N1; A/swine/Argentina/CIP051-A207/2012/H1N1	2012 Feb ; 2012 Feb	90 (HA)
2	A/swine/Argentina/CIP051/BsAs76/2009/H1N1; A/swine/Argentina/CIP051-A160/2011/H3N2	2009 Oct; 2011 Apr	100 (PB2), 86 (PB1)
Colombia			
1	A/swine/Colombia/1-01/2009/H1N1; A/swine/Colombia/1101/2009/H1N1	2009 Aug; 2009 Nov	85 (HA)
2	A/swine/Colombia/1/2011/H1N1; A/swine/Colombia/2/2011/H1N1	2011 Jun 16; 2011 Jun 16	100 (HA)
3	A/swine/Colombia/3/2011/H1N1; A/swine/Colombia/4/2011/H1N1	2011 Jul 28; 2011 Jul 28	100 (HA)
Costa Rica	A/swine/Costa Rica/000125-15/2010/H1N1; A/swine/Costa Rica/000125-3/2010/H1N1; A/swine/Costa Rica/000125-20/2010/H1N1; A/swine/Costa Rica/000125-19/2010/H1N1; A/swine/Costa Rica/000125-16/2010/H1N1 A/swine/Costa Rica/000125-14/2010/H1N1	2011 Nov 1; 2011 Nov 1; 2011 Nov 1; 2011 Nov 1; 2011 Nov 1	100 (HA), 100 (NA)
Mexico	A/swine/Mexico/SG1443/2010/H1N1; A/swine/Mexico/SG1450/2011/H1N1	2010 Oct 2; 2011 Nov 9	100 (HA), 100 (NA)

*Bootstrap support for clusters of viruses associated with introductions of pH1N1 virus from humans into swine in Latin America (online Technical Appendix Figures 4–6, http://wwwnc.cdc.gov/EID/article/21/8/14-1891-Techapp1.pdf). HA, hemagglutinin; NA, neuraminidase; PB, polymerase basic; pHN1, influenza A(H1N1)pdm09.

Reassortment with pH1N1 Internal Genes

Several additional introductions of pH1N1 were evident only on the internal gene trees (e.g., PB2; online Technical Appendix Figure 6) resulting from reassortment with HA and NA segments of human seasonal virus origin (Tables 1,2). In contrast to the genetic diversity of the HA and NA segments in swine in Brazil, the genetic diversity of the 6 internal gene segments in Brazil was restricted to pH1N1, at least for those for which sequence data were available (Table 1). This pattern indicates that the internal genes of pH1N1 viruses have reassorted with the HA and NA of other swIAVs of human seasonal virus origin circulating in Brazil.

Discussion

Our recently initiated influenza virus surveillance in Brazil uncovered multiple new lineages of swIAVs that are related to seasonal influenza viruses that circulated in humans more than a decade ago. These swIAVs have not been detected in any other country, including the well-sampled swine populations of the United States, although surveillance for swIAVs is infrequent or lacking altogether in neighboring Latin America countries. Therefore, the possibility that these swIAVs have circulated, or even originated, in other Latin America countries that do not routinely conduct surveillance in swine cannot be excluded. At this time, however, the most parsimonious explanation based on the data available is that human seasonal viruses were introduced at least 3 times into swine in Brazil: H3N2 viruses were introduced twice into swine during the late 1990s, and a human seasonal H1N2 virus was introduced during the early 2000s. Viral segments from all 3 introductions have persisted in swine at least until 2011, the most recent date of sample collection. However, the internal gene segments were replaced in the intervening years by reassortment with pH1N1 viruses transmitted from humans to swine in Brazil multiple times since 2009.

These findings highlight the importance of human-to-swine transmission in the evolution of swIAV diversity in Brazil. The detection of multiple influenza viruses of human seasonal and pandemic origin circulating within Brazil's large swine herds is entirely consistent with the frequency of human-to-swine transmission that recently has been highlighted on a global scale for seasonal (*35*) and for pandemic (*40*) influenza viruses. The swIAVs of human seasonal and pandemic origin also have been identified in Argentina in recent years (*17*,*22*). Our findings also match the globally observed pattern that new HA and NA

segments of human seasonal virus origin persist at higher rates than the internal genes, which frequently are replaced through reassortment by cccirculating swine viruses, particularly pH1N1 (*35*).

Our data provided no evidence of importation of any swIAVs into Brazil from another country's swine population. None of the triple reassortant, classical, or delta lineage influenza viruses that are prevalent in swine populations in the United States and Canada were detected in Brazil. However, it is possible that other swIAV lineages circulate in Brazil that our surveillance did not detect. Notably, our surveillance in Brazil did not identify either of the 2 swIAV lineages (North American triple reassortant or avian-like Eurasian viruses) that combined to create the pH1N1 virus associated with the 2009 pandemic. Consequently, the geographic origins of the pH1N1 virus in swine before its emergence in humans in 2009 remain unclear. Additional swIAV surveillance in unstudied and understudied regions in Latin America is greatly needed to understand the spatial origins and evolution of the swine virus that gave rise to the first pandemic outbreak in Mexico in early 2009.

The identification of swIAV diversity in Brazil, particularly in the HA and NA segments, has implications for the design of effective influenza vaccines for Brazil, which are in development. Although influenza viruses had been isolated from swine in Brazil from as early as 1974, influenza was not believed to be a major problem clinically for swine until the 2009 pandemic (*18*,*19*). Our findings suggest that H3N2 and H1N2 viruses could have circulated for many years in Brazil but were not associated with major clinical illness and have become pathogenic only since 2009, after reassortment with co-circulating pH1N1 virus internal genes. Given the relatively limited number of samples we collected compared with the large population of ≈41 million swine in Brazil, additional surveillance is critically needed to understand the diversity of swIAVs in circulation. Further surveillance is also necessary to select representative vaccine strains that will best protect against the diversity of swIAVs circulating in Brazil. Finally, further antigenic characterization is needed to assess the age-specific pandemic threat presented by these new swIAVs. The H3N2 and H1N2 viruses are most closely related to human seasonal IAVs that circulated during the 1990s and 2000s, and children may have particularly low preexisting immunity.

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Differentiation of Acute Q Fever from Other Infections in Patients Presenting to Hospitals, the Netherlands[1]

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Differentiating acute Q fever from infections caused by other pathogens is essential. We conducted a retrospective case–control study to evaluate differences in clinical signs, symptoms, and outcomes for 82 patients with acute Q fever and 52 control patients who had pneumonia, fever and lower respiratory tract symptoms, or fever and hepatitis, but had negative serologic results for Q fever. Patients with acute Q fever were younger and had higher C-reactive protein levels but lower leukocyte counts. However, a large overlap was found. In patients with an indication for prophylaxis, chronic Q fever did not develop after patients received prophylaxis but did develop in 50% of patients who did not receive prophylaxis. Differentiating acute Q fever from other respiratory infections, fever, or hepatitis is not possible without serologic testing or PCR. If risk factors for chronic Q fever are present, prophylactic treatment is advised.

Q fever is a zoonosis caused by the bacterium *Coxiella burnetii*. During 2007–2010, the southern part of the Netherlands had the largest outbreak of Q fever ever reported (*1*,*2*). Infection with *C. burnetii* is symptomatic in ≈40% of all patients (*3*). Clinical signs range from a mild influenza-like illness to pneumonia or a hepatitis-like syndrome and can differ by region (*4*,*5*). After initial infection, chronic Q fever will develop in 1%–5% of patients (*1*,*3*). Furthermore, long-lasting fatigue will develop in ≈20% of all patients with symptomatic acute Q fever (*6*–*8*) without development of chronic Q fever (*9*).

Treatment for acute infection decreases the duration of fever, increases recovery from pneumonia (*10*), and might lead to a lower percentage of patients in whom chronic Q fever will develop (*10*–*13*). In addition, several reports indicate that, in acute Q fever patients at risk for development of chronic Q fever, prophylactic treatment might prevent persistent infection (*12*,*14*). Therefore, recognizing Q fever in an early stage is a useful strategy.

The only available data on symptoms of acute Q fever in the Netherlands were obtained from a retrospective study that collected data several months after onset of disease by sending questionnaires to patients with acute Q fever (*15*). However, this method for obtaining data is limited by a high risk for recall bias. To help physicians differentiate acute Q fever from other diseases, a clear description of signs and symptoms compatible with *C. burnetii* infection is desirable.

The purpose of this case–control study was to evaluate differences in clinical signs and symptoms between patients with acute Q fever referred to a hospital and a control group of patients with signs and symptoms that led to addition of Q fever in the differential diagnosis. Furthermore, outcome of patients hospitalized with acute Q fever were evaluated, and the effect of prophylactic treatment for those patients with an indication to prevent development of chronic Q fever was analyzed.

Materials and Methods

Patients

The study group consisted of adult patients who came to the Radboud university medical center or Canisius Wilhelmina Hospital in Nijmegen, the Netherlands, during January 2007–March 2011 with pneumonia, fever and lower respiratory tract symptoms, or fever and hepatitis, and who were given a diagnosis of acute Q fever. Symptoms had to be present for <3 weeks before presentation. Exclusion criteria were chronic Q fever and a known previous acute Q fever episode. The same clinical criteria were used for the control group, but Q fever serologic results and, if available, PCR results had to remain negative. A standardized case report form was completed for every patient. According

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to national law, this study was exempt from approval by an ethics committee because of the retrospective characteristics of the study and the anonymous storage of data.

PCR and Serologic Analysis

During January 2007–March 2011, several laboratory techniques were used to diagnose acute Q fever. Because both hospitals collaborate extensively, the same microbiological laboratory techniques were used in both hospitals. The PCR used to detect DNA of *C. burnetii* in serum was an in-house, real-time PCR directed against insertion sequence IS1111a.

Serologic analysis was performed for blood samples by using the *Coxiella burnetii* (Q Fever) IgM ELISA (PanBio Pty Ltd., Windsor, Queensland, Australia), which detects IgM against phase II antigens and has a cutoff index of 1.1; a complement fixation assay (CFA) (Virion-Serion, Würzburg, Germany), which detects *C. burnetii* phase II antigens and shows a positive result if the titer is >1:10; and a Q fever immunofluorescent assay (IFA) for IgG and IgM (Focus Diagnostics Inc., Cypress, CA, USA), which detects IgM and IgG against phase I and phase II antigens and shows a positive result if the titer is >1:16.

Definition of Acute Q Fever

On the basis of the algorithm published by the Dutch working group on diagnostics of acute Q fever (*16*), the following definition of acute Q fever was used for all included patients: pneumonia, lower respiratory tract symptoms and fever, or hepatitis-like symptoms and fever, all ≤3 weeks before presentation; and 1) a positive serum PCR result ≤21 days of onset of disease; or 2) a negative serum PCR result, but a positive ELISA result for IgM against phase II antigens of *C. burnetii* and a positive CFA result for immunoglobulins against *C. burnetii*; or 3) a negative serum PCR result but a positive ELISA result and a positive IFA result for IgM and IgG against phase I and phase II antigens of *C. burnetii*; or 4) two serum samples tested by CFA or IFA during an interval of ≥2 weeks that showed seroconversion or a 4-fold increase in titer.

A blood sample for Q fever serologic analysis obtained ≥2 weeks after the first day of illness was required because it was not possible to rule out acute Q fever if serologic samples are taken only at an earlier point, even if PCR results were negative during that period (*16*). Patients were selected only if an appropriate diagnostic procedure for Q fever was performed.

Treatment

Adequate treatment for acute Q fever was defined as antimicrobial drug therapy with doxycycline (200 mg/d), moxifloxacin (400 mg 1×/d), or ciprofloxacin (500 mg 2×/d) for ≥14 days (*17*,*18*). Indications for prophylactic treatment to prevent development of chronic Q fever were patients who met the criteria for endocarditis prophylaxis according to the international guidelines of the American Heart Association (*19*); patients with a structural aortic valve defect or mitral valve defect (*12*); patients with a known aneurysm of the aorta or other large vessels; and patients with a vascular prosthesis. Adequate prophylactic treatment was defined as doxycycline (200 mg/d) and hydroxychloroquine (200 mg 3×/d) for ≥6 months.

Statistical Methods

All data were analyzed by using SPSS version 20.0 (IBM, Armonk, NY, USA). For analysis of qualitative data, the Pearson's χ^2 test was used. To evaluate the effect of prophylactic treatment, the Barnard exact test was used because this test is more powerful than the Fisher exact test for instances of smaller sample sizes (*20*). For quantitative data, the Student *t*-test was used. A p value <0.05 was considered significant.

Results

General Characteristics

A total of 82 patients with acute Q fever who fulfilled inclusion criteria for the study group and 52 patients who fulfilled criteria for the control group were included in the study (Table 1). Patients with acute Q fever were younger (mean ± SD age 52 ± 16 years vs. 59 ± 16 years; p = 0.03); had less often a history of lung disease (p = 0.001); and were immunocompromised less often (p = 0.002). Patients with acute Q fever had more history of smoking (p = 0.01) and a higher frequency of a sore throat (p = 0.008) (Table 2). Production of sputum was reported less frequently by patients with acute Q fever (p = 0.049).

Physical Examination

Of patients with acute Q fever, 18% had shortness of breath (Table 3) compared with 44% in the control group (p = 0.03). A total of 4% of patients with acute Q fever had rhonchi at pulmonary examination compared with 22% in the control group (p = 0.005). Oxygen saturation was significantly higher in patients with acute Q fever (p = 0.02).

Laboratory Values

Patients with acute Q fever had a higher levels of C-reactive protein (mean 167 mg/L vs. 117 mg/L; p = 0.02) (Table 4) and lower leukocyte counts (mean 9.0×10^9 cells/L vs. 11.5×10^9 cells/L; p = 0.006). Leukocyte counts remained significantly lower in the first 3 days after presentation (p = 0.006–0.043). At admission to the hospital, no differences were found between the groups for levels of alkaline phosphatase and γ-glutamyl transpeptidase. However, from day 1 onward, levels of alkaline

Table 1. Characteristics for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group	Control group	p value
No. patients	82	52	NS†
Male sex, no. (%)	53 (65)	38 (73)	NS‡
Mean ± SD age, y (range)	52 ± 16 (23–91)	59 ± 16 (19–85)	0.027†
Mean no. days between first day of sickness and presentation	5.5	5.4	NS†
History of lung disease	8/78 (10)	18/51 (35)	0.001‡
Immunocompromised§	5/81 (6)	13/51 (25)	0.002‡
Valvular dysfunction	8/82 (10)	3/52 (6)	NS‡
Valve prosthesis	3/82 (4)	0/52 (0)	NS‡
Aneurysm	2/82 (2)	3/52 (6)	NS‡
Vascular prosthesis	3/82 (4)	3/52 (6)	NS‡
Liver disease	1/82 (1)	1/52 (2)	NS‡
Malignancy	2/82 (2)	9/52 (17)	0.002‡
Diabetes	9/82 (11)	7/52 (13)	NS‡
Contact with cattle	29/47 (62)	8/20 (40)	NS‡
History of smoking	58/74 (78)	25/44 (57)	0.013‡
Alcohol use	17/44 (39)	12/27 (44)	NS‡
Illicit drugs	4/35 (11)	0/18 (0)	NS‡
Proton pump inhibitors¶	13/82 (16)	22/52 (42)	0.001‡
Corticosteroids¶	5/82 (6)	10/51 (20)	0.017‡

*Values are no. positive/no. tested (%) unless otherwise indicated. NS, not significant.
†By Student *t*-test.
‡By χ^2 test.
§Also includes patients using corticosteroids.
¶Only medications that differed significantly between groups is shown.

phosphatase and γ-glutamyl transpeptidase were significantly higher in patients with acute Q fever (p = 0.01–0.047 and p = 0.007–0.05, respectively).

PCR and Serologic Analysis

Serum PCR for DNA of *C. burnetii* was performed for 41 patients in the study group (Table 5). Blood samples were obtained at day 8 ± 7 (mean ± SD) of illness. The sensitivity of this PCR was 56%. For 4 patients, a second blood sample was obtained at day 12 ± 5 of illness. The sensitivity of this PCR was 25%.

ELISA was performed on samples from 33 patients with acute Q fever and 18 patients in the control group. Blood samples were obtained from the study group at day 10 ± 8 of illness and from the control group at day 7 ± 6 of illness. Sensitivity of this ELISA was 61%.

CFA, which was performed for 81 patients in the study group at day 9 ± 19 of illness and for 52 patients in the control group at day 8 ± 6 of illness, showed a sensitivity of 22% (Table 5). A total of 57 patients were hospitalized, of whom 36 were given a diagnosis of acute Q fever during their hospitalization.

Table 2. Signs and symptoms for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group, n = 82, no. positive/no. tested (%)	Control group, n = 52, no. positive/no. tested (%)	p value†
Fever	64/75 (85)	37/49 (76)	NS
Chills	31/42 (74)	16/28 (57)	NS
Myalgia	22/24 (92)	11/14 (79)	NS
Night sweats	12/19 (63)	9/17 (53)	NS
Weight loss	11/26 (42)	7/14 (50)	NS
Chest pain	11/55 (20)	13/38 (34)	NS
Dyspnea	37/65 (57)	31/43 (72)	NS
Rhinorrhea	1/12 (8)	7/14 (50)	NS
Sore throat	12/22 (55)	1/12 (8)	0.008
Cough	49/76 (64)	38/48 (79)	NS
Sputum production	18/73 (25)	20/48 (42)	0.049
Nausea	14/48 (29)	12/37 (32)	NS
Vomiting	17/47 (36)	10/39 (26)	NS
Abdominal pain	9/51 (18)	6/33 (18)	NS
Diarrhea	9/50 (18)	4/36 (11)	NS
Headache	38/54 (70)	21/27 (78)	NS
Weakness	9/21 (43)	1/9 (11)	NS
Painful joints	7/20 (35)	2/16 (13)	NS
Arthritis	0/17 (0)	1/16 (6)	NS

*NS, not significant.
†By χ^2 test.

Table 3. Physical examination results for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group, n = 82	Control group, n = 52	p value
Dyspnea	13/73 (18)	18/41 (44)	0.03†
Abnormal heart sounds	1/80 (1)	0/51 (0)	NS†
Cardiac murmur	11/80 (14)	4/50 (8)	NS†
Decreased breath sounds	6/78 (8)	7/46 (15)	NS†
Bronchial breath sounds	9/64 (14)	5/37 (14)	NS†
Crackles	36/76 (47)	19/43 (44)	NS†
Rhonchi	3/68 (4)	9/41 (22)	0.005†
Palpable liver	1/69 (1)	1/39 (3)	NS†
Palpable spleen	0/68 (0)	0/36 (0)	NS†
Exanthema	2/9 (22)	0/6 (0)	NS†
Lymphadenopathy	2/27 (7)	2/21 (10)	NS†
Temperature, °C (no. patients)	38.4 (67)	38.3 (48)	NS‡
Heart rate, beats/min (no, patients)	93 (73)	91 (50)	NS‡
Systolic blood pressure, mm Hg, (no. patients)	134 (73)	138 (49)	NS‡
Respiratory rate, breaths/ min (no. patients)	25 (24)	25 (21)	NS‡
Saturation, % oxygenation (no. patients)§	97 (57)	95 (34)	0.022‡

*Values are no positive/no. tested (%) unless otherwise indicated. NS, not significant.
†By χ^2 test.
‡By Student *t*-test.
§Saturation without oxygen.

Imaging Studies

A total of 78% of chest radiographs for patients with acute Q fever showed signs of pneumonia. A total of 54% of chest radiographs for patients in the control group showed signs of pneumonia (p = 0.003) (Table 5).

Treatment

Treatment was started before a diagnosis was made. Significantly more patients with acute Q fever started treatment with doxycycline than patients in the control group (35% vs. 15%; p = 0.001) (Table 6). For 8 patients in the study group, the duration of antimicrobial drug treatment was unknown. Of the remaining 74 patients with acute Q fever, 34 (46%) patients were given adequate treatment. The mean ± SD follow-up time for patients given adequate treatment was 11.7 ± 5 months compared with 13.3 ± 9 months for patients given inadequate treatment.

Outcomes

Hospitalization (70% vs. 94%; p = 0.001), admission to an intensive care unit (4% vs. 18%; p = 0.002), and need for respiratory support (2% vs. 16%; p = 0.001) were less common for the study group than for the control group (Table 7). Also, duration of hospital stay was shorter for patients with acute Q fever (9 ± 7 days vs. 17 ± 15 days; p = 0.001). Accurate follow-up data were available for 59 of 82 patients with acute Q fever who had a mean ± SD follow-up of 12.8 ± 8.2 months. Chronic Q fever developed in 6 (10%) patients in the Q fever group.

Sixteen patients with acute Q fever met the criteria for prophylactic treatment to prevent development of chronic Q fever (Table 8). Indications were valvular dysfunction (n = 8); cardiac valve prosthesis (n = 3); aneurysm (n = 1); vascular prosthesis (n = 3, of whom 1 patient also had a cardiac valve prosthesis); and a new cardiac murmur (n = 2). Eight (50%) of these patients received prophylactic treatment. Proper follow-up data for development of chronic Q fever were available for 14 patients with an indication for prophylaxis. Chronic Q fever did not develop in any of the 8 patients who received prophylaxis. The other 6 patients with an indication for prophylaxis for whom follow-up serum samples were available did not receive prophylaxis because the indication for prophylaxis was not recognized by the treating physician. Chronic Q fever developed in 3 (50%) of these 6 patients (p = 0.02). In the group without an indication for prophylaxis, chronic Q fever developed in 3 (6%) patients. Six (11%) of 56 patients in the study group for whom these data were available reported long-lasting fatigue.

The mortality rate during a 12-month follow-up period was 6% for the study group compared with 19% for the control group (p = 0.02). None of the patients in the study group died during the episode of acute Q fever. Four patients in the study group died because of reasons unrelated to Q fever. One patient died of consequences of an infected vascular prosthesis caused by chronic Q fever, although adequate treatment was started after the diagnosis. In contrast, 2 patients in the control group died during initial hospitalization, 1 of a *Mycoplasma* sp. infection and 1 of pneumonia without a known causative agent. Eight patients in the control group died during follow-up. One of them died of a non-Hodgkin lymphoma and 1 of consequences of an *Aspergillus* sp. infection. For the other 6 patients who died, no detailed information was available.

A total of 49 control patients were given a diagnosis of pneumonia; for 38 of these patients, no causative agent was found. For the remaining 11 patients, causative agents were *Pneumocystis jiroveci*, *Moraxella catarrhalis*, *Legionnella pneumophila*, *Chlamydia* sp., *Haemophilus influenzae*

Table 4. Laboratory values for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Laboratory value	Day†	Study group, n = 82		Control group, n = 52		p value‡
		Mean	No. tested	Mean	No. tested	
Hemoglobin, mmol/L; reference range: men 8.1–10.7 mmol/L, women 7.3–9.7 mmol/L	0	8.3	77	8.0	51	NS
	1	7.4	28	7.3	34	NS
	2–3	7.7	27	7.0	29	0.036
	4–6	7.6	27	7.0	29	NS
Leukocytes, $\times 10^9$ cells/L; reference range 3.5–11.0 $\times 10^9$ cells/L	0	9.0	80	11.5	50	0.006
	1	8.5	40	10.8	28	0.043
	2–3	8.0	34	11.1	33	0.021
	4–6	10.9	28	9.2	31	NS
Platelets, $\times 10^9$/L; reference range 20–350 $\times 10^9$/L	0	239	78	208	50	NS
	1	242	23	178	29	0.038
	2–3	229	19	172	26	0.042
	4–6	298	24	208	27	0.011
Total bilirubin, μmol/L; reference value <17 μmol/L	0	14	26	16	20	NS
	1	12	14	14	8	NS
	2–3	9	12	28	6	0.017
	4–6	8	12	9	6	NS
AP, U/L; reference value <120 U/L	0	104	75	85	50	NS
	1	127	19	75	12	0.047
	2–3	126	26	66	12	0.010
	4–6	145	23	95	15	0.036
ALT, U/L; reference value <45 U/L	0	45	76	37	49	NS
	1	64	22	58	16	NS
	2–3	66	30	40	13	0.050
	4–6	81	22	84	18	NS
γ-GT, U/L; reference value: men <50 U/L, women <35 U/L	0	74	68	65	49	NS
	1	117	21	53	12	0.030
	2–3	106	27	42	9	0.007
	4–6	112	22	66	14	0.050
CRP, mg/L; reference value <10 mg/L	0	167	79	117	50	0.015
	1	184	44	150	37	NS
	2–3	132	46	147	32	NS
	4–6	76	41	98	27	NS
Urea, mmol/L; reference value 2.5–7 mmol/L	0	6.4	79	8.6	51	0.039
	1	6.4	33	7.9	35	NS
	2–3	5.4	38	8.7	35	0.014
	4–6	5.8	34	9.3	30	0.018
Creatinine, μmol/L; reference value: men <110 μmol/L, women <90 μmol/L	0	86	80	105	52	0.042
	1	84	38	103	38	NS
	2–3	79	37	103	37	NS
	4–6	81	36	136	31	NS

*NS, not significant; AP, alkaline phosphatase; ALT, alanine aminotransferase; γ-GT, γ-glutamyl transpeptidase; CRP, C-reactive protein.
†Day 0 is the day of coming to the hospital.
‡By Student *t*-test.

(2 patients), *Mycoplasma* sp. (3 patients), influenza virus and *Mycoplasma* sp., and *Staphylococcus aureus* and *Streptococcus pneumonia*. The remaining 3 patients were given diagnoses of acute myeloid leukemia, non-Hodgkin lymphoma, and restrictive pericarditis.

Discussion

This retrospective case–control study evaluated differences in clinical signs and symptoms between patients with acute Q fever referred to a hospital and a control group. Because patients in the control group had Q fever included in the differential diagnosis, a selection bias is possible. However, differences were found between the 2 groups. In addition, because of the Q fever outbreak during that time, *C. burnetii* was considered a possible etiologic agent in many patients who came to a hospital. The higher number of patients in the study group can be explained by strict implementation of inclusion criteria for the control group.

Consistent with findings of earlier studies (*1*,*21*), we found that patients with acute Q fever more often had a history of smoking. However, a history of lung disease was found less often. A lower mean age in the study group than in the control group might explain this finding. Previous studies suggest typical signs and symptoms of acute Q fever: fever, headache, and cough (*1*,*3*,*22*). However, no difference was observed in the occurrence of fever. It has been postulated that headache is rather specific for acute Q fever (*5*,*23*). However, in our study, headache was less common in patients with acute Q fever than in the control group. Although cough was a relatively common sign in both groups, sputum

Table 5. PCR and serologic results for patients in study group with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group, n = 82	Control group, n = 52	Day of illness for study group, mean ± SD	Day of illness for control group, mean ± SD	Sensitivity, %
PCR					
First sample	23/41	0/15	8 ± 7	8 ± 7	56
Second sample	1/4	0/1	12 ± 5	30 ± 0	25
ELISA					
First sample	20/33	0/18	10 ± 8	7 ± 6	61
Second sample	15/18	0/2	20 ± 11	25 ± 8	83
CFA					
First sample	18/81	0/52	9 ± 19	8 ± 6	22
Second sample	27/34	0/28	18 ± 9	20 ± 12	79
Third sample	5/5	0/3	21 ± 6	26 ± 5	100
Culture					
Blood†	0/42 (0)	0/40 (0)	NA	NA	NA
Urine†	0/30 (0)	0/37 (0)	NA	NA	NA
Sputum‡	1/15 (7)	3/22 (14)	NA	NA	NA
Chest radiograph§	62/79 (78)	28/52 (54)	NA	NA	¶

*Values are no. positive/no. tested (%) unless otherwise indicated. CFA, complement fixation assay; NA, not applicable.
†Includes only results for first cultures obtained after coming to the hospital.
‡Includes only results for first cultures obtained after coming to the hospital. In the study group, 1 patient was positive for parainfluenza virus. In the control group, 1 patient was positive for *Moraxella catarrhalis,* 1 patient was positive for *Legionella pneumophila*, and 1 patient was positive for *Streptococcus pneumonia* and *Staphylococcus aureus*.
§Includes only first chest radiographs after coming to the hospital. Values are no. abnormal/no. tested (%).
¶p = 0.003, by χ^2 test.

production was reported less often in patients with acute Q fever. In addition, a sore throat was reported more often in the study group, which has not been previously reported.

A limitation of these results is the retrospective nature of the study because physicians probably did not include all signs and symptoms in patient charts. In general, patients with lung disease often use corticosteroids, which might explain why fewer patients in the study group were classified as immunocompromised. In contrast to medical and physical examination results, more patients with acute Q fever showed signs of an infiltrate on chest radiographs when they came to the hospital. Although acute Q fever usually is a relatively mild influenza-like disease, it has been reported that chest radiographs often shows signs of an infiltrate (*24*). Compared with our control group, fewer patients in the study group needed hospitalization, and duration of hospitalization was shorter. These findings might be explained by the lower mean age of patients with acute Q fever, assuming that they were in a more healthy condition. Furthermore, *C. burnetii* is known for its self-limiting character, in contrast to those of other pathogens found in the control group.

In the Netherlands, a Q fever hospitalization rate of 50% in 2007 was registered, which stabilized at ≈20% in later years (*25*). This rate is higher than that previously reported (2%–5%) (*5*). However, large variations in hospitalization rates for acute Q fever patients have been reported (*26*). In this study, 70% of patients with acute Q fever were hospitalized. Most patients with acute Q fever are asymptomatic or have only a mild influenza-like illness. Thus, a selection bias caused by the study design is likely.

Table 6. Initial treatment for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Initial treatment	Study group, n = 82, no. positive/no. tested (%)	Control group, n = 52, no. positive/no. tested (%)	p value†
Doxycycline	29/82 (35)	8/52 (15)	0.001
Moxifloxacin	5/82 (6)	2/52 (4)	NS
Ciprofloxacin	7/82 (9)	6/52 (12)	NS
Penicillin	7/82 (9)	1/52 (2)	0.049
Amoxicillin	13/82 (16)	5/52 (10)	NS
Amoxicillin/clavulanic acid	3/82 (4)	4/52 (8)	NS
Piperacillin/tazobactam	1/82 (1)	5/52 (10)	NS
Cephalosporin	14/82 (17)	17/52 (33)	NS
Co-trimoxazole	0/82 (0)	1/52 (2)	NS
Flucloxacillin	2/82 (2)	0/52 (0)	NS
Clarithromycin	0/82 (0)	1/52 (2)	NS
No treatment	1/82 (1)	1/52 (2)	NS
Unknown	0/82 (0)	1/52 (2)	NS
Patients with adequate treatment‡	34/74 (46)	NA	NA

*NS, not significant; NA, not applicable.
†By χ^2 test.
‡Defined as use of doxycycline (200 mg/d), moxifloxacin (400 mg 1×/d), or ciprofloxacin (500 mg 2×/d) for ≥2 wk.

Table 7. Outcome, follow-up, and prophylaxis for patients with acute Q fever and control group with negative serologic results for Q fever, the Netherlands*

Characteristic	Study group	Control group	p value
Outcome			
Hospitalized	57/82 (70)	49/52 (94)	0.001†
Need for ICU	2/57 (4)	9/49 (18)	0.002†
Need for respiratory support	1/57 (2)	8/49 (16)	0.001†
Mean ± SD duration of hospitalization, d	9 ± 7	17 ± 15	0.001‡
Mean ± SD duration of time in ICU, d	5 ± 1	14 ± 10	0.266‡
Follow up			
Development of chronic Q fever	6/59 (10)	NA	NA
Development of long-lasting fatigue§	6/56 (11)	NA	NA
Death	5/82 (6)	10/52 (19)	0.019†
Q fever–related death	1/82 (1)¶	NA	NA
Indication for prophylaxis	16/82 (20)	NA	NA
Development of chronic Q fever			
Prophylactic treatment	0/8 (0)	NA	NA
No prophylactic treatment	3/6 (50)	NA	0.018#

*Values are no. positive/no. tested (%) unless otherwise indicated. ICU, intensive care unit; NA, not applicable.
†By χ^2 test.
‡By Student *t*-test.
§Defined as persisting fatigue for >6 mo after acute Q fever in the absence of chronic Q fever.
¶This patient died of consequences of an infected vascular prosthesis caused by chronic Q fever.
#By unilateral Barnard exact test.

We found that 78% of patients in the study group had an abnormal result on a chest radiograph, which might indicate that only patients with severe symptoms were hospitalized.

Although C-reactive protein levels and leukocyte counts differed between the study group and the control group, this finding did not contribute to differentiation between *C. burnetii* and other pathogens at hospitalization because differences were small and showed much overlap. In addition, although leukocyte counts were usually within the reference range, patients with acute Q fever more often had a lower leukocyte count, which is consistent with results of other studies (*3*,*4*). In contrast to these studies, which found thrombocytopenia in patients with acute Q fever, we found slightly higher levels of platelets, all within the reference range, in the study group than in the control group. Increased levels of liver enzymes have been reported in patients with acute Q fever (*3*,*5*,*22*). However, we found no differences in these levels between both groups at hospitalization. Furthermore, creatinine levels were not increased, in contrast to results reported in a previous study (*3*).

Although antimicrobial drug treatment was inadequate in an unexplainably high percentage of patients with acute Q fever, more patients in the study group than in the control group were initially treated with doxycycline, the treatment of choice for patients with acute Q fever. The choice of antimicrobial drug treatment in patients with community-acquired pneumonia (CAP) of unknown origin in the Netherlands depends on the Confusion, Urea nitrogen level in blood, Respiratory rate, Blood pressure, age ≥65 years (CURB-65) score (*27*). In addition, although CURB-65 scores could not be calculated for all patients, fewer patients in the study group were hospitalized, needed admission to an intensive care unit, and needed respiratory support, which suggests lower CURB-65 scores in the study group than in the control group.

Although changes were made in the national guidelines for treating CAP issued by the Dutch Working Party on Antibiotic Policy in 2011 (*28*), until 2011, doxycycline was the first choice for patients with a low CURB-65 score (*29*). In addition, more patients in the study group were given a diagnosis of having an infiltrate, which suggested that initial treatment in the study group was also aimed at atypical microorganisms. Presumably, patients in the control group were treated with broader spectrum antimicrobial drugs because of higher CURB-65 scores. Also, more patients in the control group were immunocompromised, which also could have influenced the choice of treatment.

Long-term prophylactic treatment with doxycycline and hydroxychloroquine has been suggested for patients with risk factors for development of chronic Q fever (*12*,*14*). Although controversy still exists (e.g., with regard to treatment duration and patient selection), prophylactic treatment of high-risk patients after an episode of acute Q fever can be beneficial and is widely advised (*30*–*32*). In our study, not all patients who had an indication according to our definition received prophylaxis. Chronic Q fever developed in 3 of 6 patients who did not receive prophylaxis, in contrast to none of the patients who received prophylaxis, which is a difference that clearly supports findings of other studies in which prophylactic treatment was suggested to prevent development of chronic Q fever in patients with risk factors for this disease (*12*,*14*). On the basis of these results, prophylactic treatment is advised if risk factors for developing chronic Q fever exist, but potential side effects must be taken into consideration (*33*).

For 48 of 67 patients without indication for prophylactic treatment, follow-up data were available on development of

Table 8. Characteristics of 16 patients with acute Q fever with an indication for prophylaxis, the Netherlands*

Patient no.	Age, y/sex	Hospitalized	Indication at presentation for prophylactic treatment	Prophylactic treatment and duration, mo	Chronic Q fever	Died
1	42/M	Yes	Valvular dysfunction (AS)	D + H, 12	No	No
2	49/M	Yes	Cardiac bioprosthesis and vascular prosthesis	D + H, 12	No	No
3	51/M	Yes	Cardiac bioprosthesis and TOF	D 12 + H 4 (added after 8)	No	No
4	54/M	Yes	Aneurysm common iliac artery	D + H, 9	No	No
5	43/M	Yes	Valvular dysfunction (TI) and TGV	D + H, 7	No	No
6	78/F	Yes	Cardiac bioprosthesis	D + H, 1, switched to Mox, 3	No	Yes†
7	26/M	No	Vascular prosthesis	D + H, 2.5	No	No
8	81/F	Yes	Valvular dysfunction (MI)	D + H, 12	No	Yes‡
9	65/M	Yes	Valvular dysfunction (MI)	No	No	No
10	80/M	Yes	Valvular dysfunction (MI)	No	No	No
11	78/F	No	Valvular dysfunction (MI)	No	No	No
12	64/F	Yes	Vascular prosthesis	No	Yes	Yes§
13	75/F	Yes	New cardiac murmur	No	Yes	No
14	75/M	No	New cardiac murmur	No	Yes	No
15	57/F	No	Valvular dysfunction (AS)	No	Unknown¶	No
16	58/M	Yes	Valvular dysfunction (MI)	No	Unknown¶	No

*AS, aortic valve sclerosis; D, doxycycline 100 mg 2×/d; H, hydroxychloroquine 200 mg 3×/d; TOF, tetralogy of Fallot; TI, tricuspid insufficiency; TGV, transposition of the great vessels; Mox, moxifloxacine 400 mg 1×/d; MI, mitral insufficiency; CFA, complement fixation assay; IFA, immunofluorescence assay.
†This patient was rehospitalized shortly after the acute Q fever episode and died because of a reason unrelated to Q fever. The last available serologic follow-up showed no signs of chronic Q fever (negative PCR result; CFA titer 1:10, IFA IgG phase I negative result; IgG phase II titer 1:256; IgM phase I negative result; and IgM phase II titer 1:64).
‡This patient eventually died because of a reason unrelated to Q fever. The last available serologic follow-up 1 year after the acute Q fever episode showed no signs of chronic Q fever (negative PCR result; CFA titer 1:10; IFA IgG phase I titer 1:64; IgG phase II titer 1:512; IgM phase I titer 1:16, and IgM phase II titer 1:16).
§This patient was hospitalized and admitted to the intensive care unit for 5 d. She was treated with several antimicrobial drugs (penicillin, ciprofloxacin, cefuroxim, metronidazol, ceftazidim, and teicoplanin) before given a diagnosis of an infected vascular prosthesis caused by chronic Q fever. Although doxycycline and hydroxychloroquine were given after the diagnosis was made, this patient eventually died from consequences of an infected vascular prosthesis caused by chronic Q fever.
¶No follow-up with reference to Q fever was performed for this patient.

chronic Q fever. Chronic Q fever developed in 3 (6%) of these patients, which is slightly higher than expected (*1*,*34*). This finding might be explained by the fact that we included only patients who were referred to a hospital, and therefore selected patients most affected by *C. burnetii* infection. It is possible that more severely acute Q fever predisposes for development of chronic Q fever (*13*).

After having acute Q fever, patients often report long-lasting fatigue, which frequently persists for >6 months. This symptom after acute Q fever has been designated Q fever fatigue syndrome. Our data suggest a prevalence of 11%, which is lower than expected; other studies reported a prevalence of ≈20% worldwide and a higher prevalence in the Netherlands (*6*,*35*,*36*). The prevalence found in this study is presumably an underestimation because proper analysis was not performed for most patients.

Although we found some differences in clinical manifestations for patients with acute Q fever coming to a hospital compared with controls, considerable overlap between both groups hamper the use of these variables for clinical differentiation. Differentiating *C. burnetii* from other pathogens is not possible without Q fever serologic analysis and PCR in patients coming to a hospital. In disease-endemic areas or in instances in which patients have risk factors for Q fever, suspicion should remain high, and the threshold for performing Q fever serologic analysis and PCR should remain low. Because only 46% of patients received adequate treatment acute Q fever in our study, treatment for acute Q fever should be improved. Furthermore, our findings underline the recommendation that prophylactic treatment should be given to patients with risk factors for developing chronic Q fever. However, more studies are needed to develop uniform guidelines with regard to optimal prophylactic treatment.

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Susceptibility of Carrion Crows to Experimental Infection with Lineage 1 and 2 West Nile Viruses

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West Nile virus (WNV) outbreaks in North America have been characterized by substantial die-offs of American crows (*Corvus brachyrhynchos*). In contrast, a low incidence of bird deaths has been observed during WNV epidemic activity in Europe. To examine the susceptibility of the western European counterpart of American crows, we inoculated carrion crows (*Corvus corone*) with WNV strains isolated in Greece (Gr-10), Italy (FIN and Ita09), and Hungary (578/10) and with the highly virulent North American genotype strain (NY99). We also inoculated American crows with a selection of these strains to examine the strains' virulence in a highly susceptible bird species. Infection with all strains, except WNV FIN, resulted in high rates of death and high-level viremia in both bird species and virus dissemination to several organs. These results suggest that carrion crows are highly susceptible to WNV and may potentially be useful as part of dead bird surveillance for early warning of WNV activity in Europe.

West Nile virus (WNV), a flavivirus (family *Flaviviridae*) transmitted by mosquitoes, uses birds as its primary vertebrate reservoir host. WNV has an extensive geographic range that includes Europe, Africa, the Middle East, southern Asia, and Australia (*1*). In 1999, WNV emerged in North America, where it was first detected in New York, New York. The virus subsequently spread rapidly across the continent, becoming the leading cause of arboviral encephalitis in humans and horses (*2*), and it was associated with deaths among at least 326 bird species (*3*). High death rates are most frequently observed among passeriform birds, of which the family *Corvidae* comprises the most highly susceptible species to WNV (*4*). In particular, deaths among the American crow (*Corvus brachyrhynchos*) have been used to track the spread of the virus across many parts of North America (*5*–*8*).

Since 2008, WNV has been responsible for outbreaks throughout central and southeastern Europe, affecting countries such as Greece, Italy, Hungary, Romania, and Croatia and constituting a serious veterinary and public health problem. Fatalities have been reported among wild birds in Europe, such as eagles (*9*,*10*), sparrow hawks, goshawks, geese, and falcons (*11*–*13*). However, death rates among birds in Europe have been low, and no clustered death events have occurred, even when cases were associated with outbreaks of severe human and equine WNV infections (*14*–*17*). Several theories have been proposed to explain the low death rates among birds in Europe: limited or insufficient monitoring of deaths among wild birds in Europe; development of immunity among birds from infections acquired on wintering grounds (*18*); and circulation of WNV strains in Europe with reduced virulence for birds.

Experimental infection of American crows with the North American genotype of WNV (NY99) has shown that the strain has a highly pathogenic phenotype: viremia titers exceeded 9 $\log_{10}$ PFU/mL, and all infected birds died (*19*–*23*). However, the lack of WNV-associated bird deaths in Europe suggests that European birds might not be susceptible to WNV or that WNV strains from Europe are not virulent to birds. Thus, we evaluated the susceptibility of the European equivalent of the American crow, carrion crows (*Corvus corone*), which are ubiquitously present across Europe, by injecting them with selected strains of WNV circulating in Europe and with the prototypic NY99 strain. In addition, we inoculated American crows with a selection of these viruses to assess and compare the virulence of WNV strains from Europe in a bird species known to be highly susceptible to WNV. Susceptibility was assessed in terms

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of death, survival time, magnitude and duration of viremia, and spread of virus to different organs.

Materials and Methods

Source of Virus and Birds

Five different WNV strains were used in this study: lineage 1a strain NY99-4132 (NY-99) (*20*); lineage 2 strain Nea Santa-Greece-2010 (Greece-10; GenBank accession no. HQ537483.1) (*24*); lineage 1a strain Italy/2009/FIN (FIN; GenBank accession no. KF234080); lineage 1a strain Ita09 (GenBank accession no. GU011992.2) (*25*); and lineage 2 strain 578/10 (GenBank accession no. KC496015). Further details about these viruses are provided in Table 1.

Carrion crows were captured by using walk-in traps in the municipality of Rotterdam, the Netherlands, and then transported to indoor housing at the animal holding facilities at the National Institute for Public Health and the Environment, Bilthoven, the Netherlands. After being inoculated with WNV, the crows were cared for in groups of 8 in isolators under negative pressure. Only seronegative birds were used in this study. Seronegativity was determined by using a neutralization assay (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-0714-Techapp1.pdf).

American crows were captured by using cannon net traps in Bellvue, Colorado, USA; the National Wildlife Diseases Program, Animal and Plant Health Inspection Service, United States Department of Agriculture, assisted with the captures. The crows were banded and transported to Fort Collins, Colorado, where they were housed in 1-m^3 cages (2 birds per cage) at the Colorado State University Animal Disease Laboratory.

Experimental Infection and Sampling Protocol

Crows were subcutaneously inoculated in the thigh or breast region with 2,000 50% tissue culture infectious doses ($TCID_{50}$) of virus per 0.1 mL of serum-free Dulbecco's Modified Eagle Medium (DMEM) (Lonza Benelux BV, Breda, the Netherlands). Carrion crows (8 per virus) were injected with WNV strain NY99, Greece-10, FIN, Ita09, or 578/10. American crows were inoculated with NY99 (n = 6), FIN (n = 5), or Ita09 (n = 5). Approximately 0.1 mL of blood was collected from carrion crows at 2-day intervals, up to 8 days postinoculation (dpi), and 0.2 mL of blood was collected from American crows at the same time points and added to 0.9 mL of serum-free DMEM. Coagulated blood from carrion crows was centrifuged at 1,300 × *g* for 5 min in MiniCollect vials (Greiner Bio-One, Alphen aan den Rijn, the Netherlands) to separate serum, and coagulated blood from American crows was centrifuged at 3,700 × *g* for 10 min to pellet clotted cells. Serum samples were stored at −80°C until further use.

Crows were examined for clinical signs twice daily for 14 dpi and euthanized under isoflurane anesthesia upon display of clinical signs. In addition, 2 birds per group of the carrion crows were euthanized at 4 dpi.

Necropsies were performed on all euthanized carrion crows; heart, liver, spleen, kidney, bone marrow, and brain samples were collected. A small section of each tissue was collected, weighed, and homogenized by using a metal bead in 1 mL of DMEM containing 100 U penicillin and 100 μg/mL streptomycin. The remaining portion of the tissues was collected in formalin for use in immunohistochemical staining.

Determination of Virus Loads

We used quantitative real-time reverse transcription PCR (qRT-PCR) to determine virus titers in serum and tissue samples and $TCID_{50}$ titration to calculate infectious virus titers in serum only. In brief, RNA was isolated from 50 μL of serum or 100 μL of homogenized tissue by using the MagNA Pure LC Total Nucleic Acid Isolation Kit (Roche, Almere, the Netherlands) and a MagNA Pure LC automated nucleic acid robotic workstation (Roche) according to the manufacturer's instructions, and subsequently stored at −80°C. RNA copy numbers were quantified by using unmodified primers as previously described (*26*). The limit of detection of the assay was 9 (1.0 $\log_{10}$) RNA copies.

After $\log_{10}$ titration of serum samples on Vero E6 cells, cytopathic effect was determined at 5 dpi and $TCID_{50}$ infectious titers were calculated by using the Spearman–Kärber method (*27*,*28*). An initial 1:10 dilution of serum resulted in a limit of detection of $10^{1.8}$ $TCID_{50}$/mL.

Immunohistochemistry

Paraffin sections (4-μm thick) of sagittal organ were processed for streptavidin–biotin–peroxidase immunohistochemical detection of nonstructural protein (NS) 3 antigen. Sections were deparaffinized in xylene, rehydrated in

Table 1. West Nile virus strains used for susceptibility studies in carrion and American crows

Strain	Source	Passage history*	Location	Genetic lineage	Crow species inoculated
NY99-4132	American crow (brain)	V2	United States	1a	Carrion, American
Nea Santa-Greece-2010	*Culex pipiens* mosquito	V1	Greece	2	Carrion
Italy/2009/FIN	Human with neuroinvasive disease	V2, C1	Italy	1a	Carrion, American
Ita09	Human with neuroinvasive disease	V1, C1	Italy	1a	Carrion, American
578/10	Horse (brain)	V2, C1	Hungary	2	Carrion

*Viruses were propagated in Vero (V) or C6/36 insect cells (C). Numbers following passage source represent the number of virus passages.

descending concentrations of ethanol, and incubated for 10 min in 3% H_2O_2 diluted in PBS to block endogenous peroxidase activity. Antigen exposure was performed by incubation at 121°C for 15 min in citrate buffer (10 mmol/L, pH 6.0). Sections were subsequently incubated overnight at 4°C with polyclonal goat anti-WNV NS3 protease (1:100; R&D Systems, Abingdon, UK) or isotype control (goat serum, 1:100; Dako, Eindhoven, the Netherlands) and then detected with polyclonal rabbit anti-goat IgG/HRP (Dako) antibody. Sections were counterstained with Mayer hematoxylin, mounted with Kaiser glycerin-gelatin, and analyzed by using a light microscope.

Statistical Analyses

Survival curves were analyzed by using the log-rank (Mantel-Cox) test. Statistical analyses between >2 groups were performed by using Kruskal-Wallis 1-way analysis of variance; any significant differences were more closely analyzed between the groups by using the Mann-Whitney U test. A Bonferroni correction was applied to each p value, according to the number of comparisons (corrected p value of 0.05/10 = 0.005 for carrion crow peak viremia and organs of carrion crows euthanized on day 4; corrected p value of 0.05/6 = 0.008 for American crow peak viremia and organs of carrion crows euthanized due to illness). For all comparisons, each group had 6 crows, except for American crow groups that received FIN or Ita09 (n = 5).

Results

Illness and Death

Signs of illness (e.g., lethargy, unresponsiveness, anorexia, and ruffled feathers) were observed among most crows within 9 dpi. All 6 carrion crows inoculated with Greece-10 or Ita09 died, and 5 (83%) of the 6 inoculated with NY99 or 578/10 died. All 6 carrion crows inoculated with strain FIN survived (Table 2). Survival curves of the infected birds showed a significant difference in survival between carrion crows infected with Ita09, Greece-10, NY99, or 578/10 and those infected with FIN (p = 0.005) (Figure 1). The median day of death was 7 dpi for carrion crows that died from infection with NY99, Greece-10, or Ita09 and 8 dpi for birds that died from infection with 578/10. All American crows inoculated with NY99 (n = 6) or Ita09 (n = 5) died, and all 5 crows inoculated with FIN survived (Table 3).

Viremia Profiles

WNV viremia profiles were determined in terms of viral RNA (Table 2; Figure 2) and infectious virus titers in serum (Table 2; Figure 3) of infected carrion crows. In strain NY99–infected birds, the median peak viral RNA titer was $10^{8.7}$ RNA copies/mL of serum (range 10^{1}–$10^{10.0}$ [nontransformed values]), and the median peak infectious virus titer was $10^{7.4}$ $TCID_{50}$/mL of serum (range $10^{1.8}$–$10^{8.8}$); these values include 1 bird in which detectable viremia did not develop during the entire course of infection. The median peak viremia titer for Greece-10–infected birds was $10^{10.3}$ RNA copies/mL of serum (range $10^{9.8}$–$10^{11.7}$) and $10^{7.8}$ $TCID_{50}$/mL of serum (range $10^{7.3}$–$10^{9.8}$). FIN-infected birds had median peak viremia titers of $10^{2.7}$ RNA copies/mL of serum (range 10^{1}–$10^{5.9}$) and $10^{1.8}$ $TCID_{50}$/mL of serum (range $10^{1.8}$–$10^{2.5}$); however, viremia was detectable in only 3 of 6 birds, and infectious virus could be isolated from only 1 bird. The median peak viremia titers for Ita09-infected birds were $10^{9.7}$ RNA copies/mL of serum (range $10^{8.0}$–$10^{10.0}$) and $10^{7.6}$ $TCID_{50}$/mL of serum (range $10^{6.3}$–$10^{8.8}$). Birds infected with strain 578/10 had median peak viremia titers of $10^{8.4}$ RNA copies/mL of serum (range $10^{6.0}$–$10^{10.1}$) and $10^{5.1}$ $TCID_{50}$/mL of serum (range $10^{2.8}$–$10^{8.5}$).

Strain Greece-10–infected birds had median peak viral RNA titers significantly higher than those for NY99-infected (p = 0.004), FIN-infected (p = 0.005), and 578/10-infected (p = 0.004) birds. Greece-10–infected birds also had median infectious virus titers significantly higher than those for FIN-infected birds (p = 0.003), but FIN-infected birds had RNA and infectious titers lower than those for Greece-10–infected (p = 0.005 and 0.003, respectively), Ita09-infected (p = 0.005 and 0.002, respectively), and 578/10-infected (p = 0.005 and 0.002, respectively) crows.

Viremia profiles were also determined for American crows infected with 3 of the 5 different WNV strains (Table 3). NY99-infected birds had median peak viremia titers of $10^{9.6}$ RNA copies/mL of serum (range $10^{9.1}$–$10^{10.1}$) and $10^{7.2}$ $TCID_{50}$/mL of serum (range $10^{4.7}$–$10^{7.2}$). Detectable viremia

Table 2. Clinical profile for carrion crows experimentally infected with various West Nile virus strains*

Virus group	No. died/no. total (%)	Day of death, median dpi	Median peak viremia, viral RNA/mL serum (range); no. birds	Mean duration, d, of viremia ± SD; no. birds†	Mean day of peak viremia ± SD; no. birds†	Median peak viremia $TCID_{50}$/mL (range); no. birds‡
NY99-4132	5/6 (83)	7	$10^{8.7}$ ($10^{1.0}$–$10^{10.0}$); 6	5.2 ± 1.0; 5	4.3 ± 0.7; 5	$10^{7.4}$ ($10^{1.8}$–$10^{8.8}$); 6
Nea Santa-Greece-2010	6/6 (100)	7	$10^{10.3}$ ($10^{9.8}$–$10^{11.7}$); 6	5.7 ± 0.7; 6	4.5 ± 0.9; 6	$10^{7.8}$ ($10^{7.3}$–$10^{9.8}$); 6
Italy/2009/FIN	0/6 (0)	NA	$10^{2.7}$ ($10^{1.0}$–$10^{5.9}$); 6	2.7 ± 0.9; 3	6.7 ± 0.9; 3	$10^{1.8}$ ($10^{1.8}$–$10^{2.5}$); 6
Ita09	6/6 (100)	7	$10^{9.7}$ ($10^{8.0}$–$10^{10.0}$); 6	6.0 ± 1.2; 6	4.3 ± 0.7; 6	$10^{7.6}$ ($10^{6.3}$–$10^{8.8}$); 6
578/10	5/6 (83)	8	$10^{8.4}$ ($10^{6.0}$–$10^{10.1}$); 6	5.7 ± 1.8; 6	3.5 ± 0.9; 6	$10^{5.1}$ ($10^{2.8}$–$10^{8.5}$); 6

*dpi, days postinoculation; NA, not applicable; $TCID_{50}$, 50% tissue culture infectious dose.
†Based on viral RNA titers.
‡Viral titers are expressed as $\log_{10}$ $TCID_{50}$/mL of serum.

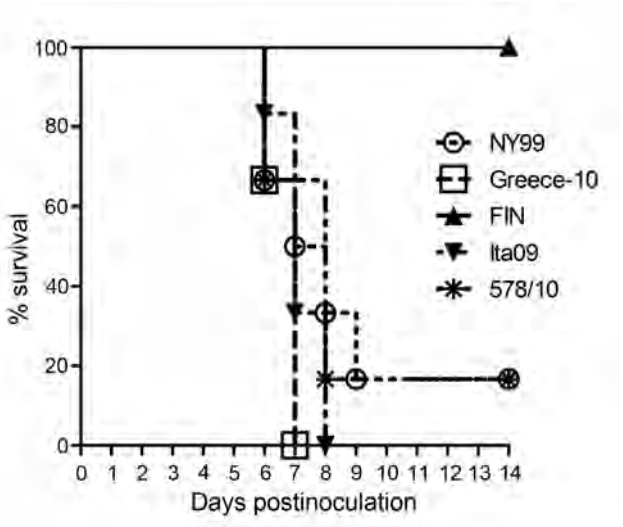

Figure 1. Survival rate for West Nile virus (WNV)–infected carrion crows after inoculation with 2,000 50% tissue culture infectious doses of WNV; each group (n = 6) was inoculated with a different strain. Crows were monitored daily for signs of disease through postinoculation day 14.

developed in only 2 of the 5 FIN-infected birds, resulting in median peak viremia titers of $10^{1.0}$ RNA copies/mL of serum (range 10^{1}–$10^{6.9}$) and $10^{1.8}$ $TCID_{50}$/mL of serum (range $10^{1.8}$–$10^{2.7}$). Median peak viremia titers for Ita09-infected birds were $10^{8.8}$ RNA copies/mL of serum (range $10^{8.0}$–$10^{9.1}$) and $10^{6.7}$ $TCID_{50}$/mL of serum (range $10^{6.0}$–$10^{7.5}$). American crows infected with strain NY99 had the highest median peak viral RNA and infectious virus titers, and FIN-infected birds had the lowest median titers (significant only when compared with each other: p = 0.008 and 0.006, respectively).

Tissue Tropism

Virus loads were determined in the heart, liver, spleen, kidney, bone marrow, and brain of all birds. To assess the spread of virus to the different organs at the approximate peak of viremia, we euthanized 2 birds per group at 4 dpi. Virus was detected in all organs from these birds. On average, the highest viral RNA titers were detected in the liver, followed by the bone marrow, spleen, kidney, and heart; the lowest titers were found in the brain (Figure 4). Between the different virus strains, viral RNA titers were the highest in the organs of birds infected with strain Greece-10 or 578/10, followed by NY99 and Ita09; titers were significantly higher than those for birds infected with strain FIN (p = 0.005 for all). Virus distribution in FIN-infected birds was not consistent; viral RNA was undetectable in the bone marrow and brain of both birds tested on 4 dpi, and for 1 of these birds, viral RNA was also undetectable in the spleen.

Birds euthanized because of illness had virus present in all organs; in most cases, the spleen, liver, and bone marrow contained the highest average viral RNA load, followed by kidney and heart; the lowest average viral RNA titers were in the brain. Viral RNA titers in organs of Greece-10–infected birds were higher than those in organs of birds infected with the other viruses, but this observation was not statistically significant (Figure 5).

The 1 NY99-infected and 3 FIN-infected survivor birds that were free of viremia throughout the 8 days of blood sampling underwent necropsy at 14 dpi. Of interest, virus was present in all organs of the NY99-infected bird (median virus load of $10^{3.1}$ RNA copies/g of tissue) and in at least 3 of the 6 organs from FIN-infected birds (median virus load of $10^{2.0}$ RNA copies/g of tissue), showing that these birds did undergo productive WNV infection.

Immunohistochemistry

Sections of organs from 2 birds necropsied at 4 dpi were stained with polyclonal anti-WNV NS3 to confirm replication of virus in the tissues and to exclude positive qRT-PCR detection due to spillover from blood at the approximate peak of viremia. Tissues most consistently positive for WNV antigen were the liver (80%), kidney (80%), bone marrow (80%), and spleen (78%); tissues least consistently positive for WNV antigen were heart (50%) and brain (10%) (Table 4). However, in terms of virus load, antigen was most abundant in the liver, bone marrow, and spleen. Overall, at 4 dpi, organs of birds most positive and most abundant for viral antigen were those infected by strains 578/10 and Greece-10, followed by NY99 and Ita09. The organs of FIN-infected birds were all negative for virus antigen at this time point.

Discussion

In this study, we assessed the susceptibility of carrion crows to different strains of WNV. First we demonstrated that carrion crows are susceptible to WNV infection by using the North American strain NY99, which has previously been shown to be highly virulent in American crows (*19–23*). In agreement with the findings in those studies, our results showed that infection of carrion crows with NY99

Table 3. Clinical profile of American crows experimentally infected with West Nile virus strains NY99-4132, Italy/2009/FIN, and Ita09

Virus group	No. died/no. total (%)	Median peak viremia, viral RNA/mL serum (range); no. birds	Median peak viremia, $TCID_{50}$/mL serum (range); no. birds*
NY99-4132	6/6 (100)	$10^{9.6}$ ($10^{9.1}$–$10^{10.1}$); 6	$10^{7.2}$ ($10^{4.7}$–$10^{7.2}$); 6
Italy/2009/FIN	0/5 (0)	$10^{1.0}$ ($10^{1.0}$–$10^{6.9}$); 5	$10^{1.8}$ ($10^{1.8}$–$10^{2.7}$); 5
Ita09	5/5 (100)	$10^{8.8}$ ($10^{8.0}$–$10^{9.1}$); 5	$10^{6.7}$ ($10^{6.0}$–$10^{7.5}$); 5

*Virus titers are expressed as $\log^{10}$ 50% tissue culture infectious dose ($TCID^{50}$)/mL of serum.

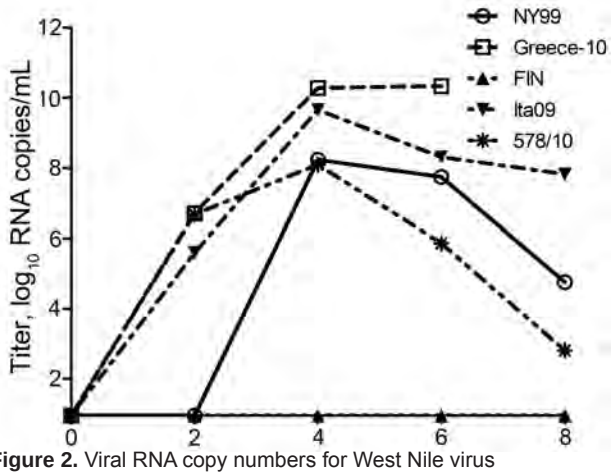

Figure 2. Viral RNA copy numbers for West Nile virus (WNV)–infected carrion crows after inoculation with 2,000 50% tissue culture infectious doses of WNV; each group (n = 6) was inoculated with a different strain. RNA copy numbers are represented as log-transformed medians. The assay had a detection limit of 9 (1.0 $\log_{10}$) RNA copies/mL of serum.

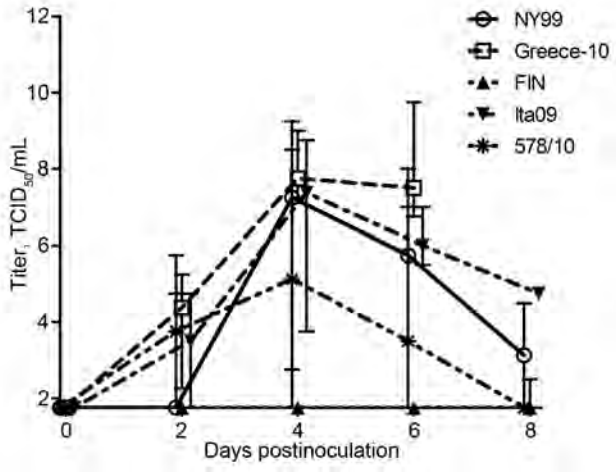

Figure 3. Infectious virus titer profiles for West Nile virus (WNV)–infected carrion crows after inoculation with 2,000 50% tissue culture infectious doses ($TCID_{50}$) of WNV; each group (n = 6) was inoculated with a different strain. Infectious virus titers were determined by $TCID_{50}$ titration and are represented as log-transformed medians; error bars indicate range. The assay had a detection limit of 1.8 $TCID_{50}$/mL.

resulted in high viremia titers and death. In addition, virus had disseminated to the organs of infected birds by 4 dpi, further demonstrating the susceptibility of carrion crows to WNV infection, which appears to be very similar to that of American crows.

Next we studied the susceptibility of carrion crows to selected strains of WNV from Europe. We found that carrion crows are highly susceptible to infection with both lineage 1 and 2 WNV strains from Europe. In addition, we showed that susceptibility is strain-dependent. Of the 5 WNV strains tested, 4 led to death for 83%–100% of infected birds and to high viremia titers and abundant antigen in the organs of euthanized birds; however, birds inoculated with FIN did not die from infection, and they had relatively low virus titers in the blood and no viral antigen in the organs at 4 dpi. A previous study describing the inoculation of carrion crows with WNV strains from France (Fr2000) and Israel (Is98) also suggested that carrion crows are susceptible to infection with WNV in a strain-dependent manner (*29*). The study showed death rates of 33% (Fr2000) and 100% (Is98) from the 2 strains, and viral RNA loads in serum, oral swab samples, and feathers of Is98-infected birds were higher than those of Fr2000-infected birds (*29*). Thus, WNV strains FIN and Fr2000 show a similar attenuation in carrion crows.

To more accurately assess the virulence of WNV strains from Europe, we inoculated American crows, a bird species known to be highly susceptible to WNV, with 2 of the 4 strains from Europe (Ita09 and FIN) and with strain NY99 from North America. Similar to what was seen with carrion crows, American crows infected with Ita09 had high peak viremia titers, and all succumbed to the infection, whereas those infected with FIN had low viremia titers, and all survived infection. Furthermore, it was demonstrated that the Greece-10 strain used in this study was also 100% lethal in American crows (A.C. Brault et al., unpub. data). In fact, American crows infected with Greece-10 (vs. the other strains used in this study) had the highest median

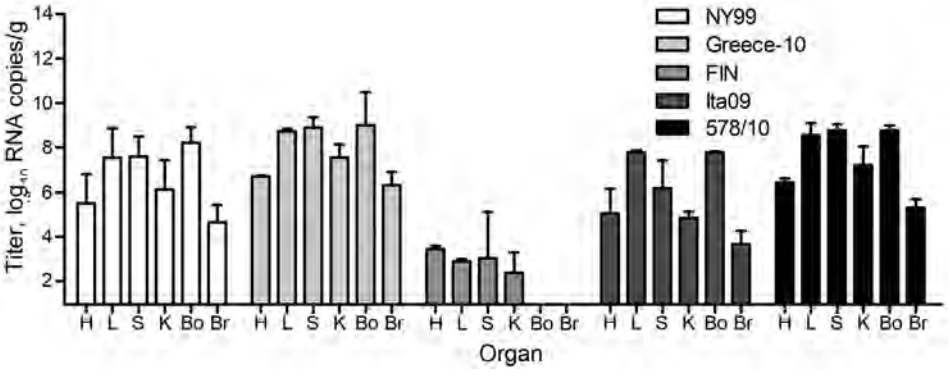

Figure 4. Viral RNA copy numbers in organs from 10 carrion crows (2 per group) euthanized 4 days after being experimentally infected with 1 of 5 different West Nile virus strains (n = 6, per group). Virus titers are represented as log-transformed medians; error bars indicate range. The assay had a detection limit of 9 (1.0 $\log_{10}$) RNA copies/g of tissue. H, heart; L, liver; S, spleen; K, kidney; Bo, bone marrow; Br, brain.

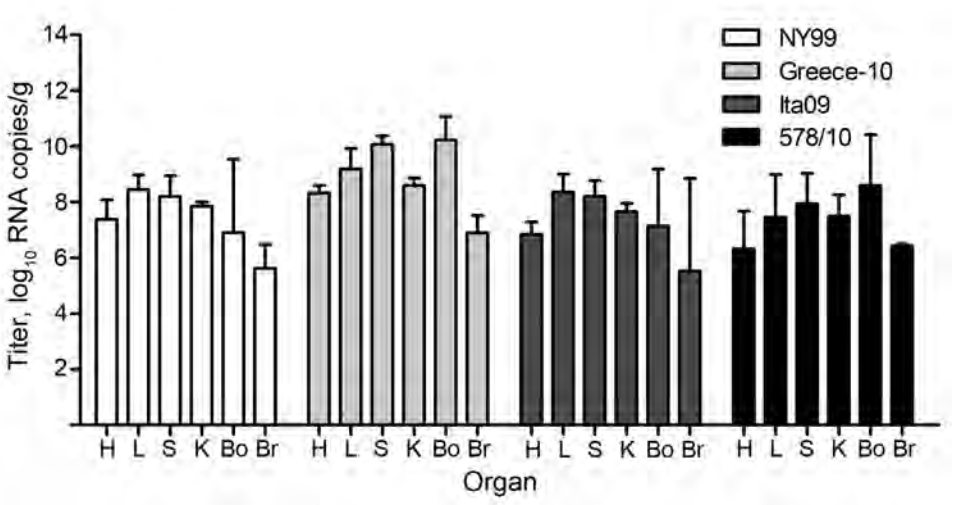

Figure 5. Viral RNA copy numbers in organs from 22 carrion crows euthanized because of illness after being experimentally infected with 1 of 4 different West Nile virus strains (n = 6, per group). Copy numbers are represented as log-transformed medians; error bars indicate range. The assay had a detection limit of 9 (1.0 $\log_{10}$) RNA copies/g of tissue. H, heart; L, liver; S, spleen; K, kidney; Bo, bone marrow; Br, brain.

peak viremia titers in terms of RNA and infectious virus (data not shown). These results show that in American crows, WNV strains (apart from FIN) from Europe are as virulent as the prototypic NY99 strain from North America.

The fact that susceptibility of birds to WNV can be strain-dependent was clearly demonstrated by the attenuated virulence phenotype of WNV strain FIN in carrion and American crows (this study) and in European jackdaws (*30*); FIN-infected crows consistently exhibited an absence of death, lower peak viremia titers, and less dissemination of virus to the organs at the approximate peak of viremia. A previous study showed that the introduction of a P249T amino acid substitution in the NS3 helicase of North American strain NY99 led to a highly attenuated phenotype, whereas a T249P substitution introduced in a low-virulence WNV strain resulted in a phenotype highly virulent to American crows (*22*). Four virus strains used in this study contain a proline at NS3-249, whereas FIN contains a threonine at this position (*31*). It is therefore likely that the attenuated phenotype of FIN is a result of this threonine amino acid at NS3-249, a mutation that could be relevant for at least 3 different species of birds in the family *Corvidae*. Studies in North American and European corvids are ongoing in order to test the relevance of the T249P substitution and several other mutations when introduced into the genome of WNV-FIN.

We have shown that bird susceptibility to WNV can be strain-dependent. However, susceptibility is also clearly

Table 4. Immunohistochemical analysis of West Nile virus antigen distribution in experimentally infected carrion crows euthanized at 4 dpi*

Virus strain, bird no.	Heart	Liver	Spleen	Kidney	Bone marrow	Brain	Total score per bird	Average score per virus strain	No. positive organs/total no. organs
NY99-4132								9.0	
1	–	++	++	+	++	–	11.0		4/6
7	–	+	+	+/–	+	–	7.0		4/6
Nea Santa-Greece-2010								12.5	
1	+/–	++	++	+	++	+/–	13.0		6/6
7	+/–	++	++	+	++	–	12.0		5/6
Italy/2009/FIN								0	
1	–	–	–	–	–	–	0		0/6
7	–	–	–	–	–	–	0		0/6
Ita09								8.0	
1	+/–	++	++	+/–	+	–	10.0		5/6
7	–	++	ND	+/–	+	–	6.0		3/5
578/10								12.5	
1	++	++	++	+/–	++	–	13.0		5/6
7	+/–	++	++	+	++	–	12.0		5/6
Score per organ	7.0	23.0	20.0	12.0	21.0	1.0			
No. positive birds/total no. birds	5/10	8/10	7/9	8/10	8/10	1/10			

*Subjective determinations of the amount of antigen in each organ were made: negative (–), minimal (+/–), moderate (+), or abundant (++). Each determination was given a score from 0 to 3: negative (0), minimal (1), moderate (2), and abundant (3). ND = not determined. dpi, days postinoculation; ND, not determined.

related to host factors. As a whole, jackdaws were less susceptible than the carrion crows to the same selection of otherwise highly virulent WNV strains, and they had lower death rates and virus loads in blood and organs (*30*). Species susceptibility has been shown to differ within various avian families (*7*), including birds in the family *Corvidae*, of which, for example, the fish crow (*Corvus ossifragus*) was less susceptible to lethal WNV infection (*23*). Although the reasons for this varied susceptibility are not well understood, potential contributing factors may include host traits, such as genetic composition, immune response, and physiologic mechanisms (*23*).

A measure of the potential for transmission of virus to feeding mosquitoes is the level of infectious virus titers produced during viremia. The median peak serum titer of infectious virus was highest in Greece-10–infected carrion crows and lowest in FIN-infected carrion crows. Studies have shown that WNV titers of $>10^5$ PFU/mL were considered infectious for *Culex pipiens* (*32*) and *Cx. quinquefasciatus* (*33*) mosquitoes. Considering this cutoff of 10^5 PFU/mL or of $10^{5.2}$ $TCID_{50}$/mL, according to a conversion factor of 1 $TCID_{50}$ to 0.7 PFU (*34*), infectious titers obtained for carrion crows infected with Greece-10, Ita09, or NY99 would be sufficient for efficient transmission of virus to feeding mosquitoes. Carrion crows infected with strain 578/10 had median peak viremia titers slightly below this threshold ($10^{5.1}$ $TCID_{50}$/mL; Table 2), suggesting that the carrion crow may not be an efficient amplifier for this WNV strain. However, a possible explanation for the apparent low viremia titers in 578/10-infected birds could be that blood sampling was conducted on alternate days, possibly missing higher peak viremia titers of infectious virus. For the American crows, median peak viremia titers for Ita09 (Table 3) were slightly lower than those for carrion crows (Table 2). However, serum samples from American crows underwent 2 repeated freeze–thaw cycles, which could have resulted in the detection of lower infectious virus titers. Nonetheless, these results show that WNV strains from Europe can produce viremia titers in American crows that could be sufficient for efficient transmission to feeding mosquitoes. Nevertheless, reservoir competence studies involving the feeding of European mosquitoes on viremic WNV-infected carrion crows are needed to determine whether the carrion crow could indeed be a potential reservoir host and contributor to the WNV transmission cycle.

We have shown that carrion crows, a species of bird ubiquitously found across Europe, are highly susceptible to WNV strains currently circulating in Europe. These birds could therefore potentially be useful as part of dead bird surveillance in the early detection of WNV in Europe. Future studies assessing the susceptibility of the closely related hooded crow (*Corvus cornix*) to WNV may also prove to be insightful, as this is the more predominant corvid species in eastern and southeastern Europe, where WNV is more common. The susceptibility of European birds to WNV has been demonstrated in multiple studies (*9*,*10*,*12*,*13*,*29*,*30*,*35*–*38*), however, it is peculiar that the number of WNV-associated deaths among birds in Europe is not as extensive as that among birds in North America. Possible explanations may be a lower reporting of bird deaths in Europe as compared with that in the United States or that other ecologic factors, such as mosquito competence, abundance, distribution or behavior, exert a limiting effect on the transmission of WNV in Europe.

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Trapping of carrion crows was performed with the assistance of the Gemeentewerken Rotterdam under permission obtained from the Ministry of Agriculture (registered under no. FF/75A/2011/031). Experimental inoculations were performed under protocol number 122-12-12 with permission obtained from the Animal Ethics Committee of Erasmus Medical Centre. All efforts were made to minimize animal suffering. Trapping of American crows was performed with the assistance of Todd Felix under US Fish and Wildlife Scientific Collecting Permit number MB-91672A. Experimental inoculations of crows were performed under Colorado State University IACUC approval number 10-2078A.

Ms. Lim is a graduate student at the department of Viroscience of the Erasmus Medical Centre. Her research interests include the pathogenesis of (neurotropic) arboviruses.

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Hospital Resource Utilization and Patient Outcomes Associated with Respiratory Viral Testing in Hospitalized Patients

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Testing patients for respiratory viruses should guide isolation precautions and provide a rationale for antimicrobial drug therapies, but few studies have evaluated these assumptions. To determine the association between viral testing, patient outcomes, and care processes, we identified adults hospitalized with respiratory symptoms from 2004 through 2012 at a large, academic, tertiary hospital in Canada. Viral testing was performed in 11% (2,722/24,567) of hospital admissions and was not associated with reduced odds for death (odds ratio 0.90, 95% CI 0.76–1.10) or longer length of stay (+1 day for those tested). Viral testing resulted in more resource utilization, including intensive care unit admission, but positive test results were not associated with less antibiotic use or shorter duration of isolation. Results suggest that health care providers do not use viral test results in making management decisions at this hospital. Further research is needed to evaluate the effectiveness of respiratory infection control policies.

In 2003, the coronavirus responsible for the severe acute respiratory syndrome (SARS) outbreak infected 774 and killed 8,096 persons worldwide (*1*). It was quickly recognized that this virus spread between close contacts, because 21% of infected case-patients were health care workers caring for patients infected with the SARS coronavirus (*1*,*2*). During the outbreak, respiratory infection control policies were developed by clinical infectious disease and public health experts, and their use was mandated in all Canadian hospitals. These measures were attributed to the eventual control of the outbreak (*3*–*6*). As a result, infection control practices, including strict hand hygiene, viral testing of patient samples, and use of isolation precautions, quarantine rooms, and personal protective equipment, were mandated for routine use with all patients who sought treatment at emergency departments (EDs) with respiratory symptoms and fever (*7*,*8*).

National guidelines suggest that patients admitted to acute care hospitals with infectious respiratory symptoms should receive screening for viral infections by answering symptom-based questionnaires, and they should be placed under droplet isolation precautions until definitive evidence rules out a transmissible respiratory illness (*7*,*9*). Viral testing in this setting is carried out with a nasopharyngeal (NP) swab sample, which is processed by direct fluorescent antibody (DFA), PCR, or both to identify a viral pathogen. Viral testing in these patients should improve diagnostic clarity, reduce the number of subsequent diagnostic tests and procedures required, and prevent infection transmission to other patients and health care workers by guiding the use of isolation precautions. However, these outcomes can only occur if physicians and infection control practitioners assess the results of the viral test and feel confident ruling out viral disease on the basis of the results.

To date, whether respiratory viral testing in patients improves outcomes or care processes has not been proven in large studies. Two small studies demonstrated that knowledge of the viral test results did not affect length of stay and subsequent antibiotic use (*10*,*11*). However, 1 previous study demonstrated reduced length of stay, mortality, and cost when using viral testing (*12*). These studies were limited by the following: relatively small sample sizes; only single winter seasons being evaluated; and utilization of hospital resources, including isolation precautions, not being assessed (*10*–*12*).

To address this gap in evidence, we set 2 main objectives for this study. First, we aimed to determine the association between the use of viral testing and subsequent hospital resource utilization (antibiotic/antiviral drugs prescribed; radiology studies conducted; cultures and bronchoscopies performed), including the duration of isolation precautions. Second, we aimed to determine whether viral testing was associated with in-hospital deaths, admission to intensive care, and length of stay in the hospital.

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Methods

Data Sources

We conducted a large retrospective observational cohort analysis based at The Ottawa Hospital (TOH), an adult academic hospital located in Ottawa, Ontario, Canada, with ≈1,100 inpatient beds. TOH is a tertiary care referral center that provides care for 1.2 million patients in the Eastern Ontario region. We created the study cohort using hospital administrative and clinical data from The Ottawa Hospital Data Warehouse, a relational database containing information from TOH's patient registration system, the clinical data repository (containing laboratory, pharmacy, radiology, and clinical notes), and the discharge abstract database. Data from these operational systems are loaded into the database on a daily basis and linked by patient unique identifiers. Extensive assessments of data quality were performed during the development of the database.

Study Population: Inclusion and Exclusion Criteria

We identified hospitalizations of adult patients from January 1, 2004, through December 31, 2012. Hospitalizations were included if the patient was admitted through the ED with any combination of cough, fever, or shortness of breath.

We excluded hospitalizations resulting from a transfer from another health institution (such as a long-term care facility or another regional hospital). If a patient had been seen in the ED with respiratory symptoms but was not subsequently admitted, the patient was also excluded from the study.

Exposure

The NP swab sample, processed by DFA or PCR, was the exposure of interest for each hospitalization. The standard of practice at our center during the study period was to process NP swab samples with DFA. However, during the 2009 influenza A(H1N1) pandemic season, multiplex PCR was used to detect viruses. The multiplex PCR can detect influenza A or B, respiratory syncytial virus, parainfluenza virus, enterovirus, adenovirus, human metapneumovirus, rhinovius, and coronavirus. We developed and validated an algorithm within our dataset to determine whether NP swab samples were analyzed, and categorized them as positive or negative on the basis of the DFA or PCR result (positive test refers to identification of a respiratory virus).

Patient Outcomes and Measures of Health Resource Utilization

The primary outcomes considered in this study were number of inpatient deaths, and length of hospital stay. Secondary outcomes included admission to the intensive care unit (ICU) and measures of resource utilization, including antibiotic and antiviral prescriptions, chest radiograph and computed tomography imaging, blood and sputum cultures, bronchoscopy, and use and duration of isolation precautions in the hospital.

Analysis and Adjustment for Confounding

The unit of analysis in this study was the patient's hospitalization. Patient characteristics were compared across groups (with and without viral testing performed) and were described by using proportions, means with SDs, and medians with interquartile range when appropriate. Using similar methods, we then compared groups with positive and negative NP swab sample results among the hospitalizations in which an NP swab sample was analyzed. We assessed the difference of means and SD for continuous variables using a 1-way analysis of variance test (ANOVA) and for differences between proportions using a χ^2 test. For all statistical tests, $p<0.05$ was considered statistically significant.

We measured patient coexisting conditions using the Elixhauser score (*13*,*14*), a validated scoring system which summarizes comorbid illness and can predict the patient's risk of death in the hospital (*14*). It was derived and validated by using hospitalization data from TOH, and the score was based on the original 30 comorbidity diagnosis groups in the Elixhauser comorbidity classification system (*13*,*14*). The Elixhauser summary score ranges from a minimum of –19 to +89, which are associated, respectively, with a 0.37% and 99.41% risk of in-hospital death (*14*). Baseline risk of death at the time of hospitalization was calculated for each hospitalization by using a regression model previously validated by data from TOH's patient population (*15*,*16*). We defined influenza seasons on the basis of dates recorded in the Public Health Agency of Canada's national surveillance system for influenza, FluWatch (*17*). We used this information to categorize hospital admissions according to whether or not they occurred during an influenza season.

We created multivariate logistic regression models to investigate whether having an NP swab sample obtained and tested was associated with probability of death and ICU admission. For each outcome in which the patient died or was admitted to the ICU, univariate odds ratios (ORs) were calculated for patient sociodemographic factors, clinical factors related to the hospitalization, patient comorbidity, and whether the patient was admitted to the hospital during influenza season. A multivariate model was created on the basis of significant predictors of death and ICU admission and was reduced by using stepwise variable selection. We used unadjusted and adjusted linear regression models to determine the change in length of stay in hospital when an NP swab sample was tested during the admission process. We used a natural logarithm transformation of the length of stay variable to adjust for the left skewed distribution of this variable.

In a secondary analysis, the same methods were used to develop multivariate logistic regression models to investigate the association between the NP swab sample testing (positive or negative test result) and odds of death and ICU admission. Multivariate linear regression was used to evaluate length of stay.

We conducted a sensitivity analysis of a subgroup of hospitalizations in which the most responsible discharge diagnosis was a pulmonary infection or exacerbation. This was done to account for the fact that patients seeking treatment for respiratory symptoms could have received a diagnosis of a noninfectious condition (such as heart failure or pulmonary embolism). We limited the discharge diagnosis to diagnoses of respiratory infections or exacerbations to determine whether there was any effect on the study outcomes. In this group, we assessed the potential association between having an NP swab sample tested and clinical outcomes (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/21/8/14-0978.pdf). All analyses were conducted by using SAS 9.2 statistical software (SAS Institute Inc., Cary, NC, USA). This study was approved by the Ottawa Health Sciences Network Research Ethics Board, and a waiver of patient consent was granted.

Results

Study Cohort Demographics

During the 8-year study period, we identified 24,567 hospital admissions in which the patient sought treatment at the ED reporting chief symptoms of fever and/or cough and/or shortness of breath. These admissions represented 17,327 unique patients. Baseline characteristics of the study cohort are described in Table 1. An NP swab sample was tested in 11% (2,722/24,567) of admissions. Overall, patients who had an NP swab sample tested were younger, more likely to be admitted during influenza season, and more likely to be female.

Description of Outcomes

Table 2 describes likelihood of deaths, ICU admission, length of stay, and use of isolation precautions in the study cohort and among hospitalizations in which the patient had a positive or negative NP swab sample. During hospitalizations in which an NP swab sample was tested, length of stay in hospital was longer (11.7 days vs. 9.5 days, p<0.001) and mean duration of isolation precautions was longer (4.8 days vs. 1.4 days, p<0.001) than in hospitalizations in which an NP swab sample was not tested. There was no significant difference in the mean number of days spent in isolation between hospitalizations in which the patient had positive or negative NP swab samples (5.16 ± 5.39 vs. 4.73 ± 7.65 days, p = 0.27).

NP Swab Samples and Resource Utilization

Table 3 describes the use of hospital resources (antibiotic drugs, antiviral drugs, chest imaging studies, cultures, and bronchoscopy) among hospitalized patients with positive and negative NP swab samples. Among hospitalizations in which the sample was positive (420/2,722) and hospitalizations in which it was negative (2,302/2,722), no significant differences were found in process of care variables, with exception of more antiviral drug use and less use of computed tomography chest scans in the group with positive swab samples. Hospitalizations in which an NP swab sample was analyzed used statistically more resources than those in which no swab sample was tested (p<0.001, for all hospital resources measured).

NP Swab Samples and Association with Odds of Death, ICU Admission, and Length of Stay

After adjustment for confounding variables, there was no association between having an NP swab sample tested in the hospital and odds of death (OR 0.90, 95% CI 0.76–1.10). We identified a significant increase in ICU admission when a patient's NP swab sample had been tested (OR 2.23, 95% CI 1.61–3.10). Finally, linear regression analysis demonstrated a nonsignificant 1-day increase in length of stay among hospitalized patients for whom a sample was tested (p = 0.55; ORs with 95% CIs are shown in online Technical Appendix Table 2). Among the hospitalizations in which an NP swab sample was tested (n = 2,722), no significant associations were found between a positive swab sample and odds of death, ICU admission, or length of stay (online Technical Appendix Table 3).

Table 1. Baseline characteristics of hospitalized adults admitted with respiratory symptoms, Ottawa, Ontario, Canada, 2004–2012*

Variable	Without NP swab samples, n = 21,845	With NP swab samples, n = 2,722	Total, n = 24,567	p value
Age at admission, mean ± SD	67.7 ± 17.14	65.99 ± 18.31	67.48 ± 17.28	<0.001
Female sex, no. (%)	10,891 (49.9)	1,420 (52.2%)	12,311 (50.15)	0.023
Admitted during influenza season, no. (%)	12,958 (59.3)	2,221 (81.6%)	15,179 (61.8)	<0.001
Baseline risk of death, mean ± SD	0.14 ± 0.15	0.14 ± 0.14	0.14 ± 0.15	0.65
Elixhauser score quartiles				<0.001
0	5,636 (25.8)	981 (36.0)	6.617 (26.9)	
1	5,877 (26.95)	762 (28.0)	6,639 (27.0)	
2	5,200 (23.8)	558 (20.55)	5,758 (23.4)	
3	5,132 (23.5)	421 (15.5)	5,553 (22.6)	

*NP, nasopharyngeal.

Table 2. Outcomes for hospitalized adults seeking treatment with respiratory symptoms, Ottawa, Ontario, Canada, 2004–2012*

Outcome variable	Study cohort, n = 24,567	With negative swab sample, n = 2,302	With positive swab sample, n = 420	p value†
Death, no. (%)	2,550 (10.4)	239 (10.4)	40 (9.5)	0.594
ICU admission, no. (%)	2,007 (8.2)	341 (14.8)	76 (18.1)	0.086
Days in ICU, mean ± SD	8.37 ± 10.64	11.22 ± 12.77	11.70 ± 14.03	0.771
Hospital isolation used, no. (%)	7,487 (30.5)	1,993 (86.6)	396 (94.3)	<0.001
No. days in isolation, mean ± SD	1.79 ± 6.79	4.73 ± 7.65	5.16 ± 5.39	0.27

*ICU, intensive care unit.
†For negative and positive swab samples.

Results of Sensitivity Analysis

In a restricted cohort of hospitalized patients in which an infectious respiratory diagnosis was made (n = 7,459), the fact that an NP swab specimen was tested was not associated with reduction in chance of death but was significantly associated with increased ICU admission. Length of stay was also significantly increased by 1 day (95% CI 0.9–1.1 days, p = 0.04), which was not the case in the primary analysis (online Technical Appendix Table 1).

Discussion

In this study, viral testing of respiratory samples during hospitalization was not associated with a significant reduction in odds of patient deaths or length of hospital stay after adjustment for critical clinical confounding factors. Viral testing, however, was associated with increased likelihood of admission to the ICU. Our study also did not find that respiratory viral testing was associated with significant reductions in antibiotic use, chest imaging studies, bronchoscopy, or microbiologic cultures among patients with infectious respiratory symptoms. Most notably, a positive viral test result did not lead to significant reductions in antibiotic use, number of chest radiographs obtained, and number of blood cultures requested. It is plausible that lack of any observable beneficial effect on these outcomes is a result of health care providers neglecting to adjust care processes on the basis of the testing results.

Although more isolation precautions were used with patients with positive viral test results than with those with negative test results (94% vs. 87%, p<0.001), the test result did not influence the duration of isolation precautions. No statistical difference was found in the mean number of isolation-days between hospitalizations in which positive and negative viral test results were obtained (5.2 days vs. 4.7 days, p = 0.27).

This finding could have several potential causes, however. First, health care providers may not be translating negative test results into the action of removing isolation precautions because of lack of infection control directives for front-line staff (nurses and physicians) to guide the safe removal of isolation precautions. As a result, patients may remain under isolation precautions for a standard fixed duration, regardless of the viral test result. Second, perhaps front-line staff fear the possibility of infection transmission (even when the NP swab sample is negative) and continue the precautions as a conservative measure.

We found that hospitalized patients for whom NP swab samples were tested had a greater chance of ICU admission, after adjustment for confounders, including admission during influenza season, isolation status, and baseline risk for death. This observation remains unexplained. It may be due to residual confounding, but it is also conceivable that obtaining the NP swab sample and subsequent isolation precautions may put some patients at risk for adverse events that require ICU admission. Abad et al. conducted a systematic review and found that isolation precautions are associated with greater adverse drug events, less physician and nurse care, and increased patient scores for anxiety and depression (*18*). In a prospective study, Stelfox et al. found that patients in isolation experienced more preventable adverse events in the hospital, made more formal complaints to the hospital about their care, were more likely to have had no vital signs done when ordered, and had more days with no physician progress notes, when compared with nonisolated controls (*19*).

Relatively few studies have evaluated the effects of respiratory viral testing on processes of care and clinical

Table 3. Laboratory, prescription, radiology, and procedure use among hospitalized patients with positive and negative NP swab samples, Ottawa, Ontario, Canada, 2004–2012*

Variable	No. (%) negative NP swab samples, n = 2,302	No. (%) positive NP swab samples, n = 420	No. (%) total swab samples, N = 2,722	p value
Antibiotic use	2,204 (95.7)	397 (94.5)	2,601 (95.6)	0.265
Antiviral use	305 (13.2)	166 (39.5)	471 (17.3)	<0.001
Blood cultures	1,813 (78.8)	340 (81.0)	2153 (79.1)	0.309
Sputum cultures	979 (42.5)	167 (39.8)	1146 (42.1)	0.291
Bronchoscopy	147 (6.4)	20 (4.8)	167 (6.1)	0.202
CT scan of thorax	599 (26.0)	83 (19.8)	682 (25.1)	0.006
Chest radiograph	1,293 (56.2)	229 (54.5)	1,522 (55.9)	0.532

*NP, nasopharyngeal; CT, computed tomography.

outcomes in hospitalized adults. Most of these studies were performed on children in the ED (*20*–*23*). However, 2 small prospective studies in adult patients have examined the effects of respiratory viral testing results on antibiotic use, whereas 1 other study also examined the effect on length of hospital stay (*10*,*11*). Hernes et al. prospectively studied the effect of respiratory viral PCR testing on antibiotic treatment and length of stay among 147 hospitalized patients >65 years of age with respiratory infections and found no difference in antibiotic use or length of stay between patients with a positive and negative viral test result (*10*). An earlier study in 2005 by Oosterheert et al. found similar results among 107 adult patients admitted for antibiotic treatment of lower respiratory tract infection (*11*). Their overall conclusion was that early knowledge of the viral test result did not significantly reduce the duration of antibiotics, or costs, when compared with those of a group in which the viral test results were not made available (*11*).

The results of these 2 small prospective studies are generally congruent with our results. However, we also found that a positive viral test result did not affect other processes of care, including whether blood and sputum cultures, bronchoscopy, and chest radiographs were obtained and, most notably, duration of isolation precautions. Our study also examined the outcomes of patient death and ICU admission, which were not addressed in the previous studies.

Our study has several strengths. It is the largest study conducted to evaluate the effects of respiratory viral testing on clinical outcomes in adult hospitalized patients. Also, our data spanned 8 years, including 8 seasons of viral infections. Given our sample size, all adjusted regression models had adequate power to evaluate the chance of death and ICU admission outcomes (*24*).

Our study also has several limitations. The retrospective nature of this study makes the results vulnerable to unmeasured confounding. We accounted for temporal confounding due to influenza seasonality and for confounding by indication using validated measures of baseline mortality risk and comorbidity in the adjusted regression models. However, we did not capture acute vital signs and other nonlaboratory clinical data at the time patients sought treatment, which may have influenced the outcomes we studied. Finally, the study was conducted by using data from a single academic center, which has implications for the generalization of these results to other medical institutions. However, the tertiary care hospital in this study follows national and international recommendations for infection control practices, which reduces the likelihood that practices would be significantly different from other major medical centers.

Our results suggest that in this academic center during the study period, respiratory viral testing did not achieve the goals of reducing antibiotic prescriptions and other diagnostic tests, nor did it result in timely discontinuation of isolation precautions. Because the duration of isolation is not guided by the viral test result, one questions whether the process of viral testing is helping reduce viral infection transmission in the hospital. We could not assess infection transmission in this study, but future work is required in this area.

Our findings should encourage hospital administrators and infection control practitioners to reevaluate current practices, so that viral test results are used appropriately to modify subsequent treatments and guide provision of isolation precautions. This study sets the foundation for further research to ensure that current policies and practices result in efficient resource utilization and prevent infection transmission in hospitals.

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Development of Framework for Assessing Influenza Virus Pandemic Risk

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Although predicting which influenza virus subtype will cause the next pandemic is not yet possible, public health authorities must continually assess the pandemic risk associated with animal influenza viruses, particularly those that have caused infections in humans, and determine what resources should be dedicated to mitigating that risk. To accomplish this goal, a risk assessment framework was created in collaboration with an international group of influenza experts. Compared with the previously used approach, this framework, named the Influenza Risk Assessment Tool, provides a systematic and transparent approach for assessing and comparing threats posed primarily by avian and swine influenza viruses. This tool will be useful to the international influenza community and will remain flexible and responsive to changing information.

Pandemic influenza remains a formidable threat to human health. Advances in national pandemic preparedness have been made during recent decades; however, the frequent infection of humans with novel influenza viruses complicates implementation of effective control measures such as vaccines (*1*). The emergence of the influenza A(H1N1)pdm09 virus (*2*), the ongoing outbreaks of highly pathogenic avian influenza A(H5N1) viruses (*3*), and, more recently, the identification of severe human infections caused by avian influenza A(H7N9) virus in China (*4*,*5*) indicate the need for a more objective, systematic, and transparent approach for evaluating newly emerging influenza viruses with pandemic potential. The Influenza Risk Assessment Tool (IRAT) was developed in response to this need and creates a framework for systematically combining input from influenza experts to support risk management decisions that have important cost implications.

Development of a Framework

The approach described here uses opinions from subject matter experts to populate a simple, multiattribute additive model (*6*) that combines information from well-established decision analysis methods (*7*,*8*) to assist in decision making and prioritization processes. Traditional risk assessment approaches (*9*) are not directly applicable to the IRAT; however, the general guiding principles still apply (Figure 1). The problem definition addressed by IRAT is captured in the formulation of 2 important questions. One question addresses the risk that the virus will achieve sustained human-to-human transmission and emerge as a pandemic virus (the emergence question) and specifically asks, "What is the risk that a virus not currently circulating in the human population has potential for sustained human-to-human transmission?" The second question, called the impact question, asks, "If the virus were to achieve sustained human-to-human transmission, what is the risk that a virus not currently circulating in the human population has the potential for significant impact on public health?" Risk analysis and interpretation involve the assessment of the potential pandemic risk of specific influenza viruses by subject matter experts, who use multiple factors or risk elements identified and defined by a panel of influenza experts as critical for a risk-based assessment. These defined risk elements provide the basis for evaluation and comparison of emerging influenza viruses and contribute to an aggregate risk score.

Identification and Definition of Risk Elements

Leading influenza subject matter experts in human and animal health, along with laboratory workers, field epidemiologists, and risk modelers, were invited to a meeting in 2011 (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-1086-Techapp.pdf) and asked to provide input into the development of the IRAT. Participants were asked to provide and refine definitions of elements used by the IRAT to evaluate the potential risk of novel influenza viruses. To distinguish among novel influenza viruses, each element must be amenable to qualitative or quantitative evaluation, independent of other elements, and must not be redundant in what is being measured. Subject matter experts agreed on and defined 10 elements to be scored that would provide the necessary critical information. Each of the 10 elements falls into 1 of 3 categories: virus properties, attributes and immune response of the human population, and the ecology and epidemiology associated with the

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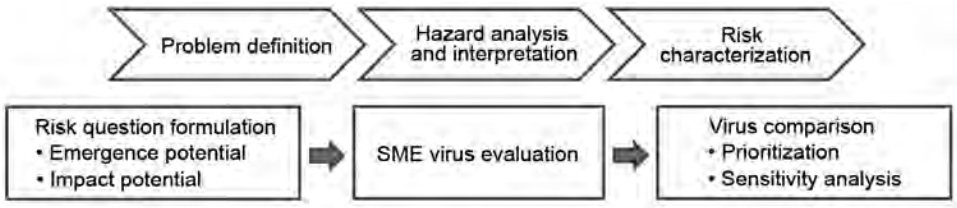

Figure 1. Alignment of the Influenza Risk Assessment Tool with a general microbial risk assessment framework. SME, subject matter expert.

novel influenza virus (Table 1). These 10 elements are meant to capture the features of the virus, the population, and the ecology and epidemiology likely to affect whether or not a specific zoonotic virus can spread widely in humans. These elements are also meant to capture factors likely to affect public health during a pandemic caused by a specific virus.

Definitions required for each element included scoring criteria that characterize viruses as low, moderate, and high risk to assist the experts in estimating a score on a scale of 1–10. For example, the definition of the element "Antivirals and Treatment Options" states, "For the purposes of the risk assessment tool, antiviral susceptibility refers to the predicted or demonstrated efficacy of available antiviral agents against animal influenza viruses." A low-risk score (*1*–*3*) for this element is defined as "no evidence of clinically relevant resistance to any of the antiviral drugs approved for human use (neuraminidase inhibitors and M2 blockers)." A moderate-risk score (*4*–*7*) is defined as "sensitive to all neuraminidase inhibitors but resistant to M2 blockers." A high-risk score (*8*–*10*) for this element is defined as "resistant to one or more neuraminidase inhibitor antiviral drugs." All 10 elements have definitions for low-, moderate-, and high-risk scores.

Weighting of Risk Elements

Because not all risk elements are equally useful in answering each risk question, a weighting system had to be determined before the final risk score for each virus was generated. A key requirement for creating a weighting system was minimizing bias regarding the weighting of input from each expert's field of study relative to that of others and reducing the difficulty of eliciting relative weights from experts when multiple attributes are compared. A surrogate weighting method was selected for this purpose (*10*). First, the expert panel was surveyed to determine a consensus rank ordering of the 10 risk elements if each risk question was asked separately. When asked to rank the elements when the "emergence question was considered," 27 of 29 subject matter experts agreed that evidence of human infections is the "most important" of the 10 elements regarding risk that a novel influenza virus can achieve sustained

Table 1. Risk element categories in the Influenza Risk Assessment Tool

Category	Risk element	Description
Virus properties	Genomic variation	Captures the degree of mutation and reassortment as a measure of the genetic diversity of a novel influenza virus; also captures presence or absence of known molecular markers denoting virulence
	Receptor-binding properties	Virus-binding preference to glycans with sialic acid in α-2,6 (human) linkage at the terminal galactose when compared with viruses that bind to sialic acid by α-2,3 (avian) linkage
	Transmissibility in animal models	Transmission of animal influenza viruses in ≥1 accepted animal models by direct contact or through respiratory droplets in the absence of direct contact
	Antiviral treatment susceptibility	Predicted or demonstrated efficacy of available (approved for human use) antiviral agents against animal influenza viruses
Host properties	Population immunity	Detection of preexisting cross-reactive serum antibodies acquired through prior infection or vaccination (examined in all age groups)
	Disease severity	Spectrum of illness with infection by a novel influenza A virus in humans or in experimentally infected animal models as surrogates for human disease
	Antigenic relationship to vaccines	Antigenic relatedness measured by hemagglutination inhibition or virus neutralization tests with postinfection ferret antiserum to emerging virus and seasonal vaccine and reference viruses
Ecology and epidemiology	Human infections	Occurrence of human infections with animal influenza viruses, frequency of these human infections, and extent of human-to human transmission of these viruses
	Infections in animals	The virus's ability to infect animal species naturally, the number and diversity of those species, ability to maintain sustained natural transmission in those populations, and potential extent of exposure between humans and those animal species
	Global distribution (in animals)	Spatial and temporal distribution of animal influenza viruses and the effect of animal production and management systems on the spread among animal populations and potential exposure to humans

human-to-human transmission. The other 2 subject matter experts believed this element was "very important." Second in importance was information regarding laboratory animal transmission studies; 26 subject matter experts thought this element was "most important" or "very important." Third in importance was receptor binding information and population immunity. Of least importance for the experts was information regarding antiviral and treatment options and disease severity; 24 of the 29 subject matter experts rated this element as "not relevant" for the question about the risk that a novel influenza virus can achieve sustained human-to-human transmission.

The second risk question addresses the potential effect of a novel influenza virus on public health in the event that the virus achieved sustained human-to-human transmission (i.e., the impact question). Subject matter experts agreed that knowledge regarding disease severity ranked first (26 ranked this element as "most important" and 3 ranked it as "very important"), followed by information pertaining to population immunity (21 ranked this element as "most important" and 8 ranked it as "very important"), human infections, and antiviral and treatment options in descending order. Of least importance were the elements related to global distribution in animal species (16 ranked this element as "not relevant") and infections in animals (11 ranked this element as "not relevant"). Although risk element definitions were the same for the emergence and impact questions, the rank order of the risk elements by experts differed for the 2 questions.

This rank ordering of risk elements for the 2 questions facilitated the calculation of surrogate weights by using the formula

$$Wk = (1/K) \sum_{i=k}^{K} \left(\frac{1}{i}\right)$$

where K is the number of elements and Wk is the calculated weight to be applied to each risk element in rank order (10). Thus, the weight for the top-ranked element of the 10 total elements was calculated as 0.2929, and the lowest-ranked element was assigned a weighting score of 0.001. By convention, the sum of all 10 weights in this example is 1.

Scoring

Using the framework of risk element definitions, scoring criteria, and rank-ordered surrogate weights, individual experts were asked to evaluate viruses by using a particular risk element within their field of expertise and to provide a point estimate score. The returned scores for each risk element were averaged and then multiplied by the corresponding surrogate weight, depending on the risk question being asked. A summation of the product of all 10 risk elements provided the final aggregate score for each virus. Tables 2 and 3 show results of subject matter experts' scoring of 3 avian influenza viruses. The H1N1 subtype virus found in North American mallards was used as an example of an influenza virus that has not infected people and appears to be wholly avian in characteristics.

Addressing Uncertainty

Inherent in any risk assessment is the need to address uncertainty. The IRAT attempts to capture this factor by asking subject matter experts to characterize uncertainty in 2 ways. First, uncertainty is captured by each expert's point estimate score on a scale of 1–10 (i.e., 1 representing the lowest level of risk and 10 representing the highest), along with an upper and lower boundary for their scores, thereby providing a range for their point scores. A second measure of uncertainty uses a confidence score from each expert; this score expresses the expert's confidence in the available data used to make the point estimate. Finally, the subject matter experts were asked to provide a basis or justification for their risk scores. This justification could include key references and data along with professional observations and experiences. This approach was based on the assumption that

Table 2. Risk scores and ranked weighting applied to 3 influenza viruses for the question "What is the risk that a virus not currently circulating in the human population has potential for sustained human-to-human transmission?"*

		HPAI H5N1 clade 1 (A/VN/1203/2004)		North American mallard influenza A(H1N1) (A/duck/NewYork/1996)		Variant H3N2 (A/Indiana/08/2011)	
Element	Wt	RS	Wt x RS	RS	Wt x RS	RS	Wt x RS
Human infection	0.2929	5.67	1.66	2.33	0.68	4.33	1.27
Transmission (laboratory animals)	0.1929	3.00	0.58	2.00	0.39	9.00	1.74
Receptor binding	0.1429	3.30	0.47	2.00	0.29	8.30	1.19
Population immunity	0.1096	8.67	0.95	3.00	0.33	3.67	0.40
Infection in animals	0.0846	7.25	0.61	2.00	0.17	8.00	0.68
Genomic variation	0.0646	4.00	0.26	3.00	0.19	8.00	0.52
Antigenic relationship	0.0479	6.00	0.29	2.00	0.10	8.00	0.38
Global distribution (animals)	0.0336	5.50	0.18	2.50	0.08	7.00	0.24
Disease severity	0.0211	8.50	0.18	2.25	0.05	6.00	0.13
Antiviral/treatment options	0.0010	4.50	0.00	2.25	0.00	2.50	0.00
Total	1.0000		5.18		2.28		6.55

*Wt, weight; HPAI, highly pathogenic avian influenza; RS, risk score. Weights are expressed to 4 decimal places because of the convention that the sum must be exactly 1. Sums for viruses may not be exact due to rounding.

Table 3. Risk scores and ranked weighting applied to 3 influenza viruses for the question "If the virus were to achieve sustained human-to-human transmission, what is the risk that a virus not currently circulating in the human population has the potential for significant impact on public health?"*

		HPAI H5N1 clade 1 (A/VN/1203/2004)		North American mallard H1N1 (A/duck/NewYork/1996)		Variant H3N2 (A/Indiana/08/2011)	
Element	Wt	RS	Wt x RS	RS	Wt x RS	RS	Wt x RS
Disease severity	0.2929	8.50	2.49	2.25	0.66	6.00	1.76
Population immunity	0.1929	8.67	1.67	3.00	0.58	3.67	0.71
Human infections	0.1429	5.67	0.81	2.33	0.33	4.33	0.62
Antiviral/treatment options	0.1096	4.50	0.49	2.25	0.25	2.50	0.27
Antigenic relatedness	0.0846	6.00	0.51	2.00	0.17	8.00	0.68
Receptor binding	0.0646	3.30	0.21	2.00	0.13	8.30	0.54
Genomic variation	0.0479	4.00	0.19	3.00	0.14	8.00	0.38
Transmission (laboratory animals)	0.0336	3.00	0.10	2.00	0.07	9.00	0.30
Global distribution (animals)	0.0211	5.50	0.12	2.50	0.05	7.00	0.15
Infections in animals	0.0010	7.25	0.01	2.00	0.00	8.00	0.01
Total	1.0000		6.60		2.38		5.42

*Wt, weight; HPAI, highly pathogenic avian influenza; RS, risk score. Weights are expressed to 4 decimal places because of the convention that the sum must be exactly 1. Sums for viruses may not be exact due to rounding.

subject matter experts using the same knowledge base will produce similar scores for each virus and narrow uncertainty ranges unless data are insufficient or the experts have a fundamental disagreement in the interpretation of available data. The upper and lower bounds of the score for each risk element can be used to determine the highest or lowest (respectively) risk score for a given virus. In addition, a sensitivity analysis wherein the rank order of 2 risk elements can be swapped and the risk score recalculated on the basis of the new weights can be used to show the effect of these changes on a virus's aggregate score. The level of uncertainty is communicated in the final report of virus scores so that decision makers using the IRAT can use this information in their considerations. Even with these efforts to measure and communicate uncertainty, the numerical score produced by the IRAT may create a sense of precision that is unfounded. Anyone making decisions by using the IRAT scores needs to be reminded that the scores are semiquantitative.

Case Illustrations

H3N2v Virus

In July 2011, the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) confirmed the first human case of an influenza A(H3N2) variant [A(H3N2)v] virus that had acquired the matrix gene from the A(H1N1)pdm09 virus. Eleven additional cases were reported during the rest of 2011, for a total of 12 confirmed cases in 5 states (*11*). A preliminary risk assessment using the IRAT was conducted on the basis of information gathered from these 12 cases and available laboratory data (Tables 2, 3). The H3N2v risk score for both the emergence and impact questions in December 2011 placed this virus in the moderate-risk category; the summary scores for both questions were >5.0.

In 2012, a total of 309 cases of A(H3N2)v virus were reported from 12 states. In most cases, the putative source was direct or indirect contact with swine (*12*), with no evidence of community transmission. Studies conducted to assess population immunity (*13–15*) provided additional information regarding age groups considered at risk for infection. In 2013, subject matter experts rescored the H3N2v virus (Figure 2) in light of these and other available data. Although 321 confirmed cases were reported over 2 years, the summary scores for the virus were lower than those for the previous scoring. The risk score for the emergence question dropped from 6.5 to 6.1, and the risk score for the impact question dropped from 5.4 to 4.5. This reduction in the scores was caused primarily by information about population immunity. This information indicated that persons at greatest risk for infection were children, especially those <10 years of age; those 20–50 years of age exhibited cross-reactive antibodies against this virus. In addition, the epidemiologic investigations of the 309 cases in 2012 showed that >90% of the patients were <18 years of age; of these cases, 16 hospitalizations and 1 death associated with A(H3N2)v virus were documented (*16*,*17*). These epidemiologic observations reinforced the population immunity data obtained in the laboratory and supported the virus's decreased cumulative risk score from its risk score in 2011.

H7N9 Virus

On March 31, 2013, the China Health and Family Planning Commission notified the World Health Organization of 3 cases of human infection with influenza A(H7N9) virus (*4*). As of April 8, 2013, the China World Health Organization Collaborating Centre for Reference and Research on Influenza, the China OIE (World Organisation for Animal Health) Reference Laboratory for avian influenza, and a provincial public health laboratory in China had identified and analyzed 7 virus isolates. The complete viral genome sequences of 6 isolates were deposited in a publicly accessible influenza database, and additional human cases continued to be reported.

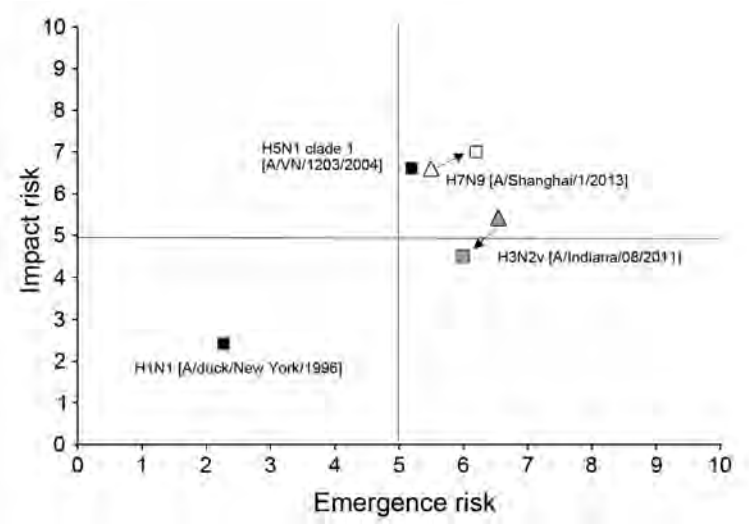

Figure 2. Influenza Risk Assessment Tool scores for 4 influenza viruses on the basis of potential risk to achieve "sustained human-to-human transmission" (emergence) and potential risk "for significant impact on public health" (impact). Black squares in top right quadrant and lower left quadrant represent risk scores for H5N1 clade 1 and H1N1, respectively. White triangle represents risk score for H7N9 in mid-April 2013; white square represents risk score for the same virus in mid-May 2013. Gray triangle represents risk score for H3N2v as of December 2011; gray square represents risk score for same virus in December 2012. Emergence risk is the risk summary score for the question, "What is the risk that a virus not currently circulating in the human population has the potential for sustained human-to-human transmission?" Impact risk is the risk summary score for the question, "If the virus were to achieve sustained human-to-human transmission, what is the risk that a virus not currently circulating in the human population has the potential for significant impact on public health?"

In April 2013, subject matter experts used the IRAT and its 10 individual risk elements to assess the potential pandemic risk for humans posed by the novel A(H7N9) virus. To address the risk of the virus to achieve human-to-human transmission (emergence question), information about the virus's ability to transmit in laboratory animals was critical. This information was not available in mid-April 2013. The absence of information for this and other elements affected the scoring of the risk elements. The scoring for the virus's potential for sustained human-to-human transmission was in the moderate-risk category, approaching 5.0 on a scale of 1–10 (Figure 2). The moderate-risk score perhaps emphasized missing information in ≥1 high-ranking element (Transmission in Laboratory Animals). For the impact question, the risk score was in the higher end of the moderate-risk range (*4*–*7*), almost 6.0. Cautionary notes regarding interpretation of the scoring in the face of data gaps were included in the subsequent report.

A month later, in mid-May, a second scoring was conducted among CDC subject matter experts (Table 4). At this time, preliminary results of studies assessing the virus's ability to transmit between animals in a laboratory setting were available. This information and updated information pertaining to the other elements were incorporated into the evaluation process. The risk score for the virus to achieve sustained human-to-human transmission was 6.2. This score was higher than the previous score based on the preliminary evaluation in April, although both scores were still in the moderate-risk range (*4*–*7*). The risk score for the virus's potential to impact public health if it were to achieve sustained human-to-human transmission is 7.0, which falls in the upper limit of the moderate-risk range and which is higher than the preliminary score of ≈6.0 in mid-April (Figure 2).

Table 4. Average risk point scores and ranked weighting applied to risk scoring of influenza A(H7N9) virus isolate A/Shanghai/1/2013 for emergence and impact questions, April 2013 and May 2013*

Emergence question				Impact question			
		Average point scores				Average point scores	
Element	Weight	Apr 2013	May 2013	Element	Weight	Apr 2013	May 2013
Human infections	0.2929	5.0	5.0	Disease severity	0.2929	9.0	8.5
Transmission (laboratory animals)	0.1929	1.0	7.0	Population immunity	0.1929	9.0	9.0
Receptor binding	0.1429	6.7	6.3	Human infections	0.1429	5.0	5.0
Population immunity	0.1096	9.0	9.0	Antivirals/treatment options	0.1096	5.4	5.8
Infections in animals	0.0846	6.0	4.7	Antigenic relationship	0.0846	6.0	3.7
Genomic variation	0.0646	8.6	8.6	Receptor binding	0.0646	6.7	6.3
Antigenic relationship	0.0479	6.0	3.7	Genomic variation	0.0479	8.6	8.6
Global distribution (animals)	0.0336	1.0	4.7	Transmission (laboratory animals)	0.0336	1.0	7.0
Disease severity	0.0211	9.0	8.5	Global distribution (animals)	0.0211	1.0	4.7
Antivirals/treatment options	0.001	5.4	5.8	Infections in animals	0.001	6.0	4.7
Weighted IRAT aggregate score		5.2	6.2	Weighted IRAT aggregate score		7.1	7.0

*IRAT, Influenza Risk Assessment Tool.

During both rounds of scoring, the subject matter experts were asked to grade their level of confidence in the available data as it applied to their scoring. They generally expressed a greater confidence in their risk scores in May. For most elements, the risk scores differed little from those in the first round. However, in May, preliminary data were available for the risk element Transmission in Laboratory Animals and informed the risk score for this element. Although the 2 scorings took place only 1 month apart, this change illustrates how a risk score can increase after availability of new data and emphasizes the necessity of reevaluating risk scores when new data become available.

Discussion

The IRAT is not a tool to assess widespread human seasonal influenza viruses that are already able to transmit from person to person; those viruses are more correctly assessed in terms of disease severity on a season-by-season basis (*18*). For seasonal influenza viruses, decisions have already been made regarding mass manufacturing and distribution of seasonal influenza vaccines. The IRAT assesses influenza viruses that are not yet readily transmissible person to person for their potential risk of emergence and potential effect on the public's health. Furthermore, the IRAT was not developed to predict the next pandemic influenza virus but rather to focus limited pandemic preparedness resources on those viruses that are believed, on the basis of current knowledge, to have the greatest potential to cause a serious pandemic.

Developing an accepted, systematic method for evaluating potentially pandemic influenza viruses to inform risk management decisions requires a framework capable of combining multiple data inputs and information. Conceptually, the IRAT follows the basic principles of microbial risk assessment frameworks but diverges from traditional methods. Microbial risk assessments typically identify and establish a working model that facilitates the analysis of dose responses and exposure estimates so that risks can be calculated and expressed in terms of likelihood, type, or magnitude. The IRAT focuses on a virologic assessment and prioritization. The development of the IRAT followed a process by which the components of a multiattribute decision analysis method were adapted to the application of evaluating influenza viruses with pandemic potential. For example, the IRAT can provide subject matter expert consensus input into decisions regarding whether to stockpile a particular influenza vaccine antigen in case it is needed. Although the IRAT can offer input into such a decision, it is not the only input.

Subject matter experts were called on to characterize, define, and capture the elements to consider when the IRAT was created. These subject matter experts, who generously brought years of experience, expertise, and familiarity with the published literature to the development of the IRAT, continue to play an integral part in its use and development. Beyond what is published, such experts in the global influenza community have insights regarding ongoing studies and research that remain unpublished. The use of subject matter experts to provide input into the IRAT is critical for addressing the inherent data gaps and uncertainty in the available information regarding emerging influenza viruses. The 2 case illustrations show how new information is readily incorporated into the analysis and reflected in the risk assessments.

This tool can also be used to guide global investments in capacity building and to highlight information gaps that can help focus research initiatives to fill these gaps. Such use was seen in the consideration of the H3N2v subtype virus, wherein studies exploring population immunity provided insight into age groups at risk and lowered the risk score of the virus once that information gap was filled. Although prediction of the next pandemic influenza virus is not yet possible, a systematic evaluation of emerging influenza A viruses with the IRAT has provided a more orderly prioritization of work on novel influenza viruses and a more judicious use of resources.

Missing information is clearly a vulnerability of the IRAT, as with any other risk assessment tool and as illustrated in the initial assessment of H7N9. Although some information may remain missing, the tool as designed incorporates weighting factors assigned to each element. To maximize the usefulness of the IRAT, information relating to the top-ranked elements, which carry the most weight, is the most helpful because these elements provide a greater proportion of the total risk score for a virus. However, even with missing or limited information, the IRAT can still provide useful insights about the potential risk posed by a novel influenza virus, particularly if the missing information pertains to lower-weighted elements.

As it is envisioned, the IRAT will continue to evolve. Current definitions of the elements are considered to be working definitions to enable users to familiarize themselves with the tool and its applications. As technology advances, the elements likely will change to reflect increasing understanding of influenza viruses. We hope that this tool will prove useful to the international influenza community yet remain flexible and responsive to increases in knowledge, understanding, and interpretation of potential risks posed by novel influenza viruses to human health.

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Dr. Trock is a veterinary medical officer in CDC's Influenza Division. Her efforts focus on animal–human interface activities, particularly as they pertain to influenza.

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Community-Based Outbreak of *Neisseria meningitidis* Serogroup C Infection in Men who Have Sex with Men, New York City, New York, USA, 2010-2013

Molly M. Kratz, Don Weiss, Alison Ridpath, Jane R. Zucker, Anita Geevarughese, Jennifer Rakeman, Jay K. Varma

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Release date: July 16, 2015; Expiration date: July 16, 2016

Learning Objectives

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

- Evaluate the general epidemiology and prognosis of invasive meningococcal disease
- Assess clinical characteristics of the outbreak of meningococcal disease described in the current study
- Determine outcomes of a meningococcal vaccination program during an outbreak of meningococcal disease
- Compare different forms of outreach to promote meningococcal vaccination during an outbreak

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In September 2012, the New York City Department of Health and Mental Hygiene identified an outbreak of *Neisseria meningitidis* serogroup C invasive meningococcal disease among men who have sex with men (MSM). Twenty-two case-patients and 7 deaths were identified during August 2010-February 2013. During this period, 7 cases in non-MSM were diagnosed. The slow-moving outbreak was linked to the use of websites and mobile phone applications that connect men with male sexual partners, which complicated the epidemiologic investigation and prevention efforts. We describe the outbreak and steps taken to interrupt transmission, including an innovative and wide-ranging outreach campaign that involved direct, internet-based, and media-based communications; free vaccination events; and engagement of community and government partners. We conclude by discussing the challenges of managing an outbreak affecting a discrete community of MSM and the benefits of using social networking technology to reach this at-risk population.

Invasive meningococcal disease (IMD) is a severe infection of the bloodstream and meninges caused by the bacterium *Neisseria meningitidis*. Although most infected persons recover, 10%–15% of cases are fatal, often within 24 hours of symptom onset. An additional 11%–19% survive with serious neurologic or other complications (*1*). *N. meningitidis* colonizes the nasopharynx and is transmitted through close or prolonged contact. Functional asplenia, complement deficiency, and infection with HIV increase sporadic IMD risk, and living in close quarters, smoking, attending bars, and kissing have been associated with IMD outbreaks (*2*,*3*).

In the United States, IMD occurs rarely and has been decreasing over the past 25 years; the estimated incidence rate for illness caused by all *N. meningitidis* serogroups in 2012 was 0.15 cases/100,000 persons, and only 0.03 cases/100,000 persons for serogroup C meningococcal disease (*4*,*5*). Infants and young children are the most at risk, followed by persons ≥65 years of age. Outbreaks, which account for only 2% of reported cases, generally involve small numbers of cases and occur in both community and institutional settings (*3*). Only 2 IMD outbreaks, both serogroup C meningococcal disease, have been reported as occurring exclusively among MSM; 1 comprised 6 cases in Toronto, Ontario, Canada, in 2001, and another 6 cases in Chicago, Illinois, USA, in 2003. Both outbreaks prompted vaccination campaigns targeting MSM; >3,850 were vaccinated in Toronto and 14,267 in Chicago (*6*,*7*).

The first outbreak of IMD in New York City, New York, in >25 years occurred during 2005–2006 and included 23 cases of serogroup C meningococcal disease among current and former illicit drug users and their contacts. The outbreak occurred in a contiguous 4-ZIP code area of central Brooklyn and was resolved after 2,763 persons were vaccinated in a targeted campaign (*8*). In 2012, the New York City Department of Health and Mental Hygiene (DOHMH) identified the third North America outbreak of serogroup C meningococcal disease occurring among MSM (a fourth outbreak was recognized in Chicago, Illinois, USA in May 2015 [http://www.bcbsil.com/pdf/education/cdph_press_release.pdf]). We report the epidemiology of the 2012 New York City outbreak and efforts of DOHMH to control it.

Methods

Surveillance and Case Investigation

The New York City Health Code requires that IMD cases be reported immediately to DOHMH. Every reported IMD case is investigated to confirm the diagnosis, identify close contacts for antimicrobial drug prophylaxis, obtain risk factor information, characterize the isolate by serogroup and by pulsed-field gel electrophoresis (PFGE) at the New York City Public Health Laboratory. Case investigations include medical record review and interviews with health care providers, family members, close contacts, and the patient when possible. Antimicrobial drug prophylaxis is offered to persons who had prolonged close contact, both sexual and non-sexual, with the patient during the infectious period. For this outbreak, the case definition used was a clinically compatible illness for serogroup C meningococcal disease meeting the 2010 Council of State and Territorial Epidemiologists case definition for a confirmed or probable case in an MSM (*9*).

After DOHMH identified the serogroup C meningococcal disease outbreak and its association with MSM in September 2012, case investigators attempted to re-interview all male IMD case-patients in New York City since 2010 to explicitly inquire about sexual identity and behavioral risk factors and to identify any additional cases. Although sexual contacts are routinely elicited during all IMD case investigations, DOHMH staff did not previously record information about a patient's sexual identity or sexual history beyond their period of infectiousness. During the outbreak period, 7 cases of serogroup C meningococcal disease were diagnosed in non-MSM; these cases were not considered to be part of the outbreak.

Vaccine Initiative and Tracking Vaccine Uptake

After the vaccine recommendation was made, DOHMH implemented an immunization initiative consisting of vaccine distribution and outreach events. DOHMH began tracking the aggregate number of doses administered at its clinics. In addition, public and private health care facilities with large MSM and HIV-infected patient populations were asked to make regular summary reports of meningococcal vaccine administration to MSM. The size of the population targeted for vaccination was estimated by using data from the New York City Community Health Survey and the HIV Surveillance Registry of DOHMH.

In the United States, quadrivalent meningococcal vaccine is used routinely and provides protection against *N. meningitidis* serogroups A, C, W, and Y. One dose is recommended for children 11–12 years of age, with a booster dose at age 16. This vaccine is also routinely recommended for first-year college students living in residential housing, travelers to disease-endemic areas, laboratory workers, and those at increased clinical risk (*10*).

Results

Outbreak

We identified 22 outbreak-related cases of serogroup C meningococcal disease during August 2010-February 2013 (Figure 1). The first case occurred in August 2010, and 8 additional cases occurred intermittently over the next 24 months. Transmission increased in September 2012; a cluster of 4 cases was diagnosed within 4 weeks. All 4 case-patients were HIV infected, and 2 reported sexual contact with each other. At that time, DOHMH formally recognized the outbreak, issued a vaccine recommendation (on October 4, 2012), and began implementing response efforts. The initial provider recommendation was to administer meningococcal vaccine to "HIV-infected men who are New York City residents and who had intimate contact with a man met either through an online website, digital application (app), or at a bar or party since September 1, 2012" (*11*). In accordance with the official Centers for Disease Control and Prevention (CDC) recommendation, those who were HIV infected and considered themselves at risk were encouraged to get a second dose of vaccine 8 weeks after the first dose (*10*).

Three additional cases of serogroup C meningococcal disease (1 in an HIV-infected person) were identified in MSM before mid-November 2012, shifting the epidemiology of the outbreak to indicate an association with certain neighborhoods in Brooklyn. This finding prompted revision of the vaccine recommendation to "men who have sex with men, regardless of HIV status, if they live in specific areas of Brooklyn and report intimate contact with a man met either through an online website, digital application (app), or at a bar or party since September 1, 2012" (*12*). Six additional cases were detected (3 in HIV-infected persons), including a cluster of 4 in early 2013, but the association with Brooklyn weakened. DOHMH further expanded the target population for vaccination in March 2013 to include all areas of the city and all HIV-infected MSM, regardless of risk behavior, in addition to MSM not infected with HIV who met the risk criteria (*13*). With no new cases identified 12 months after the final February 2013 case, DOHMH declared the outbreak over in March 2014.

Of the 22 outbreak-related cases identified, 7 (32%) were fatal. Co-infection with HIV was present in 12 (55%) case-patients, 5 (42%) of whom died. The age range of the case-patients was 21-59 years (median 31 years, mean 34 years). Case-patients lived throughout the city; the largest proportion (10 [45%]) lived in Brooklyn. Eleven (50%) case-patients were African American, and 4 (18%) were Hispanic or other. Eleven (50%) reported using recreational drugs; of the 15 case-patients for which a determination could be made, 9, including the index case-patient; reported using websites and mobile phone applications (apps) to connect with other men looking for sexual partners (Table 1).

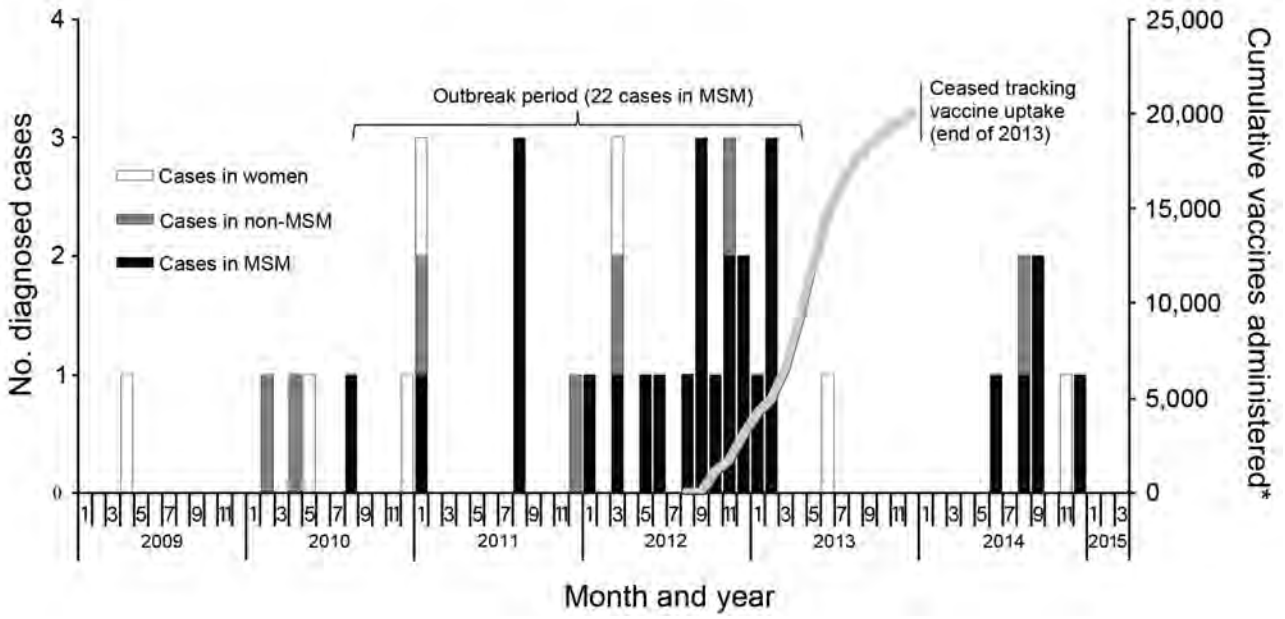

Figure 1. Monthly invasive serogroup C incidence and cumulative vaccine uptake, New York City, New York, USA, 2009–2015. *Vaccine uptake among MSM only as part of outbreak response. MSM, men who have sex with men; non-MSM, men who do not have sex with men.

Isolates from 15 case-patients were available for PFGE; 14 (93%) isolates were >85% related to the unique outbreak strain, and 9 (64%) of these were indistinguishable from it. These 15 case-patients had culture-confirmed results. The remaining 7 case-patients had culture-negative results; these 7 case-patients were given a diagnosis by PCR. During the outbreak period, an additional 7 cases were diagnosed in non-MSM; isolates from 6 were available for PFGE and only 2, both in women, were found to be >85% related to the outbreak strain. None of the cases in non-MSM were considered to be part of the outbreak.

DOHMH estimated that in 2012, MSM in New York City were 52.5 times (95% CI 19.9–137.4) more likely to contract IMD than other male residents 18-64 years of age (12.6 cases/100,000 MSM compared with 0.24 cases/100,000 unknown or non-MSM). Furthermore, HIV-infected MSM (21.2/100,000) had 88.3 times (95% CI 30.5–252.9) the relative risk for infection than unknown or non-MSM residents of New York City in 2012 (Table 2).

Vaccination Initiative

After the initial recommendation was issued, vaccine was made available, free of charge, at DOHMH clinics in 9 locations throughout New York City. DOHMH staff also identified private health care facilities with large HIV-infected and MSM patient populations and encouraged them to administer vaccine to persons at risk and facilitated procurement and distribution of vaccine when necessary. It is estimated that >20,000 men were vaccinated with ≥1 dose during October 2012-August 2013 in New York City.

DOHMH also staged free vaccine events at bars and clubs geared toward MSM to provide convenient, stigma-free opportunities for men at risk to get vaccinated. A prominent MSM party promoter was hired to host and oversee marketing of these events, including the creation of a Facebook page and a promotional YouTube video (*14*). Vaccine uptake was much lower than expected; health officials administered only 85 doses at 4 DOHMH-sponsored events. Recognizing the value of supporting established patient–provider relationships, DOHMH also supplied vaccine for events hosted by clinicians with a history of treating HIV-infected and MSM patients. These events were staged on-site at their clinical facilities, as well as off-site at MSM bars, sex clubs, and 2013 NYC Pride parades and parties. Demand was much higher at these non-DOHMH events, which facilitated the administration of >2,800 doses of vaccine.

Outreach Campaign

To promote vaccination among the at-risk subset of the MSM population in New York City, DOHMH began a multifaceted outreach campaign in October 2012. The components of the campaign can be divided into 2 general categories.

Direct and Internet-Based Outreach

DOHMH provided updates to health care providers through the Health Alert Network, mailed and posted educational materials, and resources to facilitate vaccination (e.g., screening forms, consent forms, billing information) on its website and gave presentations at academic medical centers, large clinics, and community-based organizations.

Table 1. Epidemiology and risk factors of for 22 outbreak case-patients with invasive meningococcal disease, New York City, New York, USA, 2010–2013*

Characteristic	Value	%
Age, y		
Range	21–59	NA
Mean	34	NA
Median	32	NA
Borough†		
Brooklyn	10	45
Manhattan	7	32
Bronx	2	9
Queens	2	9
Race/ethnicity		
African American	11	50
Caucasian	6	27
Hispanic or other	4	18
Asian	1	5
Risk behavior		
Using recreational drugs	9	41
Using websites or phone apps to meet sexual partners‡	9	60
HIV infected	12	55
Outcome		
Died	7	32
Died, HIV infected	5	42

*NA, not applicable.
†One case-patient was reported being homeless.
‡A determination could be made for only 15 of the 22 outbreak case-patients.

Table 2. Rates for invasive meningococcal disease for residents (all serogroups) of New York City, New York, USA, 2012*

Category	No. cases	Population at risk	Rate/100,000	Relative risk	95% CI
All New York City	25	8,175,133	0.31	NA	NA
Men	21	3,882,544	0.54	NA	NA
Women	4	4,292,589	0.09	NA	NA
All New York City, age 18–64 y	21	5,413,864	0.39	NA	NA
Women	2	2,827,956	0.07	NA	NA
Men	19	2,585,908	0.73	NA	NA
Men, not/unknown MSM	6	2,482,908	0.24	Referent	NA
MSM	13	103,000	12.6	52.5	19.9–137.4
MSM with HIV/AIDS	8	37,720	21.2	88.3	30.5–252.9
MSM without HIV/AIDS	5	65,280	7.7	32.1	9.7–103.9

*NA, not applicable; MSM, men who have sex with men.

To reach MSM at risk, DOHMH distributed >1,200 posters (Figure 2) and >70,000 palm cards at locations geared toward MSM through established community-based partners participating in the DOHMH condom distribution program. Materials were printed in English, Spanish, Chinese, Japanese, Korean, and Thai.

Beginning in October 2012, DOHMH disseminated information through Twitter and >100 websites and blogs with high MSM viewership and paid for banner and pop-up advertisements on websites and apps targeting MSM, including the app Grindr. In addition, email messages were sent en masse to all registered members of Manhunt, DaddyHunt, and Adam4Adam. When possible, communications were targeted to reach only users in New York City and, before the expansion of the vaccine recommendation, only users who identified themselves as HIV infected.

Click-through proportions, defined as the number of persons who saw the advertisement/ message and clicked to get more information divided by the number of times the advertisement/message appeared, were used to evaluate the effect of these communications. In November 2012, a total of 40,116 (0.82%) of 488,000 pop-ups ads and 2,782 (0.06%) of 463,645 banner ads were clicked on, compared with 87 (14.5%) of 605 email blasts to users of a popular hook-up website. Of 266 users surveyed, 118 (44%) recalled receiving an email about the outbreak and 77 (29%) of users recalled seeing one of the banner ads run on the site.

Engagement of External Partners and Mainstream Media Coverage

To promote vaccination, DOHMH communicated with and distributed educational materials to community organizations and elected officials representing large numbers of MSM. To address challenges with provider reimbursement, New York state regulators notified health insurers that all insurance plans must cover the cost of meningococcal vaccination. DOHMH also worked with legislators to help design a bill authorizing New York pharmacists to administer vaccine; this bill was signed into law by the governor in July 2013.

Some small-scale print and electronic publications reported the story during the first few months of the vaccine campaign, but it was not until *The New York Times* ran a comprehensive article on March 21, 2013, more than a month after the last associated case was diagnosed, that the outbreak was featured in a variety of other more high-profile publications and engendered substantial coverage on social media sites (*15*). This pattern was repeated with 2 subsequent articles in *The New York Times* that provided

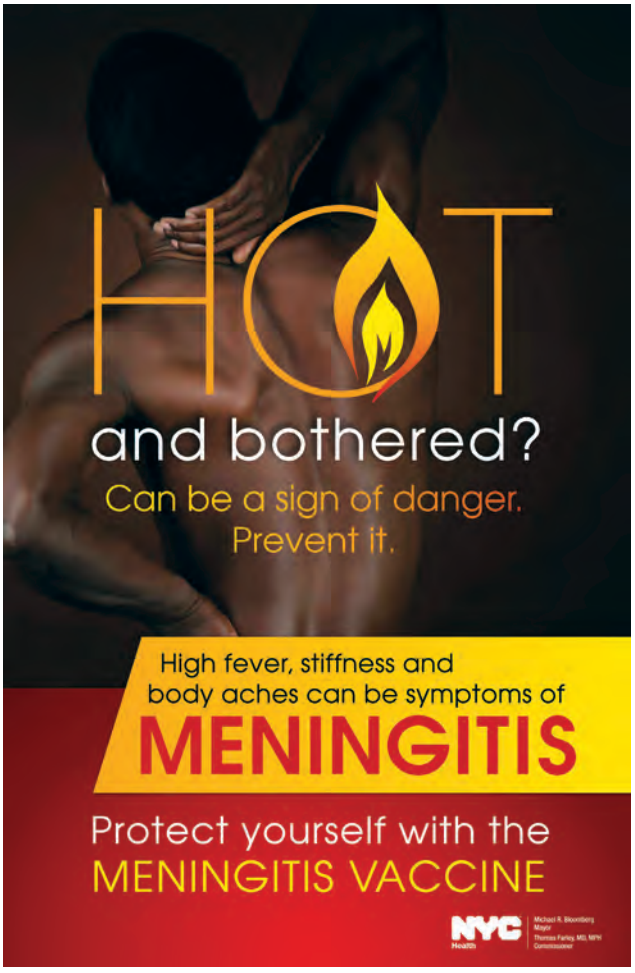

Figure 2. Meningococcal vaccination campaign outreach poster, New York City, New York, USA.

updates on the outbreak; both articles also spurred additional reporting by other media outlets (*16*,*17*). These articles appeared to have a much more substantial effect on vaccine uptake than any of the other outreach initiatives (Figure 3).

Discussion

This protracted IMD outbreak posed several administrative challenges and inspired innovative management and outreach strategies that could benefit responses to other public health emergencies. This outbreak was notable for its association with men using websites and apps to find male sex partners.

Communicable diseases spread through social networks, but because internet and mobile technologies facilitate spontaneous, often anonymous sexual encounters, they complicate understanding of these networks and identification of contacts. We were not able to link cases directly to each other, a single website or app, or a venue, hindering efforts to clearly and narrowly define the target population. Nine (60%) of the 15 cases for which case investigators could make a determination reported the risk behavior of using hook-up websites and apps to find sexual contacts; the true proportion may well be higher, considering the sensitive nature of the inquiry.

It was difficult to decide whether and when to implement a vaccination campaign in the context of a slow moving outbreak. By the end of September 2012, we had identified 13 outbreak-related cases spread out over >2 years; 4 of these cases had been diagnosed in the previous 3 months. CDC defines a community-based outbreak as having a primary attack rate of ≥10 cases/100,000 persons within a 3-month time frame (*18*). Using results from the New York City Community Health Survey of the DOHMH, we estimated the 2012 population of MSM in New York City to be ≈103,000 and calculated a serogroup C meningococcal disease attack rate of 3.9 cases/100,000 persons (July–September 2012), which is well below the CDC recommended threshold for mass vaccination. Although we were unable to make a precise determination, we proposed that the actual number of MSM at risk was a relatively small subset of the total MSM population. Thus, the true attack rate of the outbreak was probably much higher than 3.9 cases/100,000 persons. Furthermore, we considered the duration and severity of the outbreak, including the highly increased relative risk for MSM of contracting serogroup C meningococcal disease compared with that for non-MSM in New York City (Table 2) when concluding that a citywide vaccination campaign was warranted.

In constructing messages to the target population, the language had to be broad enough to reach the discrete person at risk while also being specific enough to appeal to them, and these messages were criticized for what some perceived to be a vague recommendation centered on an already stigmatized population. As the outbreak evolved, we issued 2 revisions to our initial vaccine recommendation with the intent of better capturing the MSM subset population at highest risk. However, providers and the public alike had difficulty digesting the changing message while combating fatigue and apathy toward the outbreak response efforts.

To reach those persons eligible for vaccination, we expanded on a traditional outreach approach to include more innovative, internet-based methods. When we considered click-through rates and the average cost per click, email blasts to members of hook-up websites proved a successful, comparatively cost-effective method for messaging the at-risk population. However, electronic outreach supplemented but did not substitute for traditional media. We recorded major spikes in vaccine demand after articles about the outbreak were published in *The New York Times*, a widely read publication, and uptake continued to increase as smaller-scale print and electronic publications and social media participants picked up the story. This response suggests

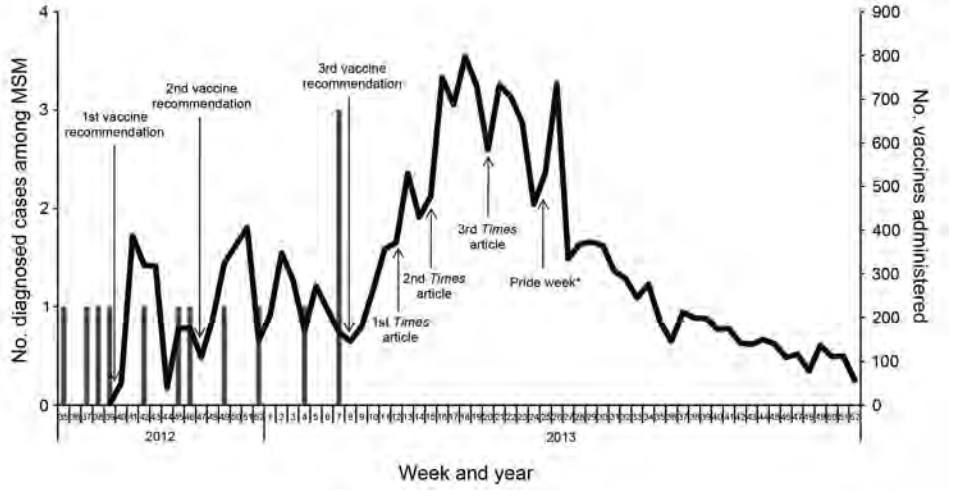

Figure 3. Weekly invasive meningococcal disease incidence among men who have sex with men (MSM) and vaccine uptake, New York City, New York, USA, August 2012–2013, encompassing the last of 13 of 22 outbreak cases. The Department of Health and Mental Hygiene ceased tracking of vaccine uptake at the end of 2013. Dates of articles in *The New York Times* are indicated. *Free vaccinations were provided at many of the parades and events during NYC Pride.

that, when used in tandem, traditional and social media can exponentially increase the effect of outreach. Traditional media can be used to penetrate mainstream society with a specific message, and social media can be used to expand and target that message to hard-to-reach populations. As the popularity of social media continues to increase, efforts to control future outbreaks of infectious disease should adapt to using technologies that are flexible, far-reaching, and cost-effective.

Our multifaceted response and outreach efforts to control the serogroup C meningococcal disease outbreak among MSM in New York City increased awareness and were associated with increased vaccine administration, which peaked after the diagnosis of the final case in February 2013. However, in June 2014, fifteen months after the last outbreak-related case was identified, another case of serogroup C meningococcal disease in MSM in New York City was reported to DOHMH (Figure 1). This case was followed by a cluster of 3 cases in late August and early September. These 3 case-patients were determined to be part of the same social network and to have had contact with each other before their onset dates of serogroup C meningococcal disease. DOHMH launched another round of outreach efforts, including ads on hook-up sites and apps, and held 3 free vaccination events, and *The New York Times* published an article covering the cluster (*19*). One additional case of serogroup C meningococcal disease in MSM was diagnosed in December 2014. All 5 of these post-outbreak case-patients were HIV infected, and PFGE testing showed the infecting strain to be closely related to the outbreak strain circulating in 2012. Two of the 5 case-patients had been vaccinated (compared with only 1 of the 14 outbreak case-patients with known vaccination status), and all survived their infections.

As of the publication of this article, the DOHMH recommendation to vaccinate all HIV-infected MSM (with 2 doses) and HIV-uninfected MSM engaging in risk behavior, first issued in March 2013, remains in effect. Since 2010, a total of 27 MSM case-patients with serogroup C meningococcal disease have been identified in New York City and 17 (63%) of them were HIV infected. Los Angeles, California; France; and Germany have also reported clusters of MSM case-patients with serogroup C meningococcal disease, many HIV infected, prompting their own vaccine recommendations (*20*–*22*). However, the behavioral and microbiologic factors leading to transmission of serogroup C meningococcal disease among MSM are still not understood and require attention as additional epidemiologic data are collected and analyzed. In addition, questions about how to protect this at-risk population deserve careful consideration, including whether national guidelines should recommend routine meningococcal vaccination of HIV-infected MSM.

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Risk for Mycobacterial Disease among Patients with Rheumatoid Arthritis, Taiwan, 2001–2011

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Increasing evidence indicates that the risk of acquiring tuberculosis (TB) and nontuberculous mycobacterial disease is elevated among patients with rheumatoid arthritis (RA). To determine the epidemiology of mycobacterial diseases among RA patients in Asia, we conducted a retrospective cohort study. We used a nationwide database to investigate the association of RA with mycobacterial diseases. The risk for development of TB or nontuberculous mycobacterial disease was 2.28-fold and 6.24-fold higher among RA patients than among the general population, respectively. Among RA patients, risk for development of mycobacterial disease was higher among those who were older, male, or both. Furthermore, among RA patients with mycobacterial infections, the risk for death was increased. Therefore, RA patients, especially those with other risk factors, should be closely monitored for development of mycobacterial disease.

The major clinical spectrum of mycobacterial diseases is caused by tuberculosis (TB) and nontuberculous mycobacteria (NTM). TB remains a major global health problem; in 2012, an estimated 8.6 million persons became infected and 1.3 million died of the disease (*1*). NTM are ubiquitous environmental microorganisms that cause chronic pulmonary and extrapulmonary infection in patients with inflammatory diseases (*2*). Several NTM strains are resistant to many antimicrobial drugs, making treatment difficult (*3*). Because reporting of NTM disease to public health administrations is not required in most countries, epidemiologic data for these countries are not available (*4*). Pulmonary diseases caused by NTM are being diagnosed with increasing frequency worldwide (*5*), including in Taiwan (*6*). In Taiwan, the incidence of TB remains high, despite extensive implementation of well-known TB control measures and use of the Bacillus Calmette-Guérin vaccine (*7*); between 2000 and 2012, a laboratory-based study indicated a trend of decreasing TB cases but significantly increasing NTM cases in Taiwan (*6*).

Rheumatoid arthritis (RA), a chronic articular inflammatory disease (*8*), affects 0.5%–1.0% of the adult population and is a major cause of disability in industrialized countries (*9*,*10*). Among RA patients, the risks of acquiring or dying of an infectious disease are increased, possibly because of disease-related immune dysfunction or the immunosuppressive effects of therapeutic agents (*11*). In Europe and the United States, an increased risk for TB among RA patients has been reported (*12*), and the risk for active TB is even higher among those receiving anti–tumor necrosis factor α (TNF-α) therapy (*13*). Previous clinical studies have shown that the prevalence of latent tuberculosis infection was higher among RA patients than among healthy controls (*14*). A recent study indicated that in the United States, the incidence of NTM disease was significantly higher among RA patients receiving anti–TNF-α therapy than among patients with other inflammatory diseases who were receiving the same treatment (*15*). The prevalence of mycobacterial diseases is higher among the general population in Asia than in the United States and Europe (*1*,*16*). However, few population-based epidemiologic studies have investigated the association of RA with mycobacterial diseases in Asia. In addition, prevalence of concurrent medical conditions is higher among RA patients (*17*,*18*), which may affect their risk for TB (*19*). However, the association of RA with concurrent medical conditions and mycobacterial infection is unclear.

In Taiwan, the National Health Insurance program is a mandatory universal health insurance program that provides comprehensive medical care for >99% of Taiwan's residents (*20*–*22*). The National Health Insurance Research Database (NHIRD) is managed by the National Health Research Institutes, and confidentiality is maintained according to Bureau of National Health Insurance guidelines (*23*). We used this nationwide database to conduct a retrospective cohort study investigating the association between RA and mycobacterial diseases in Taiwan during 2001–2011.

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[1]Deceased.

Methods

Data Source

The NHIRD includes inpatient and ambulatory care claims covering 1996–2011. The Longitudinal Health Insurance Database 2000 contains all original claims data for 1 million persons randomly sampled from the Registry for Beneficiaries of the NHIRD, which was released by the National Health Research Institutes, which confirmed that the random samples were representative of the general population in Taiwan. We systemically sampled NHIRD patient data for 2001–2011 (Figure 1). The data were de-identified forms of secondary information in an anonymous format released to the public for research purposes. All work was done at the Taichung Veterans General Hospital, Taichung, Taiwan, and the institutional review board of this hospital exempted this study from full review (no. CE13151-1).

Definitions

Patients with different diseases were identified by use of codes from the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). In addition, the NHIRD includes a registry system for catastrophic illnesses, and RA is included in this database. Therefore, the diagnosis of RA (ICD-9-CM code 714.0) was made according to the 1987 American College of Rheumatology criteria (*24*) and the NHIRD Registry of Catastrophic Illness Patient Database.

All TB and NTM cases were determined by use of ICD-9-CM codes, laboratory mycobacterium examination codes, and antimycobacterial therapy receipts; all 3 criteria needed to be met. We used the definition of TB in the published literature (*20*,*21*). First, the ICD-9-CM codes for TB were 010–018. Among TB cases, ICD-9-CM codes 010–012 and 018 are used for pulmonary TB and codes 013–017 are used for extrapulmonary TB. Second, >2 mycobacteria laboratory examination codes were used for TB identification, including those identified by acid-fast smear (13006C/13025C), mycobacterial culture (13012C/13026C), acid-fast bacillus differentiation (13013C), tuberculin test (12106C), tuberculosis test (13024C), and bronchoscopy (28006C) results (*20*). Third, we identified patients who had taken at least 1 prescription consisting of ≥3 anti-TB drugs used simultaneously for at least 120 days during a 180-day period; these drugs were isoniazid, ethambutol, rifampin, pyrazinamide, amikacin, kanamycin, streptomycin, ciprofloxacin, ofloxacin, moxifloxacin, levofloxacin, prothionamide, clarithromycin, and thioridazine (*20*,*21*).

The NTM disease definition was based on the American Thoracic Society and the Infectious Diseases Society of America guidelines (*3*), which include clinical, radiographic,

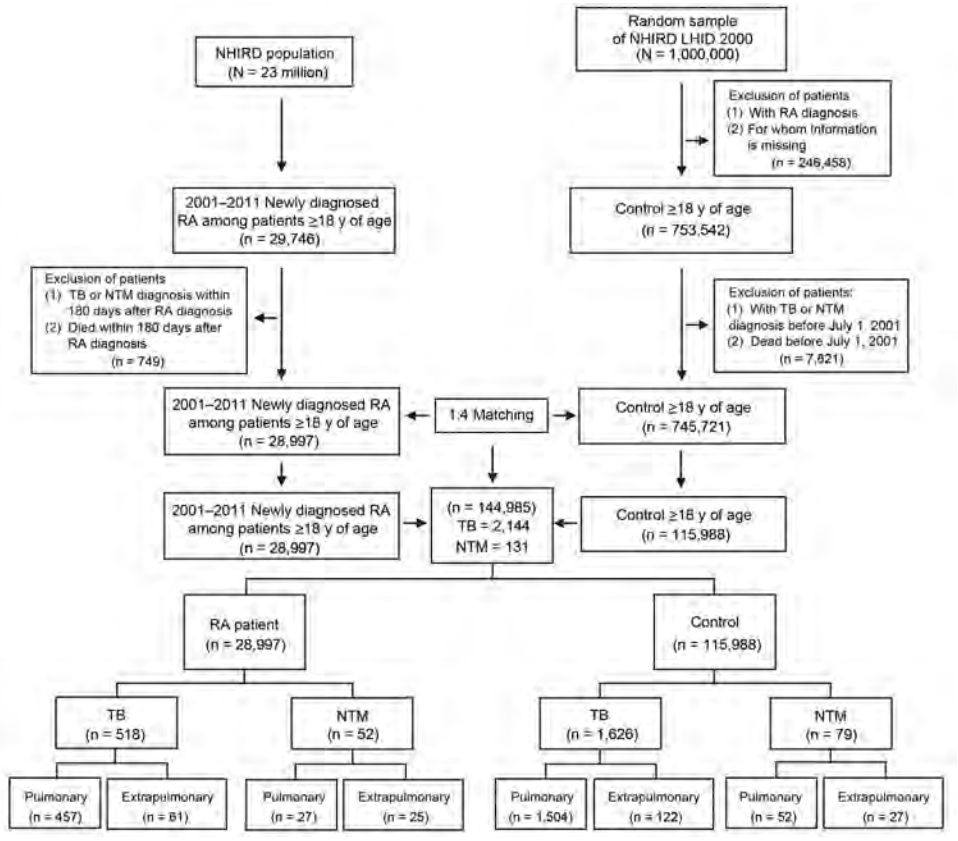

Figure 1. Flowchart of case selection for rheumatoid arthritis (RA) patients and age- and sex-matched controls (without RA) from the National Health Insurance Research Database (NHIRD). LHID 2000, Longitudinal Health Insurance Database 2000; NTM, nontuberculous mycobacteria; TB, tuberculosis.

and microbiologic criteria. All items carry equal weight and all must be met for a diagnosis of NTM lung disease (*3*) as follows. First, the patient must have been assigned an ICD-9-CM code for NTM disease (031.0, 031.1, 031.2, 031.8, and 031.9). Among NTM diseases, ICD-9-CM code 031.0 is for pulmonary NTM disease and the others are for extrapulmonary NTM disease. Second, the mycobacteria laboratory examination code criteria for NTM needed to be consistent with those for TB (20). Third, the patient should have received >2 drugs to treat NTM disease, including amikacin, cefoxitin, ciprofloxacin, clarithromycin, doxycycline, ethambutol, imipenem, levofloxacin, meropenem, minocycline, moxifloxacin, rifabutin, rifampin, tigecycline, and streptomycin (*3*). Simultaneous TB and NTM disease was defined as TB only. In addition, the first diagnosis of TB or NTM disease must have been made >6 months after the identification of the RA. The study end point was defined as the onset of new mycobacterial disease or death during the 11-year follow-up period (2001–2011).

To understand the association between RA-related concurrent conditions and mycobacterial diseases, we choose several RA-related conditions to study. The definitions of each condition were based on the following ICD-9-CM codes: cardiovascular diseases (codes 390–438), diabetes mellitus (code 250), hyperlipidemia (code 272), liver cirrhosis (code 571), and chronic obstructive pulmonary disease (codes 490–492 and 496). In addition, we also selected mycobacterial disease-related conditions (e.g., chronic kidney disease [code 585] and HIV disease [codes 042–044]).

Study Population

The study was a retrospective cohort study of NHIRD data for 29,746 patients ≥18 years of age who had received a new diagnosis of RA during January 1, 2001, through December 31, 2011. We excluded 749 patients whose TB or NTM diagnosis was made within 180 days after the RA diagnosis and/or who died within 180 days after RA diagnosis. The age-matched control group (≥18 years of age) was selected from the NHIRD Longitudinal Health Insurance Database 2000 and excluded patients for whom information regarding age or sex was missing, those with a history of RA, TB, or NTM, and those who died before July 1, 2001. Patients with newly diagnosed RA (28,997 patients) and controls who did not have RA (115,988 controls) were matched 1:4 by age and sex (Figure 1).

Statistical Analyses

Data are presented as mean ± SD for continuous variables and as proportions for categorical variables. Differences were analyzed by using the independent *t*-test for the continuous variables and the χ^2 test for the categorical variables. Incidence rates for newly diagnosed mycobacterial diseases among RA patients and controls were calculated. For each cohort, incidence rates per 1,000 person-years were also calculated according to the distribution of demographic characteristics and concurrent conditions. The multivariable Cox proportional hazards model was adjusted for age, sex, Charlson Comorbidity Index score (*25*), and urbanization (residence in urban, suburban, or rural area) before being used to identify independent factors contributing to the development of mycobacterial diseases in the RA and control cohorts. The 95% CI for each variable was also determined. All analyses were conducted by using SAS statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA). A p value of <0.05 was considered significant.

Results

Characteristics of the Study Cohort

Data for ≈99% of Taiwan residents were included in the NHIRD. Among these, 28,997 eligible persons with newly diagnosed RA were identified during 2001–2011. The follow-up period for this study population, during which risk for mycobacterial disease development was assessed; mean follow-up time was 5.1 years (range 2.1–8.3 years). The control group was matched for age and sex at a ratio of 1:4, and a total of 115,988 participants who did not have RA (controls) were identified (Figure 1). Characteristics of the enrolled participants are summarized in Table 1. RA patients were predominately (77.7%) female, and mean age was 53.9 years. Approximately 22.9% of the RA patients were ≥65 years of age. Age and sex distributions between the RA patients and controls did not differ significantly. Prevalence of concurrent conditions was higher among RA patients than among controls (56.4% vs. 32.1%, respectively; $p<0.0001$). No clinically significant difference in urbanization was found between RA patients and controls.

Characteristics of RA Patients with Mycobacterial Disease

Among the total study population of 144,985, new-onset TB developed in 2,144 (1.48%) (Figure 1). Among the 28,997 RA patients, 518 (1.79%) received a new diagnosis of TB at least 6 months after their RA diagnosis (Figure 1). Of the 518 patients with TB that we identified, the TB was pulmonary for 457 (88.22%) and extrapulmonary for 61 (11.78%). The incidence rate for TB was higher among RA patients than controls (3.4 vs. 1.4 cases/1,000 person-years, respectively; Table 2). After multivariable analysis and adjustment for age, sex, Charlson Comorbidity Index score, and urbanization, the risk for TB development increased 2.28-fold for RA patients over that for controls (95% CI 2.06–2.54; $p<0.0001$). The risk for TB development, after stratification for age, sex, and urbanization, was higher for RA patients than controls. Further analysis indicated

Table 1. Baseline characteristics for 144,985 patients with and without RA, Taiwan, 2001–2011*

Variables	With RA, no. (%), n = 28,997†	Without RA, no. (%), n = 115,988†	p value
Age at entry, y‡			
18–44	7,495 (25.9)	29,980 (25.9)	1.000
45–64	14,865 (51.3)	59,460 (51.3)	
≥65	6,637 (22.9)	26,548 (22.9)	
Sex			
F	22,524 (77.7)	90,096 (77.7)	1.000
M	6,473 (22.3)	25,892 22.3)	
Concurrent conditions			
Cardiovascular disease	8,100 (27.9)	28,327 (24.4)	<0.0001
Diabetes mellitus	2,480 (8.6)	9,749 (8.4)	0.419
Hyperlipidemia	2,951 (10.2)	8,301 (7.2)	<0.0001
Liver cirrhosis	2,451 (8.5)	7,277 (6.3)	<0.0001
COPD	2,097 (7.2)	8,247 (7.1)	0.472
Chronic kidney disease	270 (0.9)	896 (0.8)	0.007
HIV disease	1 (0.003)	9 (0.01)	0.698
Charlson Comorbidity Index score§			
0	12,653 (43.6)	78,773 (67.9)	<0.0001
1	8,708 (30.0)	20,791 (17.9)	
2	4,381 (15.1)	8,823 (7.6)	
≥3	3,255 (11.2)	7,601 (6.6)	
Urbanization			
Urban	17,167 (59.2)	68,429 (59.0)	0.668
Suburban	4,098 (14.1)	16,622 (14.3)	
Rural	7,732 (26.7)	30,937 (26.7)	

*COPD, chronic obstructive pulmonary disease; RA, rheumatoid arthritis.
†Median, mean follow-up time for patients with RA = 5.1, 5.2 ± 3.1 y and for patients without RA = 11.0, 10.3 ± 2.1 y; p<0.0001.
‡Mean (± SD) age for patients with RA = 53.9 ± 14.1 y and for patients without RA = 53.5 ± 14.9 y; p<0.0001.
§Mean (± SD) index for patients with RA = 1.0 ± 1.3 and for patients without RA = 0.6 ± 1.2; p<0.0001.

that the risk for TB development was higher for RA patients with concurrent conditions than for controls with the same conditions.

New-onset NTM disease developed in 131 (0.09%) patients (Figure 1). For 52 (0.18%) of the 28,997 RA patients, NTM disease was newly diagnosed at least 6 months after RA was diagnosed (Figure 1). Of these 52 patients with identified NTM, 27 (51.92%) had pulmonary and 25 (48.08%) had extrapulmonary disease; 27 (51.92%) had a history of previous TB. The incidence rate of NTM disease was significantly higher among RA patients than controls (0.3 vs. 0.1 cases/1,000 person-years, respectively; Table 2). Multivariable analysis indicated that risk for development of NTM disease was 6.24-fold higher among RA patients than controls (95% CI 4.24–9.17, p<0.0001). As was the case for TB, the risk for development of NTM disease after stratification for age, sex, and urbanization was higher for RA patients than controls. In addition, risk for development of NTM disease was higher for RA patients with concurrent conditions than for controls with the same conditions.

Risk Factors for Mycobacterial Disease Development

Risk factors for development of mycobacterial diseases in RA patients were older age and male sex. For patient age ≥65 years, the adjusted hazard ratio [aHR] was 5.06 (95% CI 3.72–6.88; p<0.0001) for TB and 4.70 (95% CI 1.82–12.13; p = 0.001) for NTM. For male patients, aHR was 2.35 (95% CI 1.97–2.81; p<0.0001) for TB and 2.64 (95% CI 1.52–4.60; p = 0.001) for NTM (Table 3). In addition, among RA patients, the risk for TB and NTM was positively correlated with increased age (Figure 2). No significant difference in urbanization was found for TB and NTM disease development in RA patients.

Risk for Death among RA Patients with Mycobacterial Disease

Risk for death was higher among RA patients with mycobacterial infection than among RA patients without mycobacterial infection (aHR 1.62, 95% CI 1.38–1.90, p<0.0001 for TB; aHR 3.06, 95% CI 1.53–6.12, p = 0.002 for NTM; Table 4). Furthermore, the risk for death was increased among older RA patients (≥65 years, aHR 8.81, 95% CI 7.48–10.39; p<0.0001), male patients (aHR 1.73, 95% CI 1.59–1.88; p<0.0001), and patients who lived in rural areas (aHR 1.41, 95% CI 1.29–1.54; p<0.0001).

Discussion

This nationwide population-based retrospective cohort study indicated that among the ≈20 million enrollees in Taiwan's NHIRD, the risk for development of TB was 2.28-fold greater and the risk for development of NTM disease 6.24-fold greater among RA patients than among controls. Among RA patients, significant risk factors for development of mycobacterial disease were older age (≥65 years) and male sex. Furthermore, the risk for death was higher among RA patients with mycobacterial infection than among RA patients without mycobacterial infection.

Table 2. Subgroup analysis for new-onset TB and NTM among 144,985 patients with and without RA, Taiwan, 2001–2011*

Variable	With RA			Without RA			aHR (95% CI)‡	p value
	No. events	No. person-years	Incidence†	No. events	No. person-years	Incidence†		
New-onset TB								
All patients	518	150,284.0	3.4	1626	119,2456.6	1.4	2.28 (2.06–2.54)	<0.0001
Age at entry, y								
18–44	54	42,192.3	1.3	130	321,916.2	0.4	2.54 (1.78–3.63)	<0.0001
45–64	215	78,297.6	2.7	689	627,394.5	1.1	2.23 (1.90–2.63)	<0.0001
≥65	249	29,794.0	8.4	807	243,145.9	3.3	2.28 (1.96–2.65)	<0.0001
Sex								
F	307	118,858.3	2.6	909	936,265.5	1.0	2.38 (2.08–2.74)	<0.0001
M	211	31,425.7	6.7	717	256,191.2	2.8	2.16 (1.84–2.54)	<0.0001
Concurrent condition								
CVD	204	41,104.2	5.0	671	270,893.6	2.5	1.99 (1.69–2.34)	<0.0001
DM	58	11,952.4	4.9	261	89,868.3	2.9	1.69 (1.26–2.27)	0.001
Hyperlipidemia	59	14,371.2	4.1	137	83,859.8	1.6	2.49 (1.80–3.43)	<0.0001
Liver cirrhosis	45	14,122.1	3.2	123	72,188.4	1.7	1.89 (1.33–2.69)	0.0001
COPD	74	11,232.7	6.6	343	77,702.4	4.4	1.40 (1.08–1.81)	0.011
Urbanization								
Urban	265	88,389.5	3.0	823	708,145.9	1.2	2.39 (2.06–2.77)	<0.0001
Suburban	80	21,371.3	3.7	257	169,798.3	1.5	2.16 (1.66–2.82)	<0.0001
Rural	173	40,523.2	4.3	546	314,512.5	1.7	2.20 (1.84–2.64)	<0.0001
New-onset NTM								
All patients	52	151,633.4	0.3	79	1,198,369.7	0.1	6.24 (4.24–9.17)	<0.0001
Age at entry, y								
18–44	6	42,367.8	0.1	12	322,534.9	0.0	3.70 (1.22–11.24)	<0.05
45–64	25	78,903.7	0.3	36	630,397.4	0.1	6.89 (3.93–12.07)	<0.0001
≥65	21	30,361.9	0.7	31	245,437.5	0.1	6.71 (3.67–12.28)	<0.0001
Sex								
F	30	119,677.3	0.3	55	939,708.1	0.1	5.80 (3.55–9.47)	<0.0001
M	22	31,956.1	0.7	24	258,661.7	0.1	7.08 (3.77–13.29)	<0.0001
Concurrent condition								
CVD	15	41,555.1	0.4	24	273,076.0	0.1	5.03 (2.51–10.07)	<0.0001
DM	5	12,087.8	0.4	7	90,698.9	0.1	9.84 (2.79–34.75)	<0.001
Hyperlipidemia	4	14,523.6	0.3	6	84,326.4	0.1	6.16 (1.46–26.02)	<0.05
Liver cirrhosis	4	14,219.5	0.3	7	72,638.1	0.1	4.77 (1.24–18.38)	<0.05
COPD	5	11,451.8	0.4	15	78,904.5	0.2	3.02 (1.04–8.78)	<0.05
Urbanization								
Urban	27	89,070.6	0.3	38	711,281.4	0.1	5.91 (3.42–10.21)	<0.0001
Suburban	11	21,556.7	0.5	10	170,651.8	0.1	13.85 (5.32–36.03)	<0.0001
Rural	14	41,006.0	0.3	31	316,436.5	0.1	4.51 (2.28–8.93)	<0.0001

*aHR, HR, adjusted hazard ratio; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; DM, diabetes mellitus; NTM, nontuberculous mycobacteria; RA, rheumatoid arthritis TB, tuberculosis.
†Rate per 1,000 person-years.
‡Adjusted for age, sex, Charlson Comorbidity Index score, and urbanization.

The incidence of TB (53.0 cases/100,000 population in 2012) and mortality rate (2.7 cases 100,000 population in 2012) were higher in Taiwan than in other Asian (e.g., Japan, Korea) or western countries (*7*). In this study, the incidence rate for TB was significantly higher among RA patients than controls (3.4 vs. 1.4/1,000 person-years, respectively). A population-based study in Europe showed that the risk for development of TB was 4-fold higher among RA patients than among the general population (*26*). Although the incidence rate for TB for the general population declined over time, the incidence rate for TB among RA patients was still higher than that among controls (*26*).

Our findings that male sex and older age are major risk factors for mycobacterial infection among RA patients in Taiwan are consistent with local laboratory-based data (*6*) and findings of systematic reviews in Asia (*16*,*27*). However, in Japan, South Korea, and most western countries, most NTM disease patients were female (*16*,*28*). This discrepancy may be associated with lifestyle, environmental factors, and genetic factors. More studies are required to confirm this hypothesis. We also found a history of TB for 27 (51.92%) of NTM-infected RA patients and 43 (54.43%) of 79 controls, similar to results from eastern Asia (*27*). Furthermore, our results illustrated that risk for development of NTM disease was 6.24-fold higher among RA patients than controls and that the risk for death among RA patients with NTM disease was 3.06-fold higher than that for RA patients without NTM disease. A recent population-based study in Canada demonstrated a 2.07-fold increased risk for NTM disease among RA patients and a 1.81-fold increased risk for death among RA patients with NTM disease (*29*). The risk for exposure to mycobacteria closely correlated with the prevalence of mycobacterial diseases. The prevalence of mycobacterial diseases is higher in Taiwan (*5*,*7*),

Table 3. Multivariable analysis of baseline factors for 28,997 RA patients with new-onset TB and NTM, Taiwan, 2001–2011*

Variables	aHR†	95% CI	p value
TB			
Age at entry, y			
18–44	1.00	Reference	Reference
45–64	1.96	1.45–2.65	<0.0001
≥65	5.06	3.72–6.88	<0.0001
Sex			
F	1.00	Reference	Reference
M	2.35	1.97–2.81	<0.0001
Charlson Comorbidity Index score			
0	1.00	Reference	Reference
1	1.08	0.87–1.34	0.513
2	1.14	0.89–1.46	0.305
≥3	1.19	0.91–1.54	0.202
Urbanization			
Urban	1.00	Reference	Reference
Suburban	1.09	0.84–1.40	0.525
Rural	1.19	0.98–1.45	0.087
NTM			
Age at entry, y			
18–44	1.00	Reference	Reference
45–64	2.25	0.91–5.53	0.078
≥65	4.70	1.82–12.13	0.001
Sex			
F	1.00	Reference	Reference
M	2.64	1.52–4.60	0.001
Charlson Comorbidity Index score			
0	1.00	Reference	Reference
1	1.64	0.85–3.19	0.143
2	1.31	0.59–2.94	0.509
≥3	1.01	0.39–2.64	0.983
Urbanization			
Urban	1.00	Reference	Reference
Suburban	1.34	0.65–2.75	0.425
Rural	0.85	0.43–1.66	0.631

*aHR, adjusted hazard ratio; NTM, nontuberculous mycobacteria; RA, rheumatoid arthritis; TB, tuberculosis.
†Adjusted for age, sex, Charlson Comorbidity Index score, and urbanization.

thus leading to an increased risk for infection (aHRs 6.24 vs. 2.07) and infection-related death (aHRs 3.06 vs. 1.81) among RA patients in Taiwan and Canada, respectively.

Among participants in this study, the risk for death among RA patients with NTM disease was higher than that among RA patients TB (aHRs 3.06 vs.1.62). This finding might reflect increased incidence of NTM disease in Taiwan, but awareness of NTM disease is not enough. In Taiwan, cases of TB should be reported to the public health administration, and control measures should be implemented immediately. However, cases of NTM disease do not have to be reported, and few hospitals can fully identify NTM (*6*). Furthermore, the severity of NTM disease varies, resulting in missed diagnoses for patients with mild and asymptomatic infection. Therefore, it is easy to ignore and underestimate the consequences of NTM disease. According to experience in Europe (*26*), risk for mycobacterial diseases in RA patients is reduced by increasing the awareness of hospital staff and screening for mycobacterial diseases. In addition, more laboratory data are needed for investigation of the characteristics (e.g., species distribution, invasion site, drug resistant) of clinical NTM strains.

In this study, pulmonary invasion affected most RA patients (88.22%) and controls (92.50%) with TB and slightly more than half (51.92%) of the RA patients with NTM disease. In the past few years, the incidence of NTM-associated pulmonary diseases and hospitalization has increased in Europe and the United States (*30*,*31*), and this increase is most likely associated with the global trend toward more persons being of older age, increased use of immunosuppressive medication, elevated prevalence of immune-modulating concurrent conditions, and use of advanced diagnostic techniques (*16*). In addition to pulmonary NTM disease, an increased percentage of extrapulmonary NTM disease was noted in RA patients than controls (48.08% vs. 34.18%). Similar elevated manifestations of extrapulmonary NTM (44%) were reported from another study, which found NTM disease in RA patients receiving anti–TNF-α therapy in the United States (*32*,*33*).

Our results showed that the risk for development of mycobacterial diseases was higher among RA patients with concurrent medical conditions than among controls with the same conditions. These findings were similar to those of a population-based study in Canada (*29*). Accumulating

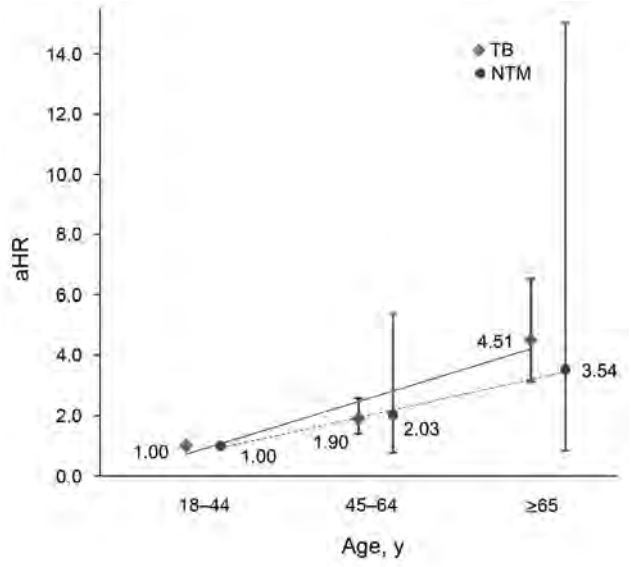

Figure 2. Adjusted hazard ratios (aHRs) and 95% CIs (error bars) for tuberculosis and nontuberculous mycobacteria infection according to age among patients with rheumatoid arthritis and matched controls. Increased risk correlated with increased age, Taiwan, 2001–2011.

evidence indicates that risk factors for TB are diabetes mellitus, liver cirrhosis, and chronic obstructive pulmonary disease (*19*). However, the relationship between other RA-related conditions (e.g., hyperlipidemia) and mycobacterial diseases remains unclear. In addition to demographic and host factors, geographic factors are associated with the development of mycobacterial diseases (*19*,*34*). A population-based study in the United States found that TB-related deaths were strongly predominant among the general population living in urban areas, but no such meaningful difference was found for NTM disease–related deaths (*34*). Our results showed that after stratification of data for urbanization, the risk for both TB and NTM disease among all RA patients was higher than that for controls, but no significant difference with regard to urbanization and mycobacterial disease development in RA patients was found. Urbanization had fewer effects on increasing the risk for development of mycobacterial diseases in RA patients in Taiwan.

The NHIRD contains complete medical information regarding patients, including prescription details, diagnoses based on the ICD-9-CM codes, registration files, and original claims records for reimbursement. However, this administrative database has several limitations. First, the NHIRD does not contain detailed information of lifestyle factors (e.g., smoking, alcohol abuse, or substance abuse) or individual health status (e.g., body mass index, malnutrition, genetic factors) that are associated with mycobacterial infection and RA (*19*,*35*). Another limitation is that the NHIRD only contains medical claims data, so the current condition of the patients is unknown. Of note, the NHIRD does not include the results of laboratory examinations. To improve accuracy of the analysis, we used not only ICD-9-CM codes but also mycobacteria laboratory examination codes and antimycobacterial therapy receipts to identify TB and NTM disease cases. Because most patients with NTM pulmonary lung disease do not receive treatment, our definition (which requires treatment) is probably relatively insensitive, missing all untreated cases. The definition is undoubtedly highly specific, but because extrapulmonary NTM is almost always treated, our definition probably resulted in a higher than

Table 4. Multivariable analysis for risk for death among 28,997 RA patients with and without TB and NTM, Taiwan, 2001–2011*

Variable	aHR†	95% CI	p value
RA without TB or NTM	1.00	Reference	Reference
RA with TB	1.62	1.38–1.90	<0.0001
RA with NTM	3.06	1.53–6.12	0.002
RA with TB and NTM	1.58	0.82–3.04	0.174
Age at entry, y			
18–44	1.00	Reference	Reference
45–64	1.91	1.61–2.27	<0.0001
≥65	8.81	7.48–10.39	<0.0001
Sex			
F	1.00	Reference	Reference
M	1.73	1.59–1.88	<0.0001
Charlson Comorbidity Index score			
0	1.00	Reference	Reference
1	1.05	0.95–1.17	0.357
2	1.25	1.12–1.41	0.0001
≥3	1.92	1.73–2.14	<0.0001
Urbanization			
Urban	1.00	Reference	Reference
Suburban	1.27	1.13–1.42	<0.0001
Rural	1.41	1.29–1.54	<0.0001

*aHR, adjusted hazard ratio; NTM, nontuberculous mycobacteria; RA, rheumatoid arthritis; TB, tuberculosis.
†Adjusted for age, sex, Charlson Comorbidity Index score, and urbanization.

expected proportion of extrapulmonary NTM cases. The major strength of this study is use of a nationwide database with medical care records, which is minimally affected by selection and recall biases. In addition, the large sample size of the NHIRD (≈20 million enrollees, including patients and the general population) and long-term records enhanced the statistical power and accuracy of this study.

Increasing evidence indicates that the elevated risk for mycobacterial diseases among RA patients is primarily attributable to the effect of immunosuppressive therapies (*15*,*32*,*33*). Because most RA patients in Taiwan were treated with disease-modifying antirheumatic drugs or biologicals, we hypothesized that the risk for development of mycobacterial diseases may be associated with immunosuppressive therapy, RA-related concurrent conditions, or immune dysfunction caused by RA itself. Further studies are required to confirm this hypothesis.

In conclusion, our results showed that risk for death is higher among RA patients with than without mycobacterial diseases. Therefore, TB- or NTM-infected RA patients should be closely monitored, especially those who are male, are ≥65 years of age, or have concurrent conditions.

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Prevalence of Hepatitis E Virus Infection in Pigs at the Time of Slaughter, United Kingdom, 2013

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Since 2010, reports of infection with hepatitis E virus (HEV) have increased in England and Wales. Despite mounting evidence regarding the zoonotic potential of porcine HEV, there are limited data on its prevalence in pigs in the United Kingdom. We investigated antibody prevalence, active infection, and virus variation in serum and cecal content samples from 629 pigs at slaughter. Prevalence of antibodies to HEV was 92.8% (584/629), and HEV RNA was detected in 15% of cecal contents (93/629), 3% of plasma samples (22/629), and 2% of both (14/629). However, although HEV is prevalent in pigs in the United Kingdom and viremic pigs are entering the food chain, most (22/23) viral sequences clustered separately from the dominant type seen in humans. Thus, pigs raised in the United Kingdom are unlikely to be the main source of human HEV infections in the United Kingdom. Further research is needed to identify the source of these infections.

Hepatitis E virus (HEV) that infects humans is composed of 4 genotypes (G1–4), each with a different geographic distribution and host range (*1*). Although G1 and G2 infect humans only, G3 and G4 infect humans and animals. HEV G3 and G4 are distributed worldwide, with G3 most commonly infecting both humans and pigs in Europe (*2*). From the observed incidence of acute HEV infection in blood donors (*3*), it is clear that HEV G3 infection in humans in England is far more common than previously thought. Realistic estimates are >100,000 infections annually.

Public Health England instituted enhanced surveillance of HEV infections in England and Wales in 2003 (*4*) and identified a recent and marked increase in the number of patients seeking treatment for HEV infections. In 2013, a total of 691 cases were identified, of which 477 (69%) were considered indigenous (occurring in persons who had not traveled outside England and Wales). Sequencing of strains from these acutely infected persons has identified an emergent phylogenetic cluster of HEV G3 infection in humans, which is likely to represent a zoonosis acquired through the consumption of undercooked meat (B. Said, pers. comm.).

In an early study in the United Kingdom of porcine samples archived during 1991–2001, antibodies to HEV were detected in 85.5% of 256 samples tested (*5*). More recent studies across Europe indicate that many pig herds show evidence of HEV G3 infection (*6*–*10*). A transient viremia in pigs is associated with dissemination of HEV into muscle and other tissues (*11*). A recent UK study found HEV RNA in 6 of 63 pork sausages tested, of which 5 were in a single batch of 11 (*12*), and a case–control study in England and Wales showed that human consumption of processed pork products is associated with an increased risk of acquiring HEV (*13*). It has long been considered plausible that the persistence of viremia in infected pigs up to the time of slaughter could provide a potential vehicle for zoonotic transmission to humans (*14*). We conducted surveillance of pigs at slaughter to investigate the epizoology of HEV in the United Kingdom and the extent of infection at the time pigs enter the food chain.

Methods

Study Details

Sample collection was undertaken during January–May 2013 as part of the 2013 Zoonoses in UK Pigs Abattoir Study, a cross-sectional study of pigs being slaughtered at 14 high-throughput abattoirs (12 in England and 2 in Northern Ireland) that together process 80% of all slaughtered pigs in the United Kingdom. The target population was all slaughtered pigs (finishers, cull sows, and boars) in the United Kingdom, excluding any condemned carcasses, pigs with a live weight <50 kg, pigs that had undergone emergency slaughter, and pigs that had been kept in the UK <3 months before slaughter (the latter was estimated to be around 2% of all slaughtered pigs) (*15*). Sampling was weighted so that the number of carcasses sampled in each of the selected abattoirs was proportional to the throughput of that abattoir and stratified by calendar month. Analysis was undertaken by using Stata statistical software version 12

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(StataCorp LP, College Station, TX, USA). The prevalence estimates were calculated by using the svy command to adjust CIs because some pigs originated from the same farm.

Samples

Whole blood taken by jugular vein stab and anticoagulated with EDTA was collected from 643 pigs for testing for antibodies to HEV and HEV RNA. Cecal contents from 638 pigs were available for testing for HEV RNA.

Paired plasma and cecal content samples were available for 629 pigs that met the study inclusion criteria. The pigs originated from 439 farms, with 1–10 pigs sampled per farm. The mode and median number of pigs per farm was 1. The geographic distribution of the pigs sampled was broadly proportionate to the UK pig population (*16*), and most (560, 89.0%) were <12 months old.

Detection, Quantification, and Characterization of HEV RNA

Plasma HEV RNA was detected in nucleic acid extracts of plasma by using a quantitative TaqMan quantitative reverse transcription PCR assay (*12*) and expressed in international units per milliliter by comparison with the World Health Organization international standard. The limit of detection, defined by Poisson titration, was 22 IU/mL. Extracts whose amplification was below the threshold level of quantification of 100 IU/mL were confirmed to contain HEV RNA through amplification in a second PCR by using inner primers JVHEVF and JVHEVR (*17*,*18*). Cecal content HEV RNA was detected in nucleic acid extracts of 10% fecal suspensions by using the TaqMan assay and a modified forward primer (JHEVF2, 5′-RGTGGTTTCTGGRGTGAC-3′), which gave a limit of detection of 250 IU/mL in cecal contents (25 IU/mL in 25% of replicates).

Phylogenetic analysis was attempted on all samples containing quantifiable HEV RNA detectable above a lower limit threshold corresponding to a cycle threshold (C_t) value of 40 and on a proportion of lower samples. HEV open reading frame 2 (ORF2) (348-bp) fragments that could be amplified by nested PCR (*19*) were sequenced as previously described (*20*). Sequences were assembled into phylogenetic trees and compared with current UK human and porcine sequences retrieved from GenBank by using MEGA 6.0 (*21*).

Detection and Measurement of Antibodies against HEV

Antibodies against HEV were detected in swine plasma samples by using the Wantai Total HEV Antibody kit (Fortress Diagnostics Ltd., Antrim, UK) in accordance with the manufacturer's protocol. To increase the dynamic range for approximate quantification of antibody, we retested samples with binding ratios (BR) ≥20 at a 1:10 dilution and adjusted the resulting BRs by a factor of 10.

Swine IgM against HEV was detected by using a modification of the human Wantai IgM assay in which the solid phase was replaced with microtiter wells coated with antibodies to the IgM-specific heavy chain domain of IgM (Bethyl Laboratories Inc., Montgomery, TX, USA). Reactivity was quantified by comparison with a calibration curve of a strong IgM-positive pig plasma arbitrarily attributed a potency of 100 pig IgM units/mL (100 AU/mL) serially diluted in HEV antibody-negative pooled pig plasma. Samples with reactivity >3.3 AU/mL in the presence of antibodies against HEV in other assays were considered to contain IgM. In the absence of HEV antibody reactivity, a more stringent cutoff of ≥10 AU/mL was used.

Swine IgG antibody was sought in an indirect immunoassay by using a modification of the Wantai IgG test for human serum samples where the labeled human IgG conjugate was replaced with labeled swine IgG (AbD Serotec, Kidlington, UK). A conservative cutoff optical density (OD) of 0.33 was used for this assay, determined by calculating the mean OD in the indirect assay of samples negative in the Wantai total assay, removing outliers, rederiving the mean negative OD, and using 1.5 × derived mean as cutoff. Samples giving reactions in excess of this were considered to contain HEV IgG.

Results

Of 629 paired samples, 14 contained detectable HEV RNA in plasma and cecal samples. RNA was detected in 22 additional plasma samples and in 93 additional cecal samples. The prevalence of current infection defined by detectable plasma HEV RNA at any level, adjusting for clustering within farms, was 5.7% (95% CI 3.9%–7.6%). Similarly, the prevalence of current infection, defined by detection of HEV RNA in cecal samples at any level, again adjusting for clustering within farms, was 17.0% (95% CI 14.0%–20.0%). Taking detection of HEV RNA in either plasma or cecal samples as evidence of infection in 129 animals, we determined that the prevalence of current infection in pigs at slaughter was 20.5% (95% CI 17.2%–23.8%).

The viral load in the plasma ranged from detectable but below the limit of quantification to a maximum of 10^6 IU/mL. Similarly, values in cecal content ranged from 40 to 7.4×10^7 IU/mL. Six plasma samples contained in excess of 10^2 IU/mL HEV RNA. Although high-level viremia in plasma samples was generally reflected by a high level of shedding in the cecal content (Table 1), most HEV RNA signals in either plasma or cecal content were present in only 1 of these paired sample types and then often only at low levels (plasma only mean C_t 40.5, range 34.3 to below levels of quantitation; cecal only mean C_t 37.2, range 24.2–45).

A total of 584 of 629 pig plasma samples were positive by manufacturers' criteria in the Wantai total antibody

Table 1. Serologic analysis of and viral RNA in cecal and plasma samples in 6 pigs ranked by viremia whose HEV plasma load at slaughter exceeded 10^2 IU/mL, United Kingdom*

Pig viral RNA			Serologic result†			
Cecal C_t‡	Plasma C_t	Viremia, IU/mL§	Wantai BR	IgG BR	IgM, AU/mL	Interpretation
24.23	23.7	5.95×10^5	161	11.9	80.76	Current acute
24.71	25.5	1.92×10^5	1.03	(0.7)	(2.91)	Early acute
33.04	34.7	4.40×10^3	198	10.3	41.71	Current acute
37.46	34.2	2.80×10^3	299	11.6	8.94	Late acute
Not detected	34.3	570	86	11.3	4.41	Late acute
39.34	37.3	270	85	7.5	4.73	Late acute

*AU, absorbance units; BR, binding ratio test/cutoff; C_t, cycle threshold; HEV, hepatitis E virus.
†Parentheses indicate BRs below cutoff.
‡C_t for amplification trace.
§Interpolated IU/mL.

assay (BR 1.0), and the pigs from which these samples were taken were considered seropositive. After adjusting for clustering of pigs within farms, we determined a seroprevalence of 92.8% (95% CI 90.7%–95.0%). Of the 584 plasma samples positive for antibodies against HEV, 276 (47.4%) also contained measurable IgM (reactivity >3.3 AU/mL), indicating a relatively recent infection. The 45 (7.2%) plasma samples from the survey that were unreactive by Wantai were tested for HEV IgM and IgG. Fifteen contained HEV IgG, albeit at low levels (mean BR 1.7, range 1.1–3.1), and 3 of the 15 contained HEV IgM (66.1, 33.4, and 18.3 AU/mL). Two of 30 that were unreactive for HEV IgG contained HEV IgM only (11.3 and 14.1 AU/mL).

Most (91%, 117/129) pigs with detectable HEV RNA at any site were seropositive, and half of the virus-positive pigs (48%, 56/117) also had detectable HEV IgM (IgM reactivity ≥3.3 AU/mL; Table 2). The remaining 220 seropositive pigs with detectable IgM did not have detectable HEV RNA. Pigs with high-level cecal content and plasma viremia had current or recent acute infection, and all had HEV IgM (Table 1). Seven of 9 seropositive pigs that were both viremic and shedding virus in the cecal content were seropositive for HEV IgM.

Twelve infections were identified in pigs that were seronegative by manufacturers' criteria in the Wantai assay in which HEV RNA was present at low level in cecal contents, plasma samples, or both (Table 2). Additional serologic testing of the 4 samples from Wantai seronegative pigs in which HEV RNA was detected in both cecal fluid and plasma (Table 3) showed 1 plasma sample to contain IgG just over the cutoff, another to contain low levels of IgM (3.4 AU/mL), and 2 samples to be reactive but below cutoff for IgG and IgM.

Amplification of RNA for sequencing was attempted on all samples containing quantifiable HEV RNA. However, due to low levels of input, virus sequences were only derived from 6 pig plasma and 21 cecal content samples. Where viral RNA could be sequenced from both plasma and cecal content, the animals had identical virus sequences at both sites (n = 4). All of the 23 unique sequences belonged to G3; all but 1 clustered within the UK human group 1 (Figure). A single cecal-derived sequence clustered within group 2 (*4*). Most viruses from UK patients with acute hepatitis clustered within group 2.

Discussion

We have demonstrated that HEV G3 RNA was present in plasma, cecal contents, or both in 20.5% of all pigs tested, which is likely to approximate the lower limit for a farm prevalence, given the modal number of pigs per farm sampled. A recent survey in France described a farm-level prevalence of 30% for HEV G3, with at least 1 RNA-positive animal detected in 27 of 90 farms sampled (*22*). Similar findings in Canada (*23*) and Italy (*24*) have been documented for other tissues and organs. In Spain, 11.5% of liver or bile samples contained HEV RNA (*25*).

Porcine HEV is acquired through ingestion of virus (*26*), and the extended duration of cecal fluid virus and, therefore, fecally shed virus in this study, exceeding that of the more transient plasma viremia (*11*), will favor porcine fecal-oral transmission. Extended fecal shedding means that older but immunologically naive animals joining the groups at finisher units may become infected closer to the time of slaughter. High cecal carriage and fecal excretion in some animals indicates the potential existence of superspreaders around the time of slaughter. The resulting

Table 2. Serologic status of 129 pigs in whom HEV RNA was detected in plasma, cecal fluid, or both, United Kingdom*

		Pig serostatus†	
RNA-positive analyte	No. pigs	No. positive (no. IgM positive)	No. negative
Plasma only	22	19 (8)	3
Plasma and cecal fluid	14	10 (8)	4
Cecal fluid only	93	88 (40)	5
Total	129	117 (56)	12

*HEV, hepatitis E virus.
†Defined by Wantai test for antibodies against HEV.

Table 3. Markers in the 4 Wantai antibody-seronegative plasma samples from pigs with concordant HEV RNA in plasma and cecal fluid samples, United Kingdom*

Cecal C_t†	Viremia, IU/mL‡	Serologic results for HEV antibody		
		Wantai BR‡	IgG BR	IgM, AU/mL
37.49	BLQ	0.02	0.4	3.40
39.73	BLQ	0.02	0.7	2.20
39.62	69	0.22	0.4	1.50
39.66	45	0.35	1.2	0.80

*AU, absorbance units; BLQ, below the level of quantification; BR, binding ratio test/cutoff; C_t, cycle threshold; HEV, hepatitis E virus.
†C_t for amplification trace.
‡Interpolated IU/mL.

environmental contamination may complicate attempts to control onward transmission of HEV in pigs.

High-level viremia was unusual, occurring in only 6 pigs, but although rare, this represents the best candidate source of potential dietary transmissions by meat ingestion (*27*). Of these 6 pigs, 1 infection was in the early acute seroconversion phase. Two were in the acute phase of the infection, with high IgM levels, and the remaining 3 were later in the acute infection, with low IgM levels. All 6 pigs had detectable plasma IgM (Table 1), which probably indicates recent infections. We postulate that plasma viremia is a good marker for possible dietary transmission by meat products. The reported absence of porcine adenovirus (another virus found in pig feces) in HEV-contaminated sausages (*12*) also implicates viremia as the source of virus rather than fecal contamination at the abattoir.

We have reported (*4*) that the viruses causing current cases of G3 hepatitis E in humans fall into 2 phylogenetically and temporally separable groups, 1 and 2. These groups derive from the analysis of a 304-nt fragment of ORF2 with levels of bootstrap support in the region of 70% depending on the number of sequences analyzed. Much stronger support for these 2 groups is obtained when a larger 1,300-nt region of ORF2 is analyzed (data not shown). Most sequences of strains in humans contemporary to this study fall within group 2 (along with reference sequence 3c; Figure). In contrast, most G3 HEV (22 of 23) sequences obtained from UK pigs fall into group 1 (along with reference sequences of 3e, 3f, and 3g; Figure). Notably, the group 1 pig viruses are almost identical to those circulating in UK pig populations a decade ago (data not shown), perhaps demonstrating a long-standing zoonosis that may be reflected in the continuing group 1 cases in humans in England and Wales. The sole group 2 G3 HEV was from a pig from Scotland and falls outside the dominant human clade, sitting among a minor grouping.

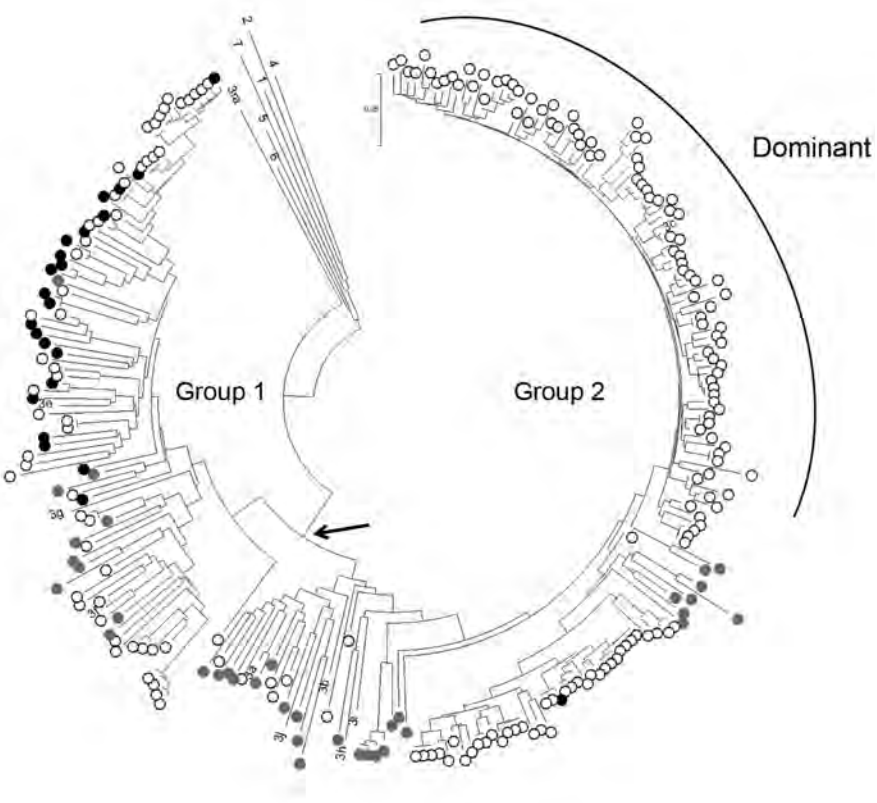

Figure. Phylogeny of genotype 3 hepatitis E viruses (HEVs) from pigs and patients with acute hepatitis in the United Kingdom. Nucleotide sequences of a 304-nt open reading frame 2 fragment (positions 5994–6297 of reference sequence M73218) from pigs at slaughter (black dots, n = 23) or from cases in persons with acute hepatitis E in England and Wales in 2013 (open circles, n = 190) were used to produce a neighbor-joining tree on the basis of maximum composite likelihood distances. GenBank accession numbers for porcine HEV sequences from this study are KP293752–774. Reference sequences were porcine sequences from Europe and North America from GenBank (gray dots, n = 36) and single examples of previously assigned HEV genotypes and subtypes for which complete genome sequences were available: 1, M73218; 2, M74506; 4, AJ272108; 5, AB573435; 6, AB602441; 7, KJ496143; 3a, AF082843; 3b, AB291955; 3c, FJ705359; 3e, AB248521; 3f, EU723514; 3g, AF455784; 3h, AB290312; 3i, FJ998008; 3j, AY115488; and 3ra, GU937805. Bootstrap support (500 replicates) for all major nodes, including those for genotypes 1–7, was weak (40%–70%), reflecting the short genome region and the large number of sequences analyzed. Arrow indicates the group 1/2 node.

In England, as in most Western industrialized countries, HEV infection in humans comprises travel-associated (G1 and G3; potentially G2 and G4) and indigenous (G3) infections. Our findings indicates that, in the United Kingdom, indigenous HEV human-to-human infection will be rare, and nontravel-related hepatitis E results from HEV G3 dietary acquisition, as shown by recent and continuing case–control studies (*13*).

Our findings suggest that slaughtered UK pigs are unlikely to be the source of most HEV G3 infections in humans in England and Wales. Although one could postulate the coexistence of group 2 viruses circulating in UK pigs, the failure to detect this virus at the time of slaughter in 22 of 23 pigs from whom virus could be sequenced would seem to render unlikely high-level viremia and possible infectivity of group 2 viruses through the contribution of UK pig meat to the food chain. We were not able to sequence most infections identified because of low viral levels in the reactive analytes. Consequent with our current understanding about infections in humans, it is instead plausible that the dominant HEV infections in humans that could be linked to pork consumption (*9*) derive from imported meat or meat products, although we are unable to establish the precise source. Other routes of transmission from hitherto unidentified animal sources to humans also cannot be excluded.

The GenBank sequences most closely related to the dominant clade of UK human HEV sequences derive from a wild boar from Germany sampled in 2006 or from pigs from Italy and France, all of which were sampled more than a decade ago. Trade of both pigs and pig products is a common practice in Europe, with imports accounting for around half of the pig meat consumed in the United Kingdom (*15*). Some of the acute hepatitis cases in UK patients associated with HEV G3 infection clustered together with most UK pig HEV isolates and the 3e reference sequence, and it is possible that UK pork is one of the sources of these infections. However, our findings conversely suggest that additional sources of pork may be responsible for further cases of human G3 HEV acute hepatitis clustering outside UK pig sequences.

The timing of HEV infection in pigs is a key consideration for informing future management options for mitigation of risk to public health. Because just over half of the pigs in this study had recently cleared infection, exhibiting antibodies against HEV and detectable IgM in the absence of detectable HEV RNA, they were likely to have been infected in the 2 months before slaughter. In this study, a few pigs remained seronegative at the time of slaughter (7.1%, or 4.5% if the 17 Wantai-negative samples reactive for IgG and IgM are considered seropositive) (Table 3). Our data indicate a lower prevalence of susceptibility (seronegativity) at slaughter in UK pigs than in continental Europe (*7–10*,*21*,*28*).

The demonstration of pigs viremic at time of slaughter explains the detection of HEV in processed food products in the absence of apparent hygiene problems. It seems likely that we are measuring by proxy, through the infection of humans, the spread of group 2 HEV G3 infection in pig populations that supply meat to the United Kingdom. The continuing annual increase in hepatitis E cases in humans in the United Kingdom may reflect changes in trade, processing, or husbandry in other countries, or a societal change in how pig meat is consumed. Consideration has to be given to developing a better understanding of this widespread zoonosis. Given the ubiquity and transmissibility of porcine HEV infection in swine, with the simultaneous absence of clinical signs, economic effects, or regulation, the elimination of HEV in pigs is unlikely in the near future.

Immunization of pigs against HEV is currently only a theoretical option because there no vaccines are on the market for pigs. In addition, one would have to ensure that any intervention is not merely delaying infection and increasing the likelihood of viremia at the time of slaughter (*29*,*30*). An alternative approach through using husbandry practices to facilitate natural immunity in early life should also be considered. Further investigations into HEV infection in humans and pigs in the United Kingdom and other countries are required to inform farming management practices to reduce active porcine infection rates at the time of slaughter.

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Estimates of Outbreak Risk from New Introductions of Ebola with Immediate and Delayed Transmission Control

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While the ongoing Ebola outbreak continues in the West Africa countries of Guinea, Sierra Leone, and Liberia, health officials elsewhere prepare for new introductions of Ebola from infected evacuees or travelers. We analyzed transmission data from patients (i.e., evacuees, international travelers, and those with locally acquired illness) in countries other than the 3 with continuing Ebola epidemics and quantitatively assessed the outbreak risk from new introductions by using different assumptions for transmission control (i.e., immediate and delayed). Results showed that, even in countries that can quickly limit expected number of transmissions per case to <1, the probability that a single introduction will lead to a substantial number of transmissions is not negligible, particularly if transmission variability is high. Identifying incoming infected travelers before symptom onset can decrease worst-case outbreak sizes more than reducing transmissions from patients with locally acquired cases, but performing both actions can have a synergistic effect.

The ongoing Ebola outbreak in West Africa, thought to have begun from a single index case in Guinea in December 2013 (*1*), has produced thousands of cases in Guinea, Sierra Leone, and Liberia (*2*). This Ebola outbreak is the largest and most widespread since the Ebola virus was discovered in 1976 (*3*), and the probability of international spread outside of West Africa is not negligible (*4*). By late April 2015, the virus had been introduced by 7 infected people traveling during their incubation or symptomatic periods to a country other than Guinea, Sierra Leone, or Liberia. Of these 7 cases, 1 led to an outbreak with 19 transmissions in Nigeria (5,6); 1 led to 2 transmissions in the United States (*7*,*8*); 1 led to 7 transmissions in Mali (*9*,*10*); and 4 led to no transmissions in Mali (*11*), Senegal (*12*), the United States (*13*), and the United Kingdom (*14*). Additionally, 20 persons who acquired infection in Africa were transferred to the United States and several European countries for treatment (*15*), leading to 1 transmission in Spain (*16*).

Although none of these introductions led to a long chain of transmissions, even a small outbreak in a new country can cause societal disruption and disproportionate costs (*17*). Furthermore, how likely it is that an introduced case will lead to a substantial number of transmissions is unclear, even in settings with a quick and vigorous public health response to new outbreaks. Gomes et al. (*4*) performed simulations of Ebola outbreaks in each of 220 countries by first estimating the risk of Ebola being exported from Guinea, Liberia, or Sierra Leone by international travelers and then simulating a stochastic Ebola transmission model conditioned on an importation. The model incorporated Ebola transmission from infected persons in the community and hospital settings and from recently deceased Ebola patients. Assumptions used in the model were that only community transmissions are relevant outside of Africa and that transmissions occur at rates corresponding to containment measures already in place. Gomes et al. provided no explicitly numerical probabilities of large outbreaks per importation, but their simulations apparently produced <100 cases in each country.

In another study, Rainisch et al. (*18*) calculated the estimated number of beds required to treat Ebola patients in the United States by using estimates of importation frequency and subsequent transmission. These researchers reported a high estimate of 7 beds (95% CI 2–13) required at any 1 time; they also provided no numerical probabilities for their estimates.

In our study, we use a branching process model to estimate the probability distribution of outbreak sizes resulting from the introduction of an Ebola case to a new country where the reproductive number R (i.e., expected number of transmissions per case) would likely be quickly, if not immediately, reduced to <1. In this scenario, theory from subcritical branching processes (*19*), also known as mortal

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branching processes (*20*), guarantees that an outbreak will eventually die out, although perhaps not before a substantial number of transmissions occur. In the modeling literature, outbreaks that die out on their own have been called minor outbreaks (*21*) or stuttering chains (*22*). Such branching process models have been used to estimate transmission parameters in the context of emerging (*22*,*23*) or reemerging (*19*–*21*,*24*) infectious diseases. However, unlike other studies, we used the outbreak final size distribution equations derived from branching process theory to calculate the risk for a large Ebola outbreak under the assumptions of immediate and delayed transmission control after an importation.

Materials and Methods

We first gathered transmission data for all Ebola patients who were documented in the ongoing West Africa outbreak and who spent all or part of their infectious periods in a country other than Guinea, Sierra Leone, or Liberia. We next fit the negative binomial distribution to the transmission data and to various data subsets according to patients' circumstances. We also applied the theory of branching processes with a negative binomial offspring distribution to estimate the probability that new introductions would lead to outbreaks exceeding various sizes. We then organized these estimates under 2 scenarios of transmission control: immediate and delayed. For each scenario, we tested the effects of different levels of variability in transmission.

In the data-gathering step, we compiled information for 56 documented Ebola patients (Table 1) who spent all or part of their infectious period in 1 of 12 countries other than Guinea, Sierra Leone, or Liberia. We broke these data into 3 subgroups: patients who traveled to 1 of the 12 countries during their incubation or symptomatic period, patients deliberately evacuated from West Africa for treatment, and patients who acquired infection in the new country after an introduction of the virus.

We fit the transmission data from patients within subgroups to the negative binomial distribution with mean R and dispersion parameter k, which characterizes individual variation in transmission, including the likelihood of superspreading events (i.e., when infected persons disproportionately transmit the virus to others) (*25*). High values of k produce low variability and a low probability of superspreading; k approaching infinity leads to a Poisson distribution, which arises when all infected persons have an equal expected number of contacts and an equal probability of transmission per contact. The value $k = 1$ corresponds to a geometric distribution, which arises when the duration of the infectious period varies among infected persons according to an exponential distribution, as is generally assumed by differential equation models; otherwise, the infected persons are homogeneous. Values of $k < 1$ produce more highly dispersed models, which occur when infected persons vary substantially in numbers of susceptible contacts or in probabilities of transmission per contact (*25*). Occurrence of high variability leads to a higher probability of superspreading.

We estimated the parameters R and k for each data subset by using the method of moments, which calculates the parameter values that produce the exact mean and variance exhibited by the data (i.e., a single estimate for R and k). We calculated associated confidence intervals by using a bias-corrected percentile method (*26*) on random samples of the data. We also used a Kolmogorov–Smirnov test (*27*) to assess goodness of fit (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/15-0170-Techapp1.pdf).

We then applied theory from branching process models, which use a discrete probability distribution, or offspring distribution, to specify the number of transmissions resulting from each infected person in a chain of transmissions. We used the negative binomial distributions fitted to the Ebola transmission data for the offspring distribution. Because our R estimates differed depending on the data subgroups representing each infected person's circumstances, we examined models representing an immediate control scenario and a delayed control scenario, each with 2 levels of control, for a total of 4 combinations of parameters.

In the immediate control scenario, the initial infected person or persons and any subsequent infected persons transmit infection according to the same distribution with $R<1$. In the delayed control scenario, the initially introduced infected person or persons have a higher expected number of transmissions (R_0, the initial or basic reproductive number) than the number expected to be transmitted from subsequent cases (R_c, the postcontrol reproductive number). The delayed control scenario can occur when an infected traveler arrives in a new country during the incubation or symptomatic period and has contact with others before the person has been identified as infected or when the person is treated in a facility that is not fully prepared or experienced in handling an Ebola patient, but any subsequent cases are identified quickly and handled more effectively by a well prepared facility. For controlled patients in either scenario, we examined 2 different levels of control; these levels are represented by 2 values of the postcontrol reproductive number: 1 derived from the average number of transmissions from evacuated infected persons and 1 from the average number of transmissions from persons who acquired infection locally. Because the k values calculated from the fitting of transmission data ranged widely, we applied a series of 3 test values (k = 0.1, 1, and 10) to each scenario to determine the effect of variability on the outcomes.

Table 1. Characteristics of Ebola case-patients reported outside Guinea, Sierra Leone, and Liberia*

Case-patients	Year and month	Country	Circumstance of infection	No. transmissions
1	2014 Jul	Nigeria	Imported by traveler	13
2–4	2014 Jul–Aug	Nigeria	Locally acquired	1
5–19	2014 Jul–Aug	Nigeria	Locally acquired	0
20	2014 Aug	Nigeria	Locally acquired	3
21–23	2014 Aug	Spain, United Kingdom, Germany	Evacuated	0
24	2014 Aug	Senegal	Imported by traveler	0
25–27	2014 Aug–Sep	United States	Evacuated	0
28	2014 Sep	France	Evacuated	0
29	2014 Sep	United States	Imported by traveler	2
30	2014 Sep	Spain	Evacuated	1
31–32	2014 Oct	United States	Locally acquired	0
33	2014 Oct	Spain	Locally acquired	0
34–35	2014 Oct	Germany	Evacuated	0
36	2014 Oct	Norway	Evacuated	0
37	2014 Oct	United States	Imported by traveler	0
38	2014 Oct	Mali	Imported by traveler	0
39–40	2014 Oct–Nov	United States	Evacuated	0
41	2014 Nov	France	Evacuated	0
42	2014 Nov	Mali	Imported by traveler	5
43–44	2014 Nov	Mali	Locally acquired	1
45–49	2014 Nov	Mali	Locally acquired	0
50–52	2014 Nov	United States, Switzerland, Italy	Evacuated	0
53	2014 Dec	The Netherlands	Evacuated	0
54	2014 Dec	United Kingdom	Imported by traveler	0
55–56	2015 Mar	United Kingdom, United States	Evacuated	0

*These data are published and publicly available as of April 24, 2015. Month/year is when patients were transferred or diagnosed. References by country: Nigeria (*5,6*); Spain (*15,16*); United Kingdom (*14,15*); Senegal (*12*);United States (*7,8,13,15*); Mali (*9–11*); other countries *(15*).

For each scenario and each set of parameter assumptions, the probability distribution of final outbreak sizes according to branching process theory can be calculated (online Technical Appendix) as examples of Lagrangian distributions (*28*,*29*). For each parameter combination, we used these equations to calculate the probability of exceeding given outbreak sizes, up to the size expected to be exceeded with a probability of ≈0.01%. To compare different scenarios, we calculated the probability of exceeding 10 and 100 total transmissions and worst-case outbreak sizes (i.e., the number of transmissions expected to be exceeded after 1% and 0.01% of introductions of Ebola). Although we show only results calculated with the assumption of 1 initial patient, the equations we provide (online Technical Appendix) generalize to any number of initial patients and can be used in situations for which multiple introductions might be of interest.

Results

Certain subgroups of patients within the dataset produced substantially different *R* estimates (Table 2). For patients who traveled to 1 of the 12 countries during their incubation or symptomatic period, we calculated $R = 2.9$ transmissions per patient; for patients deliberately evacuated from West Africa for treatment, $R = 0.05$; for patients who acquired infection in the new country after an introduction, $R = 0.3$. Estimates of k produced wide CIs within subgroups, from values <1, which are consistent with a highly dispersed distribution, to large values, which are consistent with a Poisson distribution.

Assuming $R_0 = 3$, estimated on the basis of traveler-imported cases, and $R_c = 0.3$, estimated on the basis of locally acquired cases, the chance of an outbreak with ≥10 transmissions after a single introduction ranges from ≈7%–13% across the 3 assumed values for k: 0.1, 1, and 10 (Figure 1, panel A). The chance of an outbreak with ≥100 transmissions is negligible (<0.01%) for $k = 10$ and $k = 1$ but rises to ≈0.4% under a high variability assumption of $k = 0.1$ because of increased likelihood of superspreading. We considered the effect of 2 ways to reduce the risk from those results: decreasing R_c to approach the level of control achieved among evacuated patients (*30*) and eliminating

Table 2. Summary of Ebola data and parameter estimates*

Patient group	No.	Transmissions	*R* estimate (90% CI)	*k* estimate (90% CI)
All	56	29	0.5 (0.2–1.0)	0.09 (0.03–0.2)
Traveler	7	19	2.9 (0.6–6.1)	0.4 (0.2–1.3)
Evacuated patient	20	1	0.05 (0–0.1)	∞
Patient with locally acquired Ebola	29	9	0.3 (0.1–0.5)	0.5 (0.2–∞)

*Cases were included if the patient spent any of the infectious period in a country other than Guinea, Liberia, or Sierra Leone. The 56 patients are split into 3 mutually exclusive subgroups, depending on the patients' circumstances. Parameters *R* and *k* of the negative binomial distribution are the reproductive number and dispersion parameter, respectively. Goodness of fit was not rejected by a Kolmogorov–Smirnov test (p>0.6 in all cases).

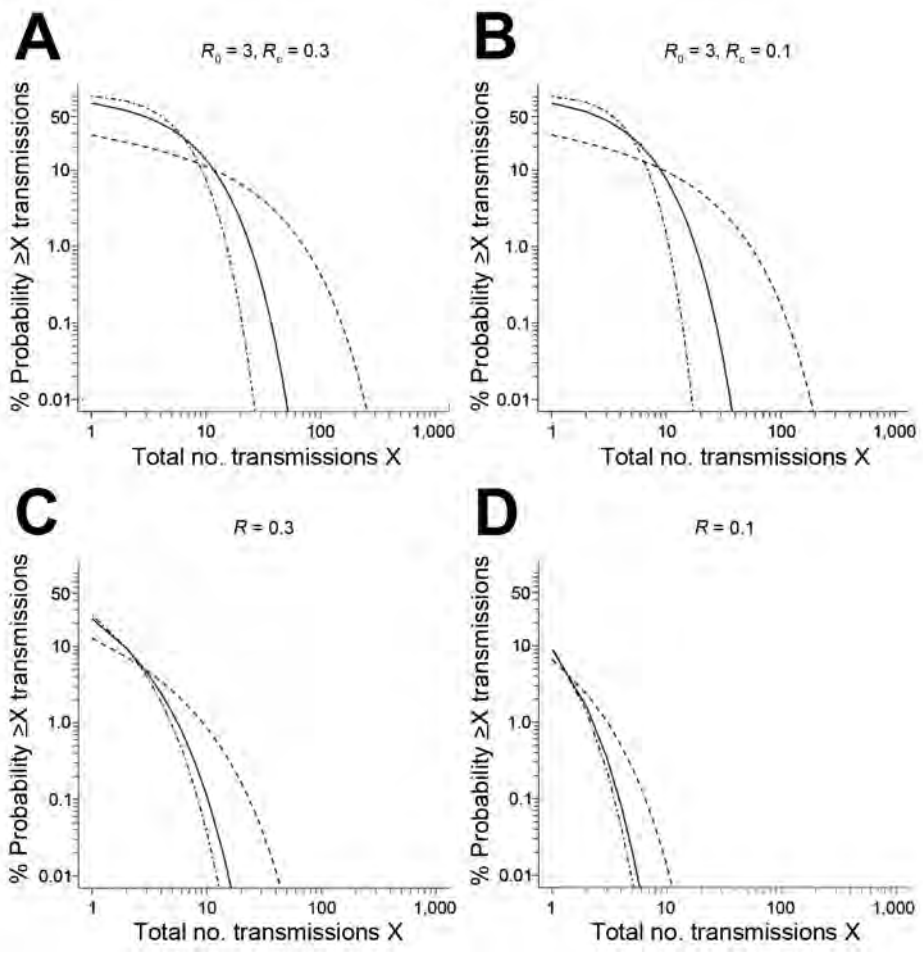

Figure 1. Exceedance risk curves for total number of transmissions in an Ebola outbreak following a single-case introduction. Solid lines, k = 1; dashed lines, k = 0.1; dash-dot lines, k = 10. A) R_0 = 3 for initial case, assumed to be traveler during incubation or symptomatic period; and R_c = 0.3 for subsequent cases, assumed to be locally acquired cases in countries other than Guinea, Sierra Leone, or Liberia. B) R_0 = 3 for initial case, assumed to be patients evacuated for treatment; and R_c = 0.1 for subsequent cases. C) R = 0.3 for all cases. D) R = 0.1 for all cases.

the initial high-average transmission step to reflect preidentification of the initial infected traveler.

When R_0 = 3 but R_c is decreased to 0.1 (Figure 1, panel B), the chance of ≥10 transmissions is 1%–10%. The chance of ≥100 transmissions is <0.01% for k = 10 and k = 1 and 0.2% with high variability. Assuming that the initial patient is identified and that transmission is controlled (R = 0.3) (Figure 1, panel C) causes a much more substantial decrease in outbreak risk, to a range of 0.04%–1% for ≥10 transmissions and <0.01% for ≥100 transmissions, even with high variability. Assuming R = 0.1 for all patients causes ≥10 transmissions to be very unlikely, with a 0.01% chance even with high variability (Figure 1, panel D).

In addition, we compared the 4 scenarios and considered worst-case outbreaks at 2 probability levels: the outbreak level estimated by the model to be exceeded in 1% of introductions (Figure 2, panel A) and the outbreak level estimated by the model to be exceeded in 1 in 10,000 introductions (Figure 2, panel B). The effect of identifying the initial patient is stronger than the effect of reducing R_c, but the combination produces a synergistic effect. For example, at moderate variability (k = 1), the 0.01% worst-case outbreak size when R_0 = 3 and R_c = 0.3 (49 total transmissions) is reduced to 73% of that value (36 total transmissions) when R_c is reduced to 0.1. The worst-case outbreak size is reduced to 31% (15 total transmissions) when the initial patient is identified. Reducing R_c (i.e., postcontrol average number of transmissions per patient) and identifying the initial patient together decrease transmission size to 10% of the worst-case value (5 total transmissions), which is greater than the expected reduction (to 22%) if each intervention was conducted independently. The worst-case risk reduction is greatest under the assumption of high transmission variability (k = 0.1); the 0.01% worst-case outbreak size is reduced from 239 total transmissions to 4% or 10 total transmissions when both intervention assumptions are applied.

We also explored the sensitivity of exceedance probabilities to additional values of R and k (online Technical Appendix Figure) and showed nuances of how higher variability can simultaneously increase the probability of the best-case scenario (no transmissions) and of worst-case scenarios (e.g., superspreading, which can lead to large outbreaks).

Discussion

The outbreak size distributions produced by our models are comparable to those of Gomes et al. (*4*), although their results also encompassed frequencies of case exportations from Guinea, Sierra Leone, or Liberia into each particular country, which our analysis did not include. None of their simulations appears to have produced >100 cases in any particular country, indicating that our scenarios result in more pessimistic outcomes. For example, our delayed-control, high-variability ($k = 0.1$) scenario produced a 0.4% probability of >100 transmissions after a single introduction. The assumed transmission probabilities of Gomes et al. appear to be more comparable to our immediate-control and lower-variability scenarios.

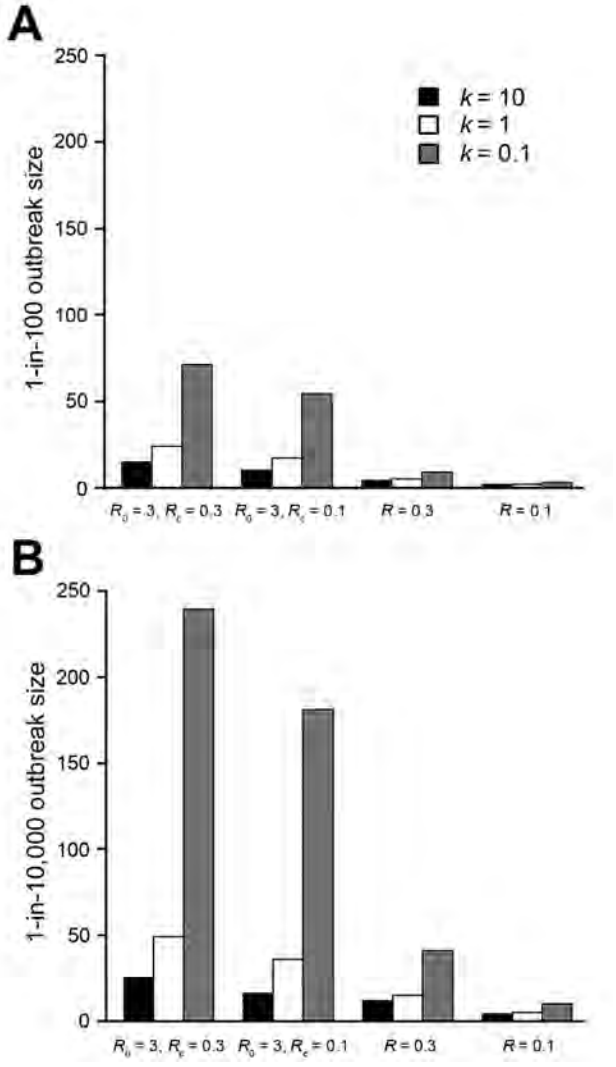

Figure 2. Comparison of worst-case Ebola outbreak sizes after a single-case introduction under different scenarios. Comparisons of the outbreak size expected to be exceeded after A) 1% of introductions and B) 0.01% of introduction of a single initial case, under different assumptions for the reproductive number R and dispersion parameter k. In all cases, higher transmission variability (lower k) leads to higher worst-case estimates. From the $R_0 = 3$, $R_c = 0.3$ case, reducing R_c to 0.1 for cases after the initial case has less effect than reducing the initial case R_0 to 0.3. Reducing both the initial and subsequent cases' R to 0.1 has a synergistic effect.

Our estimates also assign greater potential probabilities of large outbreaks than those provided by Rainisch et al. (*18*), whose highest estimate of the number of beds required at a given time to treat Ebola patients in the United States was 7 (95% CI 2–13). The difference between this result and ours is farther widened because their result includes the possibility of multiple simultaneous introductions caused by a cluster of infected travelers, whereas our results were based on a single introduction. Their worst-case estimate is lower than ours because Rainisch et al. assumed a maximum of 2 additional cases caused by secondary transmission per imported case, whereas our fitted distributions of possible transmissions reach >2, sometimes with substantial probability. For example, in our scenario of a single unidentified traveler's Ebola introduction with $R_0 = 3$ and subsequent cases transmitting with $R_c = 0.3$ and with moderate transmission variability ($k = 1$), our model estimates a 50% chance of >2 transmissions occurring after a single introduction. However, under the assumption of immediate transmission control ($R_c = 0.1$), our model estimates ≤1% chance of >2 transmissions.

The contrasting relationships between the parameter k and different measures of outbreak risk reflect the unpredictable outcome of high variability in transmission: a low frequency of outbreaks after a new introduction but a relatively high probability of an explosive outbreak when an outbreak occurs. This situation was seen in countries experiencing introduced cases of severe acute respiratory syndrome, for which estimated values of k were ≈0.1 (*25*). A recent study (*31*) produced a similar estimate ($k = 0.18$; 95% CI 0.10–0.26) when the negative binomial distribution was fitted to data from large Ebola transmission chains in Guinea (*32*); this result suggests that the high variability assumption may be appropriate, but whether or not the assumption of high variability is an appropriate characterization for potential Ebola outbreaks in new countries is unclear. Attempting to estimate k by using transmission data (Table 1) produced wide ranges of uncertainty. However, the Ebola case that resulted in 13 transmissions in Nigeria suggests that assuming a low value of k, at least for the delayed control scenario, is justified. In Nigeria, the average number of transmissions from those 13 and all subsequent cases was <0.4. This low average number of transmissions was assisted by health authorities' rapid implementation of control measures (*5*). Little evidence exists for high transmission variability from these or other locally acquired or medically evacuated Ebola cases.

Our framework quantitatively characterizes worst-case Ebola outbreaks resulting from an introduced Ebola case to a region with relatively effective control measures. Our results

can be used by public health officials engaging in risk-benefit analyses of potential decisions affecting Ebola case introductions, such as decisions to evacuate infected or potentially infected persons from West Africa, policies on travel surveillance measures, and strategies for handling identified importations. Initial public health assessments at the local level include the risk of importing a case to that geographic area (*4*,*18*,*33*,*34*), the cost versus benefit of identifying potential cases through traveler screening (i.e., in major international ports of arrival), or surveillance in health care facilities (that serve populations at risk from travel or exposure). Either way, the tried and tested methods of early detection and isolation appear to be of primary importance in controlling ongoing Ebola outbreaks in West Africa (*35*) or potential new outbreaks caused by imported cases elsewhere.

Our framework also provides a simple method to quantify the individual and synergistic effects of different control strategies. Our results stress the paramount importance of surveillance measures to identify international travelers who may have been recently exposed to Ebola virus because a higher reproductive number from initially introduced cases can drastically increase the risk of a large outbreak, even if effective control measures are immediately put in place to reduce transmission from subsequent cases. Surveillance, combined with measures to reduce transmission from local cases to the low average achieved among evacuated cases (*30*), can reduce the probability of all but a handful of transmissions to negligible levels.

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Genomic Assays for Identification of Chikungunya Virus in Blood Donors, Puerto Rico, 2014

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A newly developed transcription-mediated amplification assay was used to detect chikungunya virus infection in 3 of 557 asymptomatic donors (0.54%) from Puerto Rico during the 2014–2015 Caribbean epidemic. Viral detection was confirmed by using PCR, microarray, and next-generation sequencing. Molecular clock analysis dated the emergence of the Puerto Rico strains to early 2013.

Chikungunya virus (CHIKV), a mosquitoborne alphavirus, family *Togaviridae*, causes an acute illness, manifested as fever and severe arthralgia (*1*). CHIKV infections are associated with global epidemics, and cases reemerged in the Americas in December 2013 after an ≈200-year absence (*1*,*2*). The initial cases were reported from the island of Saint Martin in the Caribbean, with autochthonous cases reported across 9 islands by April 2014. By March 2015, >1,250,000 suspected or confirmed cases had been reported in the Americas (http://www.paho.org/hq/index.php?Itemid=40931), including ≈3,200 cases in North America, most (89%) in returning travelers.

Risk for transfusion-transmitted infection (TTI) of CHIKV is currently unclear. However, several factors raise concern about possible CHIKV TTI, including a 10%–25% asymptomatic infection rate and high viremic titers in asymptomatic persons (*3*). Recently, a probable TTI case from Ross River virus (RRV), an alphavirus related to CHIKV, was reported in a person who had received RRV-positive donor blood, and a clinically compatible illness developed with subsequent seroconversion (*4*).

The Study

A prototype CHIKV transcription-mediated amplification (TMA) assay was used to screen blood donors from Puerto Rico during the peak of the 2014 Caribbean epidemic (Table). After routine blood donation to the American Red Cross April 4–August 14, 2014, frozen surplus plasma samples from all donors were de-identified and retained for study (all collected during the peak weeks of the 2014 CHIKV outbreak; http://www.salud.gov.pr/Estadisticas-Registros-y-Publicaciones/Pages/Chikungunya.aspx) (Figure 1, panel A). Each retained sample tested negative for pathogens on all required donation screening tests and was also negative for investigational dengue virus (DENV, types 1–4) RNA by TMA (*8*). Passive reporting was encouraged by use of a donor information sheet describing signs/symptoms of DENV and CHIKV infection. No donor reported any symptoms of arbovirus infection from the time of collection through 12 days following donation. The 557 samples were screened with a candidate screening real-time TMA CHIKV assay with a 95% limit of detection of 16.27 RNA copies/mL (95% CI 11.10–29.56 copies/mL) on the high-throughput automated Panther system (Hologic, Inc., San Diego, CA, USA). Each sample was tested in singlet; reactive samples were diluted 1:16 and logarithmically from 10^{-2} to 10^{-8} and retested in triplicate. Three samples (0.54%) were CHIKV RNA–reactive by TMA, with estimated viral loads ranging from 2.9×10^5 to 9.1×10^7 copies/mL (Table). One sample corresponded to a donor who had a confirmed diagnosis of CHIKV infection when contacted after the 12-day reporting period (7.6×10^5 copies/mL); the other 2 donors remained asymptomatic.

For confirmation, we performed blinded orthogonal panviral microarray (ViroChip, University of California San Francisco, San Francisco, CA, USA) and PCR testing of 6 samples, the 3 positive for CHIKV and 3 randomly selected negative controls. (ViroChip is a DNA-detection microarray containing 57,444 probes, and the latest version (v. 5.0) represents all viruses in GenBank as of December 2010 [*9*]). Nucleic acid extraction was performed from 400 μL of TRIzol-inactivated donor serum by using the Direct-zol RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA), and on-column treatment was performed with Turbo DNase (Life Technologies, Carlsbad, CA, USA). After microarray processing, ViroChip hybridization patterns were analyzed by using hierarchical clustering and z-score analysis (*6*). Each of the 3 TMA-positive samples was positive for CHIKV by ViroChip by one or both analysis methods (Figure 1, panel B), whereas all 3 controls tested negative by ViroChip. Given the presence of sparse cross-hybridization artifacts in

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Table. Asymptomatic blood donors testing positive for CHIKV infection, Puerto Rico, 2014*

Collection date, 2014	Initial testing, undiluted	Prototype CHIKV real-time assay on Panther system, dilution†‡ Confirmatory testing 1:16	1:100	1:1,000	$1:10^4$	$1:10^5$	$1:10^6$	$1:10^7$	$1:10^8$
Jul 15									
Reactive/total no. tested	1/1	3/3	3/3	3/3	3/3	2/3	0/3	NT	NT
Estimated copies/mL				2.9×10^5					
Jul 16§									
Reactive/total no. tested	1/1	3/3	3/3	3/3	3/3	3/3	2/3	NT	NT
Estimated copies/mL				7.6×10^5					
Aug 14									
Reactive/total no. tested	1/1	3/3	3/3	2/2	3/3	3/3	3/3	3/3	3/3
Estimated copies/mL				9.1×10^7					

*CHIKV, chikungunya virus; NT, not tested.
†For the real-time CHIKV, transcription-mediated amplification assay, plasma samples (0.5 mL) were tested on the fully automated Panther system which performs magnetic target specific capture, amplification, and real-time detection in the presence of an internal control. During the target capture step, the hybridized target is captured onto magnetic micro-particles that are separated from the specimen in a magnetic field. Wash steps remove extraneous components from the reaction tube. Target amplification occurs by using 2 enzymes, MMLV (Moloney murine leukemia virus) reverse transcription and T7 RNA polymerase. Detection is achieved using single-stranded fluorescent labeled nucleic acid probes that are present during the amplification of the target. The time for the fluorescent signal to reach a specified threshold is proportional to the starting CHIKV RNA concentration. The primers, detection probes, and target capture oligonucleotides hybridize to highly conserved regions of CHIKV RNA genome and were designed to detect all 3 major CHIKV lineages. The cutoff for reactive reactions was set by the investigators at 1,000 relative fluorescent units. Estimated copies per mL were calculated relative to the emergence time of the emitted fluorescence of a calibration curve generated by logarithmic dilution of a CHIKV in vitro synthetized transcript.
‡Dilutions were performed in defribrinated, pooled plasma, passed through a 0.2-μm filter, dialyzed to approximate a human serum profile, delipidated for clarity/stability, and prescreened as nonreactive for CHIKV.
§CHIKV-positive donor retrospectively reported postdonation fever and joint pain at 2 d postdonation.

individual microarray probes (Figure 1, panel B), we further tested the samples using a previously reported CHIKV PCR assay (*7*), which generated results 100% concordant with those of TMA (Figure 1, panel C).

We then used unbiased metagenomic next-generation sequencing (NGS) (*9*) as a pan-pathogen screen and to recover the viral genome from the 3 CHIKV-positive samples (Figure 1, panel D). NGS libraries were constructed by using the Nextera XT kit (Illumina, San Diego, CA, USA) and validated as described (*10*), followed by 161-bp, single-end sequencing on an Illumina MiSeq instrument. Raw NGS data (3.2–32.4 million reads per sample) were analyzed for reads corresponding to known pathogens by using the sequence-based ultrarapid pathogen identification (SURPI) computational pipeline (*10*). After computational subtraction of human host reads, alignment was performed against all microbial sequences in the National Center for Biotechnology Information nucleotide database and the best hit selected on the basis of percentage of mapped read coverage and pairwise identity. A Caribbean strain of CHIKV from the British Virgin Islands (accession no. KJ451624) (*11*) was identified by SURPI as the closest matching viral genome; 95%–100% genome coverage was obtained for the 3 CHIKV-positive donors (Figure 1, panel D). Phylogenetic analysis of the 3 Puerto Rico CHIKV genomes, together with all 188 publicly available sequenced CHIKV genomes in the reference database, placed the Puerto Rico strains in the Caribbean clade (Figure 2, panels A, B). Molecular clock analysis revealed that this clade, an offshoot of the Southeast Asian/Pacific lineage, possibly recently emerged in the Western Hemisphere in early 2013, with the 3 Puerto Rico viruses diverging from the other Caribbean strains 1.7 years ago (Figure 2, panel C).

Conclusions

We employed several orthogonal genomic-based assays to detect CHIKV infection by real-time TMA testing in 3 asymptomatic donors during the peak of the 2014–2015 Caribbean epidemic (*1*,*2*). We confirmed this finding using specific PCR, microarray, and NGS analyses (*10*) and tracked the emergence of CHIKV in the Western Hemisphere to early 2013 by NGS-based whole-genome sequencing and molecular clock analysis. The rate of CHIKV positivity in donors from Puerto Rico (3/557, 0.54%) is slightly higher than that previously reported in donors from the French West Indies (4/2,149, 0.2%; p = 0.16 by Fisher 2-tailed exact test) (*14*). In that study, 2 of 4 CHIKV-positive donors had febrile illness 12–24 hours postdonation, whereas fever and joint pains developed in 1 of 3 CHIKV-positive donors in our study. The level of viral RNA in the Puerto Rico donor from with the highest titer, 9.1×10^7 copies/mL, who remained asymptomatic, is comparable to the median viral titer observed previously in symptomatic CHIKV patients (5.6×10^5 PFU/mL, or $\approx 5.6 \times 10^7$ copies/mL) (*3*). No cases of CHIKV-associated TTI have been confirmed to date, although potential transmission by that route of related alphaviruses such as RRV has been documented (*4*). Nevertheless, our results indicate that high-titer asymptomatic CHIKV infections, if transmissible by transfusion, can readily elude routine screening based solely on postdonation reporting of febrile illness.

New genomic-based technologies have utility for outbreak investigation, bloodborne pathogen screening, and

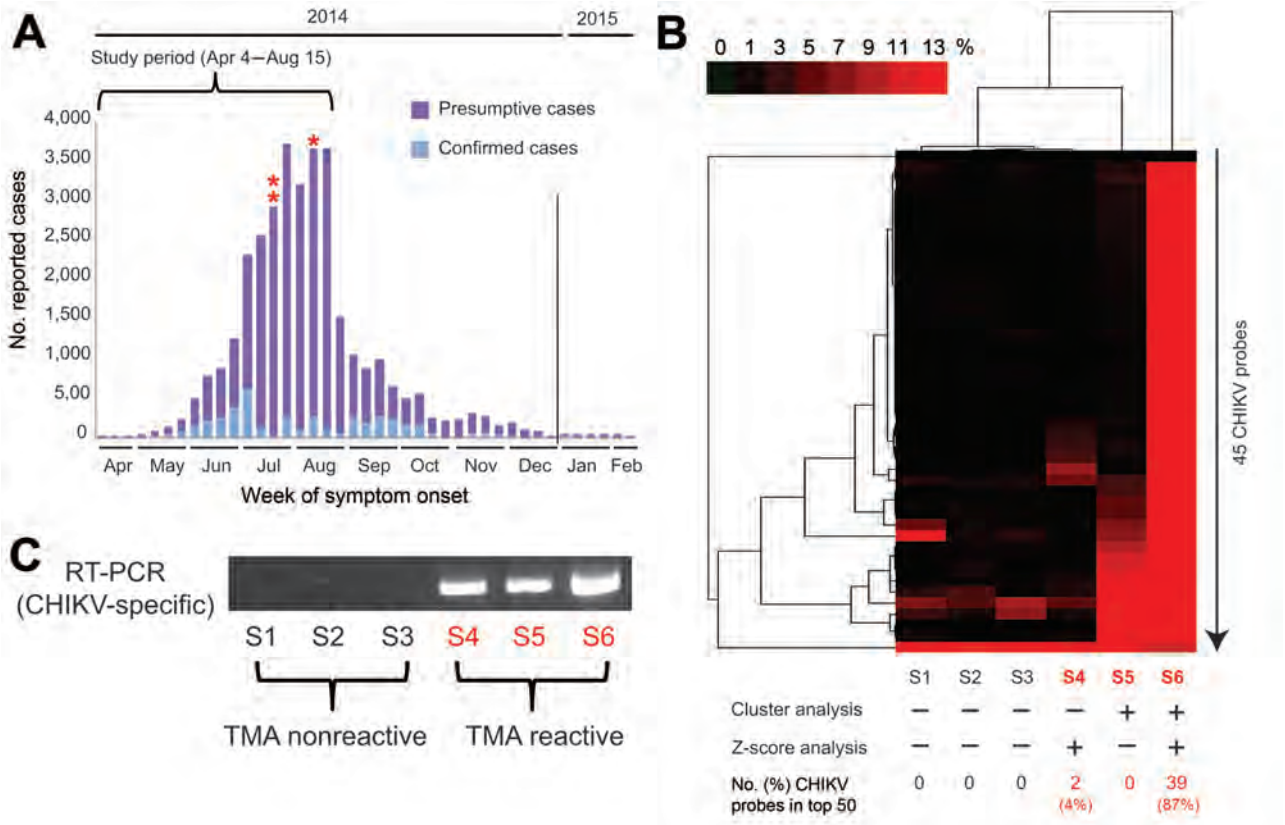

Figure 1. New genomic tests for chikungunya (CHIKV) infection in blood donors. A) Epidemic curve of reported cases in Puerto Rico, April 2014–February 2015. For 2014, 30,983 presumptive cases and 4,275 laboratory-confirmed cases were reported to the Secretary of Health in Puerto Rico. Three CHIKV-positive case-patients (asterisks) of 557 tested were identified by transcription-mediated amplification (TMA) screening of plasma samples during the study period. B) Heat map (cluster analysis) of 6 ViroChip (University of California San Francisco, San Francisco, CA, USA) microarrays corresponding to 6 donor plasma samples, 3 CHIKV positive and 3 CHIKV negative. Only microarray probes derived from CHIKV are plotted because signatures for other bloodborne viral pathogens were absent (data not shown). A sample is called ViroChip positive for CHIKV if at least 10% of the CHIKV probes on the heat map have a normalized probe intensity of >10% by cluster analysis (*5*) and/or if >1 probe is detected within the top 50 by z score analysis (*6*). Red bar denotes the magnitude of hybridization intensity normalized across the 45 CHIKV probes on the microarray. ViroChip microarray data have been submitted to the Gene Expression Omnibus database repository (accession no. GSE67234). C) Reverse transcription PCR (RT-PCR) testing for CHIKV and visualization of the PCR amplicon by 2% agarose gel electrophoresis confirm the transcription-mediated and ViroChip microarray results (*7*). A complete version of this figure, including panel D, showing coverage plots of mapped NGS reads to the "best hit" viral genome, is found online at http://wwwnc.cdc.gov/EID/article/21/8/15-0458-F1.htm. The consensus whole-genome sequences obtained from the coverage plots are used for the subsequent phylogenetic and molecular clock analyses (Figure 2). NGS reads with human sequences removed have been deposited in the Sequence Read Archive (BioProject accession no. PRJNA282046; SRP accession no. SRP057614). The 3 CHIKV genome sequences have been deposited in GenBank (accession nos. KR264949–KR264951).

disease surveillance (*9*). The availability of a high-throughput TMA assay will facilitate screening for CHIKV and more precisely establish the risk of transfusion-associated transmission. Panviral microarrays are useful for broad surveillance of bloodborne pathogens (*9*), yet rigorous individual probe validation across multiple targets is needed because of potential cross-hybridization artifacts. Metagenomic NGS (*9,10*) is an unbiased diagnostic method that identifies all potential pathogens simultaneously on the basis of uniquely identifying DNA sequences. In our study, metagenomic NGS and SURPI analysis facilitated rapid identification and whole-genome recovery of 3 Puerto Rican CHIKV strains directly from primary samples without the need for viral culture.

Recovery from CHIKV infection appears to confer lifelong immunity, and thus an unknown but potentially large fraction of the population of the Puerto Rico may be immune. However, the ongoing threat to returning travelers and spread of the mosquito vector to immunologically naive populations (e.g., in United States and Mexico) underscore the need for continual donor surveillance (*15*). Increased use of microarrays and NGS in the future would be anticipated, given its suitability for detecting threats from multiple emerging vector-borne diseases such as chikungunya and dengue (*2*).

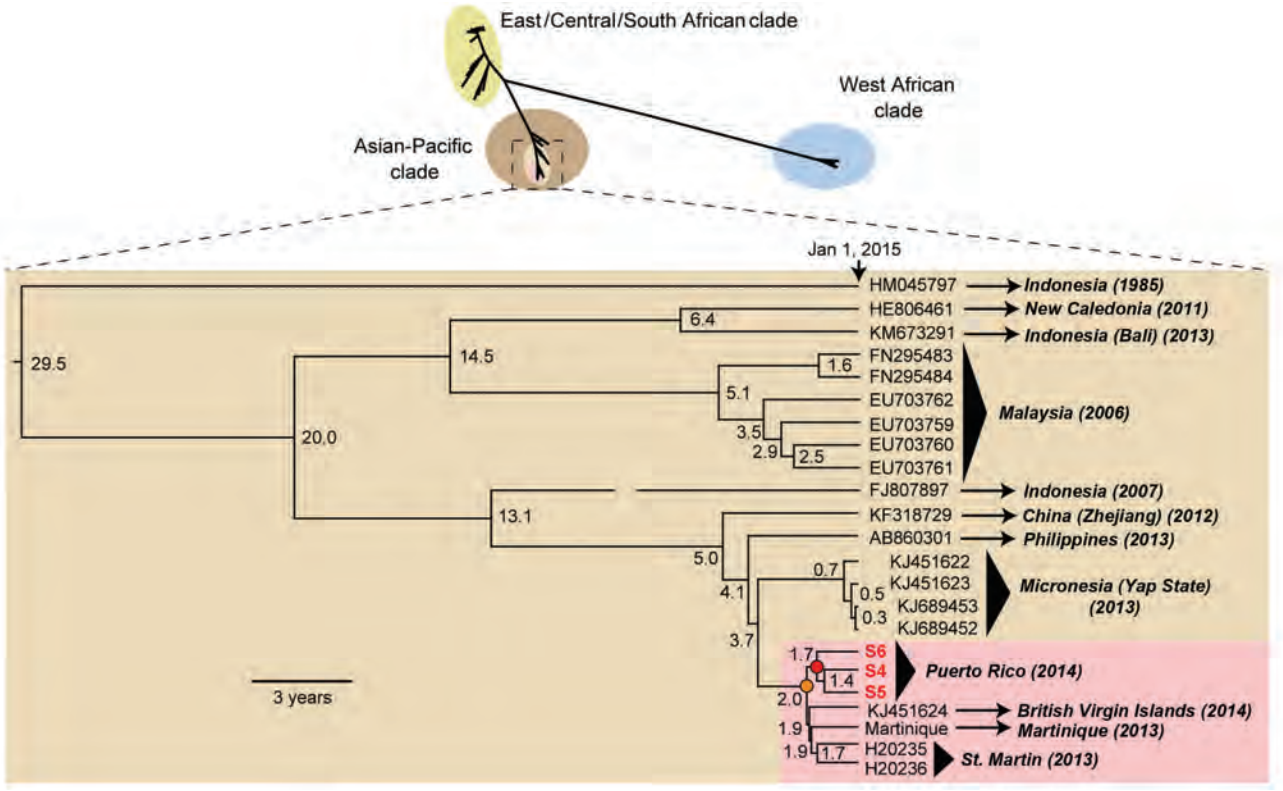

Figure 2. Phylogeny of chikungunya virus (CHIKV). (Upper panel) All 188 nearly-full or full genome CHIKV sequences available in the National Center for Biotechnology Information nucleotide database as of March 2015, including the 3 new genomes from Puerto Rico recovered in this study (red boldface) were aligned by using the multiple alignment fast Fourier transform (MAFFT) algorithm, and phylogenetic trees were constructed by using the MrBayes algorithm in the Geneious software package (*12*). Branch lengths are drawn proportionally to the number of nucleotide substitutions per position, and support values are shown for each node. (Lower panel) Molecular clock analysis of the Southeast Asian/Pacific branch containing the Caribbean sublineage (pink) was performed by using BEAST software (*13*). Branch lengths are drawn proportionally to the number of years before January 1, 2015, and the number of years is shown for each node. The 3 major lineages and Caribbean-associated sublineage are shown in different colors, and the nodes corresponding to the Caribbean (orange) and Puerto Rico (red) offshoots are highlighted.

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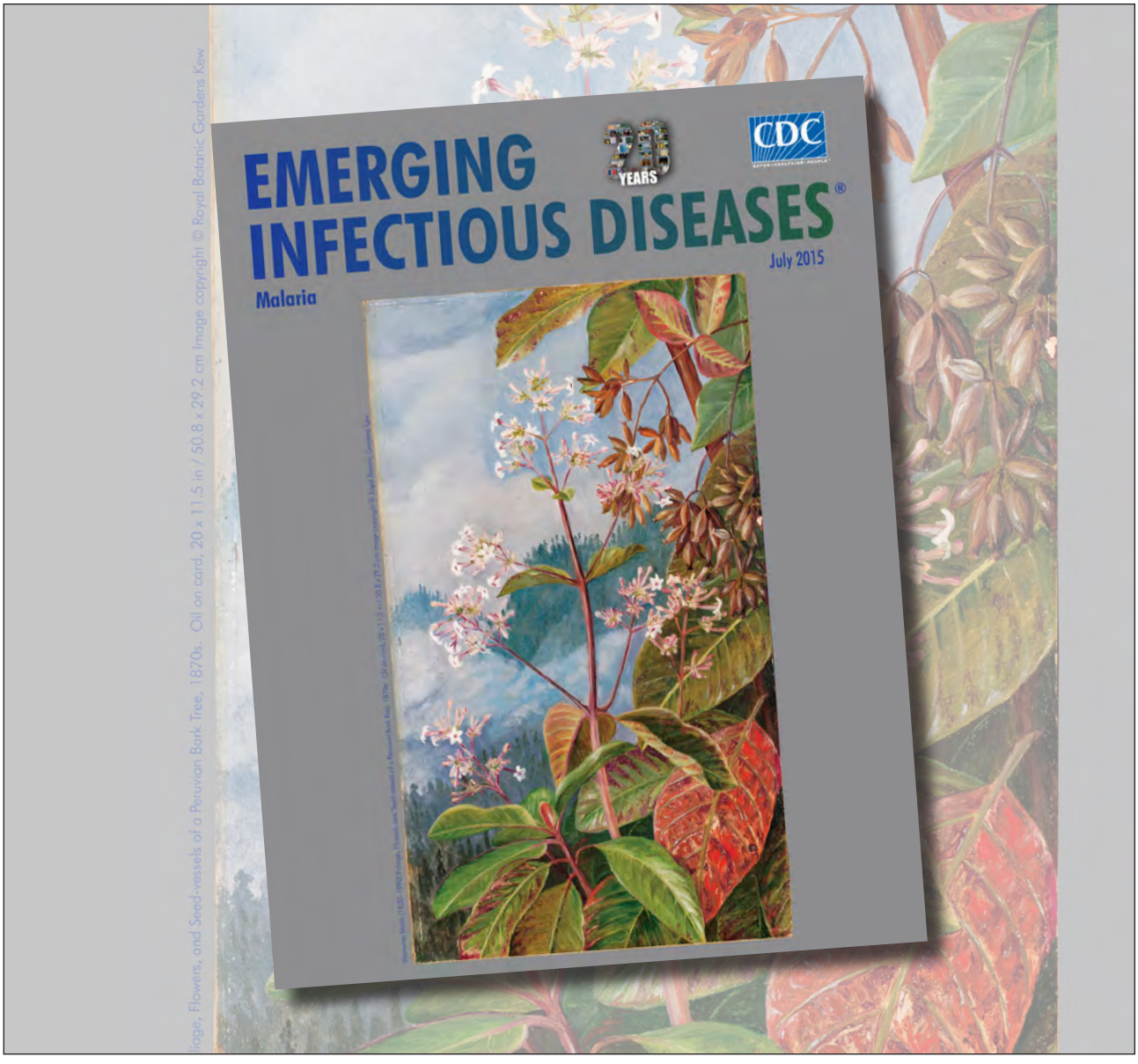

Seasonal Patterns of Buruli Ulcer Incidence, Central Africa, 2002–2012

Jordi Landier, Guillaume Constantin de Magny, Andres Garchitorena, Jean-François Guégan, Jean Gaudart, Laurent Marsollier, Philippe Le Gall, Tamara Giles-Vernick, Sara Eyangoh, Arnaud Fontanet, Gaëtan Texier

To determine when risk for Buruli ulcer is highest, we examined seasonal patterns in a highly disease-endemic area of Cameroon during 2002–2012. Cases peaked in March, suggesting that risk is highest during the high rainy season. During and after this season, populations should increase protective behaviors, and case detection efforts should be intensified.

Buruli ulcer (BU) is a severe infection caused by *Mycobacterium ulcerans*. Most affected are rural populations living in tropical areas with abundant wetlands (*1*). BU causes extensive, damaging skin lesions and often results in severe disabilities. Of the 4,000 cases reported to the World Health Organization by 14 countries in 2011, >95% originated in African countries around the Gulf of Guinea (*2*).

Much remains unknown about the mode of *M. ulcerans* transmission and the epidemiology of BU (*1*). Specifically, although the spatial distribution of BU in several settings has been addressed (*3*,*4*), most studies have examined only temporal variations of BU incidence in terms of yearly trends (*5*). Several observational studies have reported seasonal changes in the monthly number of cases and have hypothesized that cases are linked with rainfall variation (*6*–*8*). One spatiotemporal study in Australia showed that BU incidence was associated with rainfall variability with a 5-month lag and with total rainfall with a 19-month lag (*4*). However, none of these studies provided quantitative evidence of seasonal changes in BU incidence and their relationship with seasonal environmental changes. Indeed, a formal demonstration of such evidence requires a sufficiently long time series, large numbers of cases from a defined source population, and use of signal analysis techniques adapted to the constraints of BU disease surveillance and environmental data (*9*) (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-1336-Techapp1.pdf). Therefore, we investigated the seasonality of BU case incidence during 2002–2012 in Akonolinga District, located in the highly BU-endemic region of the Nyong River valley in Centre Region, Cameroon.

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The Study

Relying on previous spatial analysis of BU incidence in Akonolinga District, we analyzed a series of cases that occurred in the highest BU-risk area of the district, located along the Nyong River upstream of Akonolinga (*3*). This area includes 24,469 inhabitants of the town of Akonolinga and 24 surrounding villages. We analyzed 562 new cases of BU that originated in this area from January 2002 through May 2012, after aggregation by month of diagnosis. Biological confirmation was obtained from the National Reference Centre for Mycobacteria for 354 (63%) cases. The BU incidence rate remained stable over the 10-year period at 2.2 cases/1,000 person-years.

Median BU incidence peaked in March, and a second peak occurred in September (online Technical Appendix Figure 2), but monthly medians did not differ significantly (Kruskal-Wallis test, $p = 0.149$). Given the specificities (nonstationarity) of the BU case series, wavelet analysis was the appropriate method for analysis (online Technical Appendix). A 1-year periodic signal was identified in the BU-case time series from 2005 to 2011, and this periodicity was statistically significant from mid-2005 to the beginning of 2009 (Figure 1).

Next, we analyzed the links between BU and seasonal changes by using wavelet association and phase analyses between BU case incidence and total monthly rainfall (in mm) or mean Nyong River flow (in cubic meters per second). Strong seasonality was found in the series of monthly total rainfall and of monthly mean Nyong River flow; a 1-year period and a weaker 6-month period corresponded to the 2 rainy seasons separated by a period of lesser rainfall (the small dry season, mid-July to mid-August) (Figure 1; online Technical Appendix Figure 3). Because of

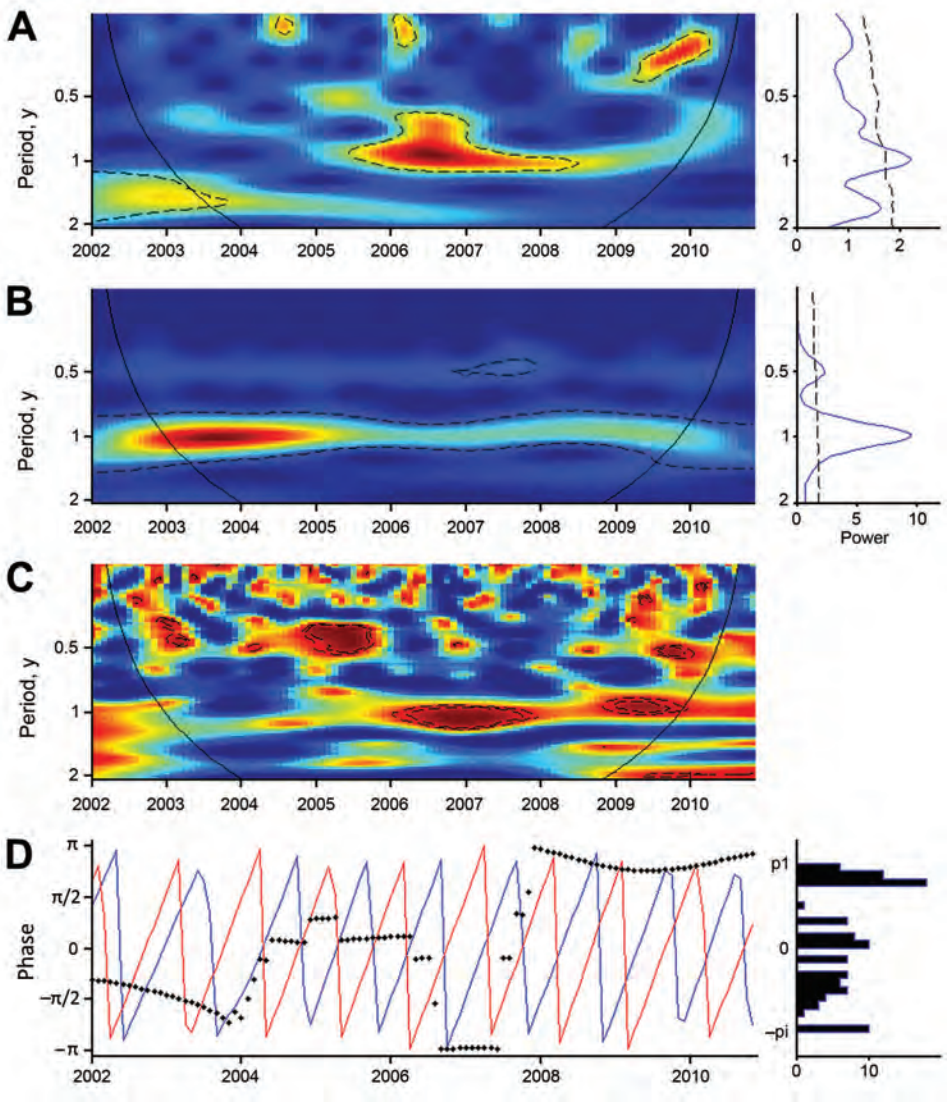

Figure 1. Wavelet analysis of Buruli ulcer (BU) case series and Nyong River flow, January 2002–December 2010. A, B) The color gradient indicates how well the wavelet of a given period adjusted with the series (power). The detection of periodic signals was performed within a confidence cone, which excluded the beginning and the end of the series where edge effects would be too likely (black solid line). Statistically significant zones are circled with dashed lines, indicating detection of significant periodic signals during the corresponding years. A) Wavelet power spectrum for the time series of BU cases: a seasonal signal with a 1-year period was detected from 2005 to 2011 (green to black), and this period was statistically significant from mid-2005 to the beginning of 2009 (dashed contour lines). B) Wavelet power spectrum for the Nyong River flow: the Nyong River flow series exhibits a statistically significant 1-year periodic signal during the whole period. C) Wavelet association between BU incidence and the Nyong River flow signal. The color gradient translates the association between the 2 signals. The dashed lines indicate statistically significant association, and the black line the confidence cone. D) Phase analysis for the 1-year period (expressed in multiples of π); BU cases are represented in blue and Nyong River flow variables in red.

its shape, the wavelet detected yearly rainfall oscillations between a minimum in December (dry season) and a maximum in July (the middle of the rainy period) instead of the maximal rainfall months of October and November (online Technical Appendix Figure 3).

We assessed the association of the incident case signal with environmental variables (online Technical Appendix). The 1-year periodic signal of the BU case series was associated with Nyong River flow from the end of 2005 to the end of 2009 (Figure 1) and with rainfall from the end of 2005 to the beginning of 2011 (online Technical Appendix Figure 3). Under the assumption that changes in the environment preceded changes in BU incidence, phase analysis indicated that cases lagged 6 months behind Nyong River flow oscillations (Figure 1). When the 2 signals were associated, a 9-month lag behind rainfall oscillations was observed (online Technical Appendix Figure 3).

Conclusions

In the BU-endemic focus of Akonolinga, Cameroon, significant 1-year seasonal variations in BU incidence occur. The incubation period for BU has been estimated to be ≈4.5 months when data from Australia are used (*10*) and ≈3 months when data from Uganda are used (*7*). The median delay between symptom onset and health care seeking was reported to be 5 weeks in Akonolinga (interquartile range 3–12 weeks), yielding a delay between infection and diagnosis of 5–6 months. Given this delay and a finding of BU diagnosis peaks during March–April, the number of infections would therefore be highest from August through October (Figure 2). Such a pattern was observed in the 1970s in Uganda (*6*,*7*) and Cameroon (*11*) and more recently in Côte d'Ivoire (*5*). In low BU-endemicity French Guiana, an overseas territory located near Brazil at the same latitude as Cameroon, periodic peaks after the 2 rainy seasons have been reported (*12*).

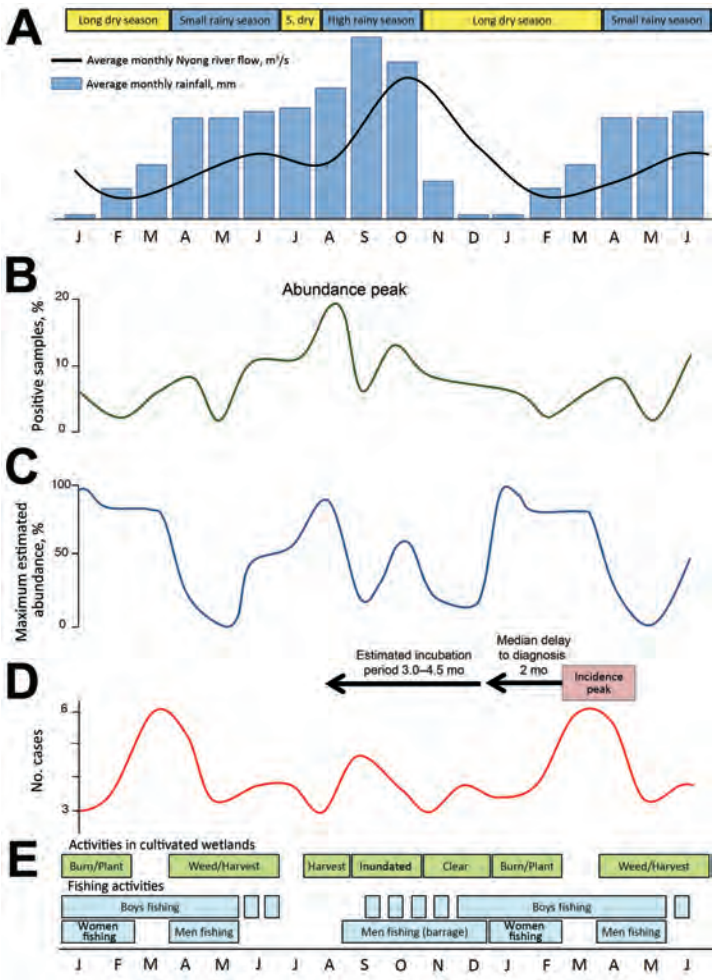

Figure 2. Schematic representation of the seasonal changes and possible links between the environment, *Mycobacterium ulcerans* presence, human exposure, and Buruli ulcer (BU) incidence in the Akonolinga district and the Nyong River valley, Cameroon, 2002–2012. For better visualization of delays, 18 months are shown. A) Average monthly rainfall and mean Nyong River flow (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-1336-Techapp1.pdf). S dry, short dry season. B) *M. ulcerans* prevalence in the aquatic environment (percentage of *M. ulcerans*–positive samples) (*14*). C) Estimated abundance of *M. ulcerans*–positive hemipterans (expressed as % of maximum abundance) (*14*). D) Monthly median number of BU cases detected in the Akonolinga district, 2002–2012 (this study). E) Selected activities involving contacts with environments in which risk for BU is high (T. Giles-Vernick, pers. comm., 2015).

Variations in BU incidence result from variations in population exposure (*13*) combined with variations in environmental presence of *M. ulcerans* (*14*). We hypothesize that one of the main drivers of these variations is the seasonal flooding of the Nyong River, which rises 3–5 m from April through November, creating temporary bodies of water and swamps on a vast surface, deeply affecting the ecosystem.

Although *M. ulcerans* was identified year-round in specific environments such as permanent swamps, its presence and abundance were maximal during the rainy months, July–October (*14*) (Figure 2). Prevalence of *M. ulcerans* in rivers was high at the beginning of the rainy season and was high in flooded areas during the following small dry season and high rainy season (*14*).

Human activity patterns follow these seasonal changes, resulting in seasonal variations in exposure to *M. ulcerans*. In Uganda, the contribution of permanent swamps to BU risk and the increased risk associated with temporary swamps during the rainy season have been documented (*8*). According to residence, age, and/or sex, the inhabitants of the Akonolinga district face varying exposures to aquatic environments; during the period identified as high risk, populations frequent seasonally flooded environments for water collection, fishing, and harvest of dry season cultures (Figure 2) (*15*).

During the study period, the intensity of the association between BU incidence and rainfall or Nyong River flow varied. The seasonal signal was detected over 5 consecutive years and was strongest when yearly variations in the Nyong River flow were lower (2005, 2006, 2008), which could indicate transient forcing of BU incidence by seasonal phenomena. Assessment of the effects of lower frequency climatic events, such as El Niño Southern Oscillation, is needed. In French Guiana, where BU endemicity is low, such events were shown to affect BU incidence dynamics (*12*).

We showed that BU incidence in this region varies significantly by season and linked these variations to the fluctuations of *M. ulcerans* occurrence in the environment, which are probably driven by the dynamics of freshwater ecosystems of the Nyong River. In Akonolinga, during the

high rainy season when risk for *M. ulcerans* transmission seems to be highest, populations should increase their protective behaviors, and case detection efforts should be intensified in subsequent months to ensure early diagnosis and access to care.

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We thank the staff of the Akonolinga district hospital, who provided diagnoses and delivered treatment to BU patients, and the staff of the Service de Mycobactériologie at Centre Pasteur du Cameroun, who provided laboratory confirmation of diagnoses.

Dr. Landier specializes in infectious diseases epidemiology. While working on his PhD degree at the Institut Pasteur in Paris, he focused on BU epidemiology and *M. ulcerans* transmission in Central Africa.

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Human–Bat Interactions in Rural West Africa

Priscilla Anti, Michael Owusu, Olivia Agbenyega, Augustina Annan, Ebenezer Kofi Badu, Evans Ewald Nkrumah, Marco Tschapka, Samuel Oppong, Yaw Adu-Sarkodie, Christian Drosten

Because some bats host viruses with zoonotic potential, we investigated human–bat interactions in rural Ghana during 2011–2012. Nearly half (46.6%) of respondents regularly visited bat caves; 37.4% had been bitten, scratched, or exposed to bat urine; and 45.6% ate bat meat. Human–bat interactions in rural Ghana are frequent and diverse.

Bats are increasingly being recognized as hosts for pathogens that affect humans and livestock (1). The 2014–2015 outbreak of Ebola virus disease in West Africa demonstrates how human–bat interactions in even remote locations can trigger infection chains that affect global public health and strain the national health care systems in Africa (2). One of the major challenges to preventing bat-related diseases is lack of knowledge about the frequency of, circumstances surrounding, and motivations for human–bat interactions in rural African communities. Only a few quantitative records are available in the scientific literature, and most are not specific for Africa (3).

In Ghana, bats carry potentially zoonotic viruses including lyssa-, corona-, henipa-, and filoviruses (*4*–*6*). Although anecdotal knowledge exists with regard to human contact with bats and bat roosts within rural communities and information about the ubiquitous bush meat trade (*7*), little information is available about the intensity and circumstances of exposure (*8*). We therefore studied the cultural practices, sociodemographic factors, and religious activities that determine human–bat contact in remote rural communities from which new disease outbreaks have repeatedly emerged (*9*). Specifically, we studied the sociocultural association of humans with bats in rural communities in Ghana, focusing on potential routes of virus transmission.

The Study

The study was conducted in 3 communities in Ghana: Kwamang (population 8,000), Forikrom (population 3,800), and Buoyem (population 3,900). Kwamang is part of the Ashanti Province; Buoyem and Forikrom are in Brong Ahafo Province (Figure 1). Ethics approval was obtained from the Committee for Human Research, Publications and Ethics of Komfo Anokye Teaching Hospital and School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi.

In each of the 3 communities, in-depth interviews of local leaders were conducted. Buoyem leaders described an activity called the Yam Festival, a hunting festival during which men took ladders to caves on Wednesday evenings and caught bats as they returned from feeding. These bats were described as fruit bats and thus were possibly *Rousettus aegyptiacus* bats, the species most commonly identified in Buoyem caves. The night's catch was collected by the women; menstruating women were excluded from participation

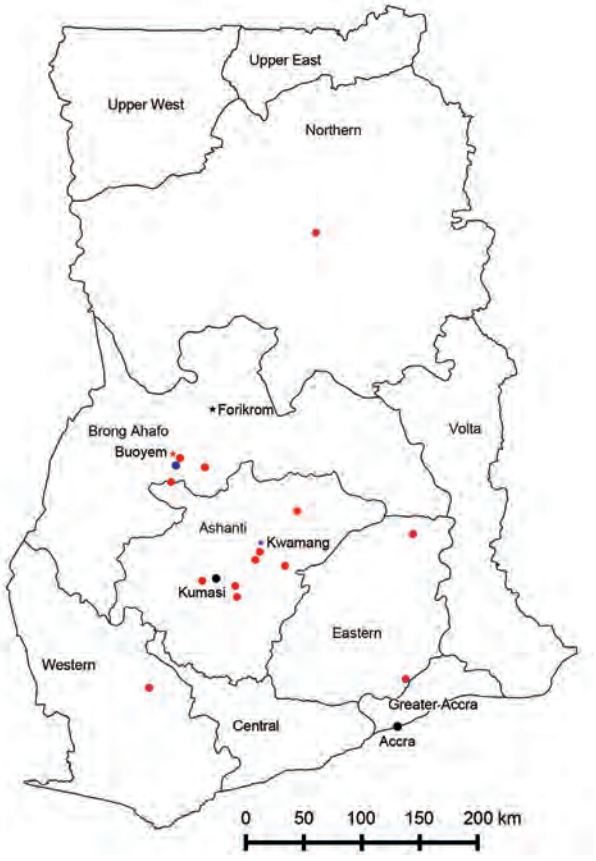

Figure 1. Human–bat interaction study locations and provinces within Ghana, 2011–2012. Asterisks indicate the study sites, Kwamang, Forikrom, and Buoyem. Red circles indicate sources of bush meat. The main Techiman market is situated in the Techiman municipality (blue circle); this market is ≈15 km from Buoyem and is the largest and most economically active market in the Brong Ahafo region. Accra and Kumasi, the largest cities in Ghana, also receive supplies of bat meat from the Techiman market.

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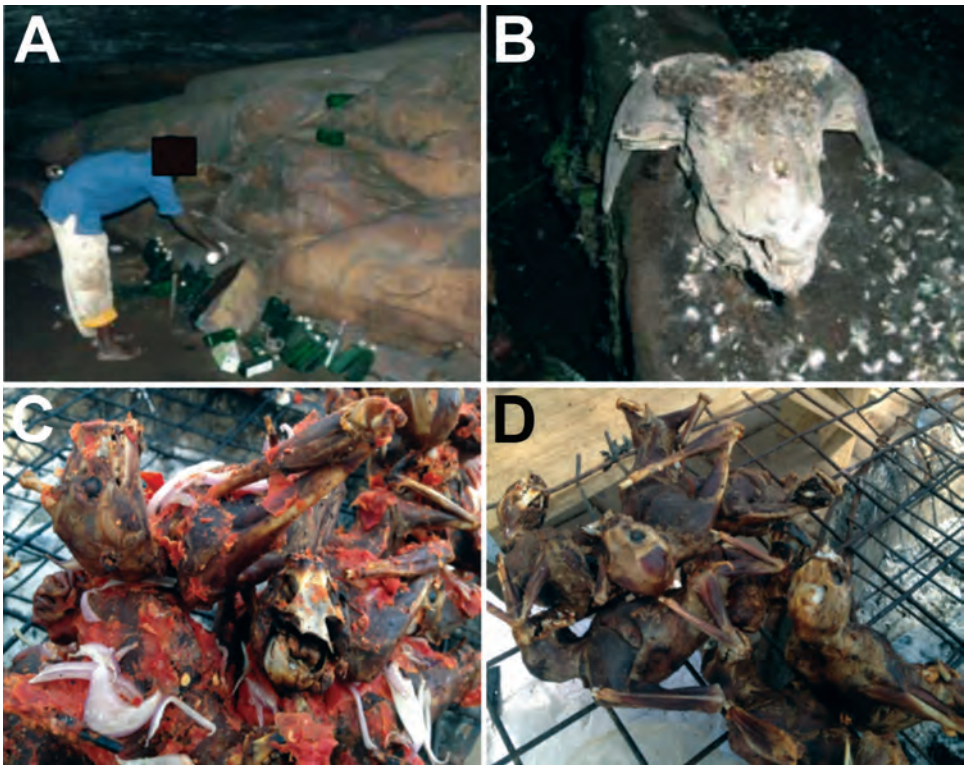

Figure 2. Typical situations in which direct and indirect bat–human contact occurred in Ghana, 2011–2012. A) Religious activity at the Mprisi cave in Buoyem. The man is pouring libation to the natural gods. The liquid poured before entering the cave is liquor. Note the number of deposited empty bottles, indicating the frequency of cave entries. B) Goat sacrificed for natural gods at the Mframmabuom cave in Kwamang. C, D) Typical examples of roasted bats widely offered and consumed in markets and public places in Ghana. Photographs provided by and published with permission from H. Baldwin.

in Yam activities for reasons explained as cleanliness. In recent years, Yam activities had been discontinued because of chieftaincy disputes and conflict over ownership of cave lands. Traditional authorities in Kwamang and Forikrom did not report similar cultural activities in connection with bats.

Regular human activities were directly observed at all cave sites, including the Mprisi (Figure 2, panel A) and Dwamerewa caves in Bouyem, Boten cave in Forikrom, and Mmframabuom and Ohene Abutia caves in Kwamang (Figure 2, panel B). The Ohene Abutia cave served as one of the major water sources in the Kwamang community. Several caves served as spiritual sanctuaries. Focus group discussions were conducted in all communities (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-2015-Techapp1.pdf).

Structured household survey questionnaires were received back from 1,274 respondents: 32.3% from Buoyem, 28.4% from Forikrom, and 39.2% from Kwamang. Contact with bats was reported by 841 (66%) respondents; bat bites, scratches, or urine exposure was reported by 476 (37.4%) respondents. Almost half (594 [46.6%]) of respondents visited bat caves frequently; 217 (17%) reported coming into contact with bats only in their normal living or work environment (Table). The proportion of respondents who deliberately visited caves was significantly higher than the proportion exposed only in their living and work environments ($p<0.001$).

Bat species identification was based on observations and standard illustrated field guides (*10*). Focus group participants identified bats species by using standard images of species recorded from each study site. Observed insectivorous bats included *Nycteris* spp. (Nycteriidae), *Hipposideros jonesi*, *H.* aff. *Ruber*, *H. gigas*, and *H. abae* (Hipposideridae); observed fruit bats included *Hypsignathus monstrosus*, *Rousettus aegyptiacus*, and *Eidolon helvum* (Pteropodidae). These bat species are known to carry coronaviruses (particularly Hipposideridae bats) (*11*); hantaviruses (particularly Nycteridae bats) (*12*); paramyxoviruses, including henipavirus (*13*); and filoviruses (*14*).

Trading of roasted and fried bats was widely observed in market places (Figure 2, panel C, and Figure 1, panel D). Initial information about the supply routes of bat meat obtained from hunters and members of the indigenous community led to investigation of the bat meat trade at the main market in Techiman. Hunters from the surrounding communities supplied most traded bats. Information gathered from traders showed that the supply route of bat meat extends far beyond the Brong Ahafo region to other regions in Ghana and neighboring countries (Figure 1). Some places mentioned by the traders as sources of bat meat include towns and villages in the Ashanti region. Some of these were Duamo (3 km from Kwamang), Adobomam, Kyekyebon, Kumawu, Deduako, Agogo, and the zoological gardens in Kumasi, where migratory *E. helvum* bats roost

Table. Modes of human–bat contact and purposes of cave visitation, Ghana, 2011–2012

Contact	Community, no. (%)		
	Buoyem, n = 412	Forikrom, n = 362	Kwamang, n = 500
Respondents reporting bat contact	263 (63.8)	244 (67.4)	334 (66.8)
In houses through broken ceilings	69 (16.7)	51 (14.1)	65 (13)
In bat roosts on farms	41 (10)	28 (7.7)	63 (12.6)
In caves	129 (31.3)	161 (44.5)	187 (37.5)
At work places	0	1 (0.3)	0
In school buildings	24 (5.8)	3 (0.8)	5 (1)
In other areas	0	0	14 (2.8)
Respondents visiting bat caves	181 (43.9)	178 (49.3)	222 (44.4)
For religious activities	19 (4.6)	79 (21.8)	5 (1)
For recreation	58 (14.1)	73 (20.2)	46 (9.2)
To collect bat guano	0	14 (3.9)	2 (0.4)
To fetch water	1 (0.2)	0	123 (24.6)
To hunt for bats	102 (24.8)	6 (1.7)	10 (2)
To farm	9 (2.2)	17 (4.7)	33 (6.6)
For other reasons	2(0.5)	5 (1.4)	14 (2.8)

*Data based on focus group discussions and stratified household surveys (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-2015-Techapp1.pdf).

seasonally (*13*). Other areas were in Techiman, Nkoranza, Tanoso, and Tuobodom in the Brong Ahafo region; Afram Plains and Akuapem in the Eastern region, and Accra in the Greater Accra region. Some supplies came from the Northern region and beyond the borders of Ghana from Côte d'Ivoire.

Of the 1,274 respondents, 581 (45.6%) reported having consumed bats. Among these, 257 (44.2%) respondents were from Buoyem, 141 (24.2%) from Forikrom, and 183 (31.5%) from Kwamang (online Technical Appendix Table 1). Of the 581 respondents who ate bat meat, 237 (40.8%) obtained bats from caves, 123 (21.1%) caught bats on farms with bat roosts, 114 (19.6) bought bats from community markets, and 60 (10.3%) bought bats from restaurants as part of meals served. Most respondents described the consumed animals as "big bats," suggesting that most were fruit bats (Pteropodidae).

To identify the factors associated with bat consumption, we compared determinant variables for the 581 respondents who consumed bats and the 690 who did not (online Technical Appendix Table 2). Bat meat was eaten by a significantly higher percentage of men than women ($p<0.001$) and a significantly higher proportion of farmers than those with other occupations ($p<0.001$). To determine the variables that significantly influenced the consumption of bat meat, we entered all significant variables into a logistic regression model. The odds of consuming bat meat were higher for men (odds ratio 2.47; 95% CI 1.93–3.17) than for women and for respondents >25 years of age (odds ratio 4.14; 95% CI 2.91–5.89) than for those ≤25 years of age (online Technical Appendix Table 3).

A second multivariate analysis, conducted to determine factors that predict visitation of bat caves, indicated that older age and male sex were significantly associated with visitation of bat caves (online Technical Appendix). The association between cave visitation and bat consumption was significant ($\chi^2 = 75.6$; $p<0.001$); odds of eating bat meat were twice as high among respondents who visited bat caves (odds ratio 2.74) than among those who did not.

Conclusions

The deliberate entry into bat caves represents a prevalent behavior that could be influenced by community-level education in the aftermath of the ongoing outbreak of Ebola virus disease in West Africa. Another obvious target is the widespread bat meat trade and consumption. Further research will be necessary for understanding belief systems and developing acceptable guidance for rural communities exposed to bats because of traditional and spiritual reasons.

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Occupational Exposure to Dromedaries and Risk for MERS-CoV Infection, Qatar, 2013–2014

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We determined the presence of neutralizing antibodies to Middle East respiratory syndrome coronavirus in persons in Qatar with and without dromedary contact. Antibodies were only detected in those with contact, suggesting dromedary exposure as a risk factor for infection. Findings also showed evidence for substantial underestimation of the infection in populations at risk in Qatar.

Since Middle East respiratory syndrome coronavirus (MERS-CoV) was first detected in 2012, approximately 1,000 human infections have been reported to the World Health Organization, all linked to residence in or travel to countries on the Arabian Peninsula (*1*). Dromedaries (*Camelus dromedarius*) are thought to play a central role in MERS epidemiology because widespread evidence of MERS-CoV–specific antibodies and virus shedding in camels was found (*2*), and highly similar viruses have been detected in humans and dromedaries at the same location (*3*,*4*). These data suggest a direct zoonotic risk for MERS-CoV infection among persons in contact with camels. We describe a comparative serologic investigation in Qatar among persons with and without daily occupational exposure to dromedaries.

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The Study

We used 498 anonymized serum samples from persons in Qatar with and without dromedary contact (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/15-0481-Techapp1.pdf) and control serum from Europe (National Institute for Public Health and the Environment, Bilthoven, the Netherlands; and University of Bonn, Bonn, Germany). Sampling in Qatar was cleared by the Ethics and Institutional Animal Care and Use Committees of the Medical Research Center, Hamad Medical Corporation (permit 2014-01-001). Samples from the Netherlands were used in accordance with the Dutch Federation of Medical Scientific Associations' code of conduct for proper use of human tissue. Samples from Germany were used in accordance with German national laws.

Of the 498 samples, 294 were from persons with daily occupational contact with dromedaries (cohorts A–D) and 204 were from persons without camel contact (cohorts E–G). Cohort A consisted of 109 healthy workers (5 camel slaughterers [subcohort A1] and 104 sheep slaughterers [A2]) at the central slaughterhouse in Doha, Qatar. All workers lived together and had contact with camels and sheep at the central animal market (CAM). Cohort B consisted of 8 CAM workers. Cohort C consisted of 22 healthy men living and working at the Al Shahaniya barn complex near the international dromedary racing track, and cohort D consisted of 155 healthy men living and working on a dromedary farm in Dukhan, western Qatar; molecular data showed ongoing circulation of MERS-CoV in dromedaries in these locations (online Technical Appendix). Cohort E consisted of 56 random samples from construction workers in Qatar. Cohort F consisted of 10 samples from persons working and living at a complex with 200 sheep barns in northern Qatar. Cohort G consisted of 138 samples for confirming specificity of the testing algorithm (66 samples from the Netherlands and Germany from persons with recent human CoV infection [subcohort G1] and 72 samples from the Netherlands obtained for routine testing from persons with suspected *Bordetella pertussis* infection [G2]).

We used microarray technology as described (*3*,*5*,*6*) to analyze samples for the presence of IgG reactive with MERS-CoV S1 antigen (Table). To avoid overinterpretation of data, we set the reactivity cutoff at 30,000 relative

[1]These first authors contributed equally to this article.

Table. Results of MERS-CoV serologic testing of humans with and without dromedary contact, Qatar, 2013–2014*

Exposure type, cohort	Country	Serum samples tested by: S1 assay, no. positive/no. tested	Serum samples tested by: $PRNT_{90}$, no. positive/no. tested† S1-positive	Serum samples tested by: $PRNT_{90}$, no. positive/no. tested† S1-negative
Dromedary contact		20/294	10/20	1/35
A, slaughterhouse workers				
A1, camel slaughterers	Qatar	4/5	2/4 (40, 20)	NT
A2, sheep slaughterers (contact with camels/camel slaughterers)	Qatar	3/104	2/3 (20, 20)	1/16 (20)
B, central animal market workers	Qatar	1/8	0	NT
C, barn workers at international camel racing track	Qatar	4/22	3/4 (40, 40, 20)	NT
D, camel farm workers	Qatar	8/155	3/8 (40, 40, 20)	0/19
No dromedary contact		0/204	NA	0/48
E, construction workers	Qatar	0/56	NA	0/48
F, sheep farmers	Qatar	0/10	NA	NT
G, specificity controls				
G1, recent infection with a common hCoV	GER, NL	0/66	NA	NT
G2, suspected infection with *Bordetella pertussis*	NL	0/72	NA	NT

*GER, Germany; hCoV, human coronavirus; MERS-CoV, Middle East respiratory syndrome coronavirus; NA, not applicable; NL, the Netherlands; NT, not tested; $PRNT_{90}$, 90% plaque-reduction neutralization test; S1, MERS-CoV S1 antigen.
†Nos. in parentheses are reciprocal antibody titers in $PRNT_{90}$.

fluorescent units for subsequent analyses (*6*). Samples from 20 of 294 persons with camel contact were reactive; no control or noncontact samples were reactive. Among camel handlers at the Al Shahaniya and Dukhan locations, 4 of 22 and 8 of 155, respectively, had antibodies to MERS-CoV S1. At the CAM, 1 of 8 handlers had antibodies. At the slaughterhouse location, 3 of 104 sheep slaughterers and 4 of 5 camel slaughterers were antibody-positive (Figure).

Samples from subcohort G1 (n = 66) and from all camel-contact cohorts were tested for antibodies to CoV OC43 S1, a common human CoV; all showed high seropositivity (range 89%–100%) (Figure). All 498 samples were tested for reactivity to severe acute respiratory syndrome CoV S1; none reacted (Figure).

We used a 90% plaque-reduction neutralization test ($PRNT_{90}$) to confirm the presence of MERS-CoV–specific antibodies in serum samples from camel handlers. For testing, we used the 20 samples that were reactive to MERS-CoV S1 and a random selection of nonreactive samples from camel-contact (n = 35) and noncontact (n = 48) cohorts. Results were positive for 10 of the 20 MERS-CoV S1 antibody–positive samples (reciprocal titers of 20 or 40) (Table).

All but 1 of the 35 samples from persons with camel contact who had negative S1 ELISA screening results were negative by $PRNT_{90}$; the positive sample had a reciprocal titer of 20 (Table). All 48 samples from the noncontact cohorts were negative by $PRNT_{90}$. This finding may indicate an underestimation of MERS-CoV seroprevalence by S1 testing. Furthermore, 6 samples from S1-positive and 2 from S1-negative persons with camel contact showed a reciprocal titer of 10, but titers of 10 were not observed in the noncontact cohorts. Five of these 8 reactive samples were also positive in a whole-virus MERS-CoV immunofluorescence assay at dilution 1:100; however, we regarded these as negative to avoid overinterpretation of data (data not shown).

Conclusions

We detected MERS-CoV neutralizing antibodies in healthy persons who had daily occupational contact with dromedaries but not in persons without such contact. Only limited evidence is available regarding the presence of MERS-CoV antibodies in the general human population or in specific population cohorts. However, an overall seroprevalence of 0.15% was found in a cross-sectional study in Saudi Arabia, and among slaughterhouse workers, neutralizing antibodies were detected in 5 of 140 participants (*7*). This finding is similar to our finding among slaughterhouse workers: 7 of 109 were MERS-CoV antibody–positive. Four other studies lacked serologic evidence of MERS-CoV infection in humans with occupational exposure to dromedaries (*8*–*11*). However, only 1 of those studies documented actual MERS-CoV circulation in dromedaries during human contact, and it was concluded that MERS-CoV was not highly transmissible from camels to humans, although only 7 persons had regular contact with only 1 herd (*8*). On several occasions, the percentage of camels shedding MERS-CoV was high (60%) at the CAM and slaughterhouse (C.B.E.M. Reusken, unpub. data). Thus, locations with a continuous flow of dromedaries with different places of origin and different immune statuses may enable prolonged circulation of MERS-CoV and sustained exposure of dromedary handlers to the virus; in Qatar, such locations would include the CAM, slaughterhouse, and barns near the international racing tracks.

In this study, $PRNT_{90}$-derived antibody titers were relatively low compared with those from earlier studies of MERS patients and dromedaries (*2*); B.L. Haagmans, unpub. data). The lower titers might reflect the apparent asymptomatic manifestation of MERS-CoV infection, individual differences in susceptibility, or both (*2*). Also, primary infections may result in a short-lived antibody peak followed by a rapid waning of antibody, depending on virus

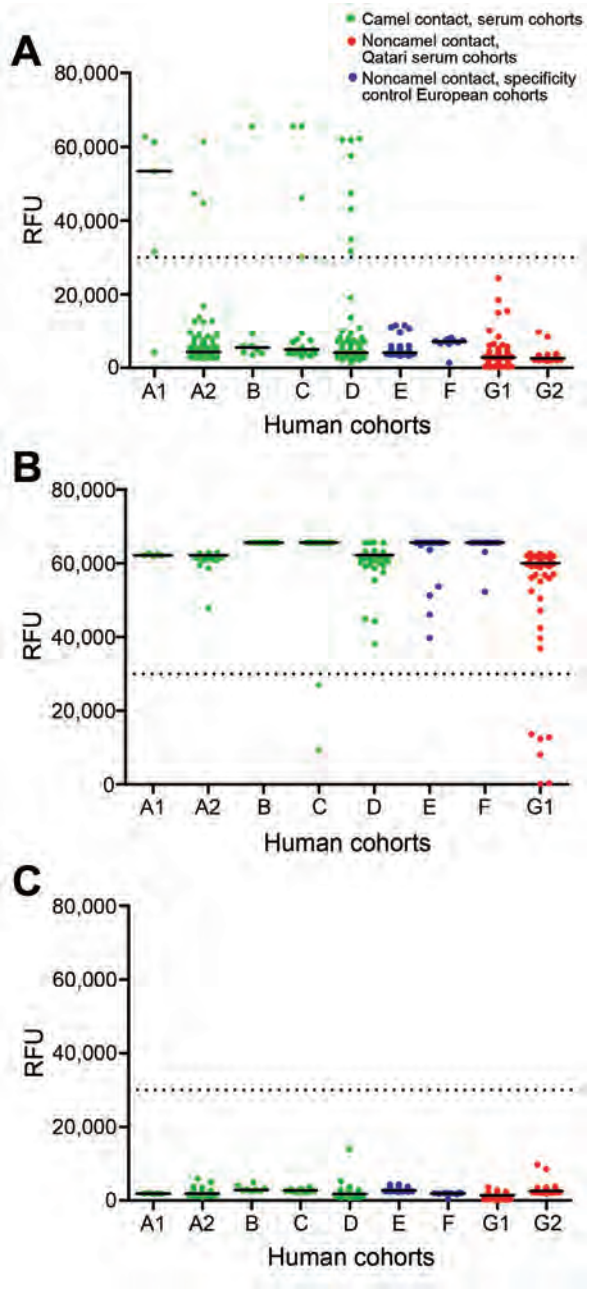

Figure. Reactivity of human serum samples, from persons with and without dromedary contact, with S1 antigens of various coronaviruses (CoVs), Qatar, 2013–2014. A) Middle East respiratory syndrome CoV S1; B) human CoV OC43 S1; C) severe acute respiratory syndrome CoV S1. Relative fluorescent units (RFU) are shown at a serum dilution of 1:20. Black lines indicate median; dotted black lines at 30,000 RFU depict cutoff for analysis. Human cohorts: A1, camel slaughterers; A2, sheep slaughterers who had contact with dromedaries and camel slaughterers; B, workers at the central animal market; C, barn workers at the international camel racing track; D, workers on camel farms; E, construction workers; F, sheep farmers; G1, persons recently infected with a common human CoV (serum samples from the Netherlands and Germany); G2, persons with suspected *Bordetella pertussis* infection (serum samples from the Netherlands).

and host properties (*12*), as seen in influenza A(H5N1) virus infection: antibody levels are higher in symptomatic than asymptomatic H5N1-infected persons, and antibodies wane more quickly during asymptomatic infection (*13*). MERS-CoV antibody kinetics and the persistence of antibodies detected by different serologic methods are not known. Such parameters are needed to estimate the force of infection on the basis of serologic data (*14*).

MERS-CoV–seropositive participants in this study did not report severe health problems, giving evidence for frequent unrecognized human infections. Assuming the health histories are accurate, this finding implies that the current overall MERS-CoV–associated death rate of 37.1% (*1*) is most likely an overestimation of the actual rate and that most infections may be asymptomatic or mild. A major issue to be resolved is whether, and to what extent, asymptomatic cases contribute to the spread of MERS-CoV; it is well recognized that variability in disease transmission exists among humans (*15*).

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Cutaneous *Legionella longbeachae* Infection in Immunosuppressed Woman, United Kingdom

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We report a rare case of cutaneous *Legionella longbeachae* infection in a patient receiving long-term corticosteroids for immune thrombocytopenia. Such infections cannot be identified by using *Legionella* urinary antigen testing but are commonly seen after exposure to commercial potting compost, particularly in immunocompromised patients.

A 70-year-old woman in whom stage A0 chronic lymphocytic leukemia had been diagnosed in 2004 by immunophenotyping was monitored with a watch-and-wait strategy for 8 years until she started treatment for anemia and splenomegaly. She was given 4 cycles of fludarabine, cyclophosphamide, and rituximab until her illness went into clinical remission.

Twelve months later, the woman sought treatment for extensive purpura. She had a platelet count of 1×10^9/mL (reference range 150–400 $\times 10^9$/mL) but an otherwise unremarkable complete blood count, peripheral blood smear, and bone marrow aspirate and trephine biopsy result. Immune thrombocytopenia was diagnosed, and 1 mg/kg of oral prednisolone daily was initiated, to which the illness responded well. Over 8 weeks, steroids were weaned to 20 mg once daily. During this time, the woman had a colonoscopy to investigate 6 weeks of persistent diarrhea. Cultures of fecal samples and multiple colonic biopsies were unremarkable.

During a routine outpatient review in August 2013, three discrete erythematous nodules were found on the ventral surface of her right forearm. The lesions were tender and nonpurulent, contained no punctum, and were distinct and separate from the site where an intravenous cannula had been placed for her colonoscopy. She had no history of tick or animal bite, recent foreign travel, or trauma. Her diarrheal illness had resolved before this review. Cellulitis was presumptively diagnosed, and the woman was started on a course of oral flucloxacillin.

Two weeks later, she was admitted to the hospital with worsening pain and swelling of the right forearm. She remained systemically well. Laboratory testing indicated a leukocyte count of 9.6×10^9 cells/L (reference range 4.0–11.0 $\times 10^9$ cells/L), with neutrophils 8.7×10^9 cells/L (reference range 1.8–7.5 $\times 10^9$ cells/L), lymphocytes 0.4×10^9 cells/L (reference range 1.5–4.0 $\times 10^9$ cells/L), monocytes 0.4×10^9 cells/L (reference range 0.2–1.0 $\times 10^9$ cells/L), eosinophils 0.1×10^9 cells/L (reference range 0–0.4 $\times 10^9$ cells/L), and basophils 0×10^9 cells/L (reference range 0–0.4 $\times 10^9$ cells/L). C-reactive protein level was 17 mg/L. She was treated empirically with intravenous flucloxacillin.

Initial blood culture results were negative. Cultures of wound swab samples were negative for methicillin-resistant *Staphylococcus aureus* but showed evidence of gram-negative bacilli. Results from urinary *Legionella* and pneumococcal antigen tests were negative. Surgical review prompted incision and drainage of the lesions, the largest of which was 4 cm × 5 cm (Figure). Histopathologic examination of the lesions showed only granulation tissues; no malignant cells were seen.

Lack of clinical improvement prompted a change in antimicrobial drug therapy to intravenous tazocin and clindamycin. Results from serial bacterial cultures of blood, feces, and tissue failed to yield further positive results. Results from PCR for herpes simplex virus types 1 and 2 and varicella zoster virus were negative. Serum β-D-glucan was elevated at 262 pg/mL (reference range <80 pg/mL); however, the patient did not receive antifungal treatment because of the lack of clinical suspicion of fungal infection and the test's high false-positive rate.

A dermatology review suggested that the lesions represented a sporotrichoid lymphocutaneous infection, possibly caused by an atypical mycobacterium. Results from an extended culture for mycobacterium, auramine stains for acid-fast bacilli, and further histopathologic staining for fungal hyphae were negative. Despite antimicrobial drug therapy, the lesions persisted for 2 weeks; repeat incision and drainage was performed.

A pus sample was sent to Great Ormond Street Hospital (London, UK) for PCR. Species-specific real-time PCR for *S. aureus* and *S. pyogenes* were negative. Subsequent analysis by broad-range 16S rDNA PCR (*1*) gave a strongly positive result, and the amplicon sequence exactly matched 16S rDNA sequences from 2 *L. longbeachae*–type strains, leading to a diagnosis of cutaneous infection with *L. longbeachae*.

The woman was started on a 6-week course of triple antimicrobial drug therapy with ciprofloxacin, azithromycin, and rifampin. By her 3-month follow-up, her lesions had resolved completely. Although she was not a keen gardener, she reported that, a week before her lesions

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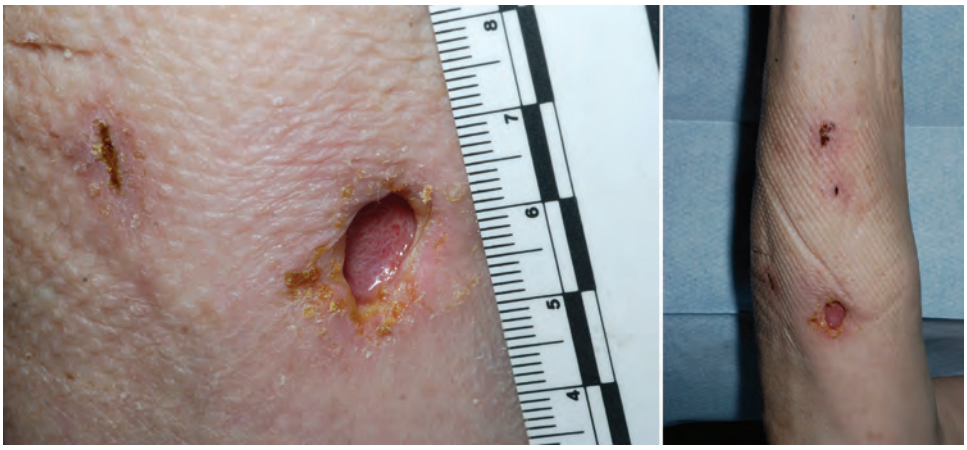

Figure. Forearm lesion after incision and drainage in immunosuppressed woman with cutaneous *Legionella longbeachae* infection, United Kingdom.

appeared, she had handled a leaking potted plant without thoroughly washing her hands afterward.

Legionella longbeachae was first described in Long Beach, California, USA, in 1981; it was isolated from respiratory tract specimens from 4 patients with pneumonia (*2*). A second serogroup was identified later that year (*3*). Reported cases of *L. longbeachae* infection are rare in Europe. However, in New Zealand, Australia, and Japan, they are as common as infections with *L. pneumophila* and often cause legionellosis and Pontiac fever, a nonpneumonic, self-limiting illness characterized by influenza-like symptoms (*4*,*5*). Only testing of urinary antigens can identify *L. pneumophila* serogroup 1, whereas *L. longbeachae* can be detected by serologic testing, culture, or PCR (*6*).

Unlike infections with *L. pneumophila*, which have been linked to water systems in the built (i.e., human-created) environment, infections with *L. longbeachae* are most commonly associated with the use of commercial potting compost (*4*,*7*,*8*). *L. longbeachae* was first isolated from potting mix after an outbreak of infections in South Australia in 1989 (*9*). Since then, outbreaks have been linked to the use of potting compost in Australia, New Zealand, Japan, the Netherlands, and most recently Scotland, where 4 cases of *L. longbeachae* infection were confirmed during 2008–2009 (*4*,*5*,*7*,*10*). A 2013 study of compost in the United Kingdom found that 15 of the 24 samples tested contained *Legionella* species, of which 4 were identified as *L. longbeachae* serogroup 1 (*11*).

A 2006 case–control study (*7*) showed that risk factors that predicted *L. longbeachae* infection included poor hand hygiene after gardening and proximity to dripping hanging flower pots, the latter indicating that ingestion might be an alternate possible route of transmission to aerosolization (*4*,*7*). These risk factors suggest a possible route of infection for the patient reported here; she reported handling a large, leaking potted plant and admitted to poor hand hygiene before meals. However, *L. longbeachae* cannot be confirmed as the source of her infection because her potting soil was not tested. An alternative route of infection would be through the site of the cannula that was inserted for her colonoscopy. Other reported risk factors include smoking, preexisting respiratory disease, and immunosuppression (*4*,*6*,*7*,*12*). The primary host defense mechanism in *L. longbeachae* infection is cell-mediated immunity, depression of which through the use of corticosteroids or immunosuppressive drugs may predispose patients to legionellosis. A 2007 report describes a case of *L. longbeachae* pneumonia after corticosteroid therapy for chronic immune thrombocytopenia (*6*).

The clinical picture in *L. longbeachae* infection is typically similar to that of infection with *L. pneumophila*; however, as in this case, variations have been reported. A 2012 case report described a patient in whom *L. longbeachae* endocarditis developed 6 months after a bioprosthetic aortic valve replacement (*13*). Several cases of cutaneous infection secondary to *Legionella* species have been reported (*14*); 7 of the 13 confirmed cases occurred in immunocompromised patients. Because so many cases occurred in immunocompromised patients, we recommend use of broad-range 16S rDNA PCR to detect *L. longbeachae* in immunosuppressed patients with respiratory or influenza-like symptoms who report a history of exposure to commercial potting compost.

Dr. Grimstead is a Foundation Programme doctor at Torbay Hospital, South Devon Healthcare Foundation Trust. He has a special interest in hematology.

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Bartonella spp. and *Coxiella burnetii* Associated with Community-Acquired, Culture-Negative Endocarditis, Brazil

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We evaluated culture-negative, community-acquired endocarditis by using indirect immunofluorescent assays and molecular analyses for *Bartonella* spp. and *Coxiella burnetii* and found a prevalence of 19.6% and 7.8%, respectively. Our findings reinforce the need to study these organisms in patients with culture-negative, community-acquired endocarditis, especially *B. henselae* in cat owners.

Worldwide, *Bartonella* spp. and *Coxiella burnetii* endocarditis have varied prevalences and clinical effects (*1*,*2*). Detection is difficult in routine blood cultures, so different diagnosis methods are needed. Our study investigated the frequency of and the risk factors for *Bartonella* spp. and *C. burnetii* infection in cases of culture-negative, community-acquired endocarditis.

The Study

During January 2004–January 2009, the Infection Control Team from the university hospital at São Paulo, Brazil (Instituto do Coração–Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo) used active surveillance to identify 369 patients with endocarditis. The study focused on community-acquired endocarditis caused by fastidious bacteria. Patients >18 years of age with confirmed endocarditis were included as a prospective inception cohort of patients (*3*). Excluded were patients with health care–associated endocarditis (i.e., patients with prosthetic valve endocarditis in the first postoperative year, hemodialysis patients, and nosocomial endocarditis patients) (*4*).

Indirect immunofluorescence assays (IFAs) were performed for all patients with negative blood cultures ≤7 days after admission at a referral center for rickettsial infections (Adolfo Lutz Institute, São Paulo). The same observer analyzed all assays; IgG titers ≥1:800 for *B. henselae* and *B. quintana* (*5*) and anti–phase I IgG titers ≥1:800 for *C. burnetii* (*6*) were considered positive (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-0343-Techapp1.pdf). New diagnostic criteria for Q fever endocarditis were used (*6*).

Immunohistochemical and molecular methods were applied to valve tissue specimens and serum samples of patients whose serum samples were positive for *Bartonella* spp. or *C. burnetii*. DNA from paraffin-embedded valve tissue specimens and serum samples were extracted. Samples positive for *Bartonella* by IFA were analyzed by using 5 different PCRs to 4 distinct regions. Tissue and serum DNA from patients positive for *C. burnettii* by IFA were tested by quantitative PCR (online Technical Appendix Table 1) (*7*).

Of the 369 identified endocarditis patients, 221 (59.9%) were included in the study; median age of included patients was 53 years. Of included patients, 144 (65.2%) were male; 107 (48.4%) had prosthetic valves; 209 (94.6%) had left-sided endocarditis; and 152 (68.8%) had concurrent conditions. Of patients with concurrent conditions, 62 (40.8%) had hypertension, 17 (11.2%) had diabetes, 53 (34.9%) had heart failure, and 36 (23.7%) had other conditions. Of the 221 patients included in the study, microorganisms were identified in 170 (76.9%); specimens from 51 (23.1%) patients were culture negative.

A standardized questionnaire regarding exposure to cats, ectoparasites, or farm animals was administered to patients with culture-negative endocarditis. For the 170 samples in which microorganisms were found, the most commonly identified bacteria were *viridans*-type *Streptococci* (81 [47.6%]), *Streptococcus bovis* (17 [10.0%]), *S. pneumonie* (6 [3.5%]), *S. agalactiae* (2 [1.2%]), *S. pyogenes* (2 [1.2%]), *Enterococcus fecalis* (13 [7.6%]), *E. faecium* (3 [1.8%]), other enterococci (4 [2.4%]) and *Staphylococcus aureus* (14 [8.2%]).

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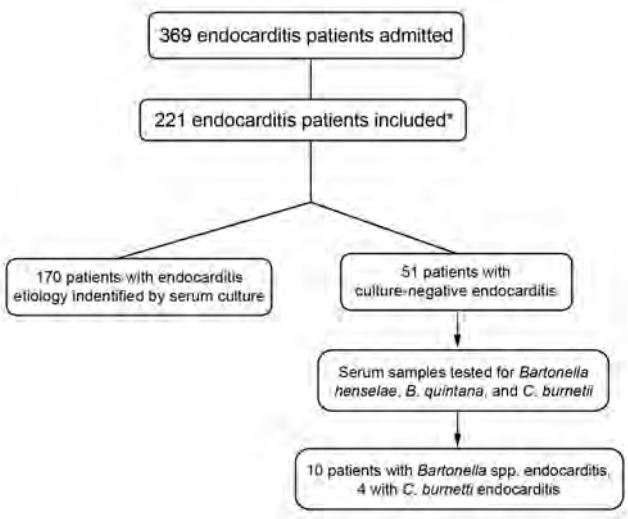

Figure. Distribution of patients etiologically diagnosed with endocarditis and admitted to the heart institute (Instituto do Coração) at the University of São Paulo Medical School, Sao Paulo, Brazil, January 2004–January 2009. *A modified Duke criteria (*3*) was used to determine inclusion of 221 patients. Excluded were 148 patients: 58 with unconfirmed endocarditis, 28 with endocarditis caused by cardiac implantable electronic devices, 47 with nosocomial endocarditis, and 15 hemodialysis patients.

For the 221 patients in the study, findings from 10 (4.5%; 95% CI 3.96%–5.09%) patients (Figure) showed *Bartonella* spp., and 4 (1.8%; 95% CI 1.58%–2.04%) showed *C. burnetii* endocarditis. For the 51 culture-negative endocarditis patients, *Bartonella* spp. was found in cultures from 10 (19.6%; 95% CI 9.8%–33.1%), and *C. burnetii* was found in 4 (7.8%; 95% CI 2.2%–18.9%). The Table shows the immunohistochemical and molecular biology analyses for patients with positive IFA results. *Bartonella* spp. DNA was detected with ≥1 PCR in all 6 patients whose paraffin-embedded valve tissue samples were found positive for *Bartonella* spp. For the other 4 patients with *Bartonella* spp., DNA was detected in 2 serum samples. Amplicons were sequenced, and their analyses showed that the cultures from 2 patients had 100% similarity with *B. quintana* (GenBank accession no. BX897700.1); cultures from 4 patients had 100% similarity with *B. henselae* infection (GenBank accession no. BX897699.1). Cultures from 2 patients were positive for *Bartonella* spp. by using IFA but negative by using PCR.

All patients used antimicrobial drugs for 7 days before sample collection. All endocarditis patients whose cultures were found to be positive for *C. burnetii* by using IFA were also positive by using quantitative PCR: 3 by serum samples and 2 by paraffin-embedded valve tissue specimens (online Technical Appendix Table 2).

Clinical and follow-up findings from *Bartonella* spp. and *C. burnetii* endocarditis patients are shown in online Technical Appendix Table 3. *Bartonella* spp. infection was associated with low levels of C-reactive protein on admission and chronic symptoms related to endocarditis (online Technical Appendix Table 4). Three (75%) of 4 patients with *Bartonella henselae* endocarditis were associated with a cat living in the patient's home, compared with 6 (12.8%) of 47 patients with culture-negative *Bartonella henselae* negative endocarditis (p = 0.015 by Student *t*-test). Hydroxychloroquine was unavailable in our facility; therefore, we used a second-line therapy for *C. burnetii* endocarditis. Hydroxychloroquine was replaced with ciprofloxacin, and treatment was extended for 72 months (*8*). Subsequently, symptoms resolved, and antibody titers reduced substantially, considered a favorable response (*9*) (online Technical Appendix Table 3).

Conclusions

In this study, the systematic use of IFA detected a 4.5% (10/221) prevalence of community-acquired endocarditis due to *Bartonella* spp. and a 1.8% (4/221) prevalence due to *C. burnetii*. For the 51 culture-negative endocarditis patients, IFA enabled recognition of the endocarditis etiology in 14 (27.5%) patients (*Bartonella* spp. in 10 [19.6%] and *C. burnetii* in 4 [7.8%]). Some of these patients have been recognized as having the first cases of endocarditis caused by these microorganisms in Brazil (*10*,*11*).

Prevalences of *Bartonella* spp. endocarditis vary worldwide by region studied (*1*). In a broad series of 759 culture-negative endocarditis patients in France, serum samples showed high sensitivity for detection of *C. burnetii* and *Bartonella* spp. infections, compared with other diagnostic tools, such as PCR, cell culture, and immunohistochemical analysis (*2*). In Brazil, studies of *Bartonella* spp. infection among culture-negative endocarditis patients have shown varied results. A retrospective case series of 51 surgically treated, culture-negative endocarditis patients found 2 cases of *Bartonella* spp. and 1 case of *C. burnetii* by using PCR on valvular tissue (*12*). Another series of 46 culture-negative endocarditis patients from the city of São Paulo used PCR to investigate *Bartonella* spp. in blood and found 13 (28%) patients with positive results (*13*).

We found an association between *B. henselae* endocarditis and the presence of a cat living at a patient´s home, a risk factor indicating that clinicians should consider this infection when assessing endocarditis patients. The relatively small sample of patients with endocarditis caused by *Bartonella* spp. and *C. burnetii* limited the statistical analyses of factors associated with these infections. Serologic investigations of infections by these agents were applied only to patients with negative cultures. Although rare (*2*,*14*), co-infection by these microorganisms in culture-positive endocarditis is possible, so frequency of *Bartonella* spp. and *C. burnetii* infections in

Table. Serologic, immunohistopathologic, and molecular test results for patients with infective endocarditis caused by *Bartonella* spp. or *Coxiella burnetii*, Brazil*

Patient no., by infection type	Serum IgG ≥800 by IFA†	Immunohistochemical analysis of cardiac valve vegetation		Microorganism by histologic analysis	PCR‡	Species of *Bartonella*
		Bartonella spp.	*C. burnetii*			
Bartonella spp.						
1	+	+	Neg	Gram-negative coccobacilli	+	*B. quintana*
2	+	+	Neg	Gram-negative coccobacilli	+	*B. henselae*
3	+	+	Neg	None	+	*B. henselae*
4	+	NA	NA	NA	Neg	NA
5	+	+	Neg	Gram-negative coccobacilli	+	NA
6	+	+	Neg	Gram-negative coccobacilli	+	*B. quintana*
7	+	NA	NA	NA	+	*B. henselae*
8	+	NA	NA	NA	+	NA
9	+	NA	NA	NA	Neg	NA
10	+	+	Neg	Gram-negative cocci	+	*B. henselae*
	Serum anti–phase I IgG	Immunohistochemical analysis of cardiac valve vegetation		Microorganism by histologic analysis	PCR‡	Q fever endocarditis (*6*)
C. burnetii	≥800 by IFA§	*Bartonella spp.*	*C. burnetii*			
11	+	Neg	+	Small gram-negative coccobacilli	+	Definite 2A criteria
12	+	Neg	+	None	+	Definite 2A criteria
13	+	Neg	+	None	+	Definite 2A criteria
14	+	NA	NA	NA	+	Definite 2B criteria¶

*IFA, immunofluorescence assay; +, positive; Neg, negative; NA, material not available for analysis.
¶Serologic result >6,400 and vegetation on ecocardiography.
†*B. henselae* or *B. quintana.*
‡Serum or tissue sample.
§*C. burnetii.*

these patients may be higher than shown. Our study indicates that systematic serologic research for *Bartonella* spp. and *C. burnetii* in community-acquired, culture-negative endocarditis may be clinically useful, particularly in screening for *B. henselae* in cat owners.

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Detection and Full-Length Genome Characterization of Novel Canine Vesiviruses

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Vesiviruses have been detected in several animal species and as accidental contaminants of cells. We detected vesiviruses in asymptomatic kennel dogs (64.8%) and symptomatic (1.1%) and asymptomatic (3.5%) household dogs in Italy. The full-length genome of 1 strain, Bari/212/07/ITA, shared 89%–90% nt identity with vesiviruses previously detected in contaminated cells.

Members of the family *Caliciviridae* are small (30–35 nm in diameter), nonenveloped viruses with a single-stranded, positive-polarity RNA genome of 7.4–8.3 kb. The family consists of the genera *Vesivirus*, *Lagovirus*, *Norovirus*, *Sapovirus*, and *Nebovirus* as well as unassigned caliciviruses (*1*,*2*).

Vesiviruses were originally identified in 1932 in California, USA, in domestic swine with vesicular disease. Since then, vesiviruses have been described in several animal species and humans, and they have been associated with a variety of clinical signs and lesions, including abortion, hepatitis, respiratory disease, diarrhea, myocarditis, encephalitis, mucosal ulcerations, vesicular lesions, and hemorrhagic syndromes (*3*–*5*). Unlike other caliciviruses, vesiviruses appear to readily cross host species barriers, and the marine ecosystem is believed to constitute a large reservoir of vesiviruses for terrestrial animals (*5*).

Vesiviruses have occasionally been detected in dogs with diarrhea and, in some instances, in dogs with glossitis, balanitis, or vesicular vaginitis (*6*–*9*). However, with the exception of a canine calicivirus, strain 48 (*9*), the caliciviruses detected in dogs have been feline viruses (*6*,*7*). The prototype canine calicivirus (CaCV) strain 48 was identified in Japan in 1990; the virus was isolated from a 2-month-old pup with intermittent watery diarrhea (*9*). Strain 48, which is antigenically and genetically unrelated to feline caliciviruses, was tentatively proposed as a true CaCV in the *Vesivirus* genus (*10*). Antibodies to strain 48 have been detected in 57.0% of dogs in Japan (*11*) and 36.5% of dogs in South Korea (*12*), but no information is available regarding the circulation of analogous viruses in dogs elsewhere.

In 2003, a novel vesivirus (strain 2117) genetically similar to CaCV strain 48 was accidentally isolated as a contaminant in Chinese hamster ovary (CHO) cell cultures by a pharmaceutical company in Germany (*13*). These cells are mostly used by biotech companies for the production of recombinant drugs; possible sources of contamination included reagents used for cell cultivation, such as porcine-derived trypsin or fetal bovine serum.

The limited information available does not clarify whether vesiviruses play a role as enteric pathogens in dogs. Considering the ability of vesiviruses to cross the host species barriers (*5*) and the close social interactions between humans and dogs, it is essential to determine whether dogs harbor viruses with a zoonotic potential. To further investigate the molecular epidemiology of vesiviruses, we screened fecal specimens from asymptomatic dogs and from dogs with diarrhea in Italy.

The Study

In 2007, we collected 385 samples from dogs in Bari, Italy. A total of 183 samples were fecal specimens from household dogs (1–6 months of age) hospitalized with signs of mild to severe gastroenteritis (collection A); 88 were rectal swab specimens from clinically healthy juvenile and adult dogs housed in 4 separate shelters (collection B); and 114 were fecal swab specimens from asymptomatic household dogs (1–6 months of age) receiving routine care at 2 veterinary clinics (collection C).

By using reverse transcription PCR with the broadly reactive consensus primers for caliciviruses, p289/p290 (*14*), and strain 48–specific primers 493F–526R (Table), we detected vesivirus RNA in 1.1% (2/183), 64.8% (57/88), and 3.5% (4/114) of collection A, B, and C samples, respectively. Partial RNA-dependent RNA polymerase sequences, obtained by using the primers p289/p290 (*14*), were determined for 10 samples. The sequences shared closest nucleotide identity (90.7%–92.6%) with vesivirus strains 2117, Geel/2008, Allston/2008/USA, and Allston/2009, which were identified as contaminants in CHO cells; the

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Table. Primers used in study of the detection and full-length genome characterization of novel canine vesiviruses

Primer	Position*	Sequence	Sense	Use†
493F	5127–5150	GGT TTG CCA TCT GGC ATG CCG CTA	+	Detection
526R	5793–5816	AGC CAT VGC TCA RTT CTC AAA CAC	–	Detection
p289	5271–5292	TGA CAA TGT AAT CAT CAC CAT A	–	Detection
p290	4962–4984	GAT TAC TCC AAG TGG GAC TCC AC	+	Detection
501F	554–577	GTC TTG TGC TCT VTA CGA CAM ATG	+	Sequencing
VN3T20	3′-poly A	GAG TGA CCG CGG CCG CT_{20}.	–	3′ RACE
502F	1215–1234	ATG ATW ATT GAH AAC CAY GA	+	Sequencing
509F	2904–2923	TAC GAT ATG GCY TGG GCY CT	+	Sequencing
510F	3411–3432	GAT GAT GAG TAC GAT GAR TGG A	+	Sequencing
511F	3588–3607	GAA GAC GTC ACC RTA ATT GG	+	Sequencing
513F	3876–3898	GTT ACG TTC RAT GGY GAA TTG GC	+	Sequencing
515F	4314–4338	CAC GTG TCA CCA GCA CAC RTD GAT G	+	Sequencing
504R	1599–1618	ACC ACG CTY TCR TTS GAC CA	–	Sequencing
528R	2887–2909	CTT GTC ATC TTA GTG TAC AAT GA	–	Sequencing
506R	1680–1702	AG GTT GGT RAC NGC RTC AAT GTC	–	Sequencing
508R	1974–1993	GT GTA GGC RYC GTG GTG GTC	–	Sequencing
512R	3588–3608	GCC AAT TAY GGT GAC RTC WTC	–	Sequencing
536F	714–732	TAC GAT CTT GCA ATC AAT G	+	Sequencing
GE72F	1915–1935	CCT ATG CCA TTG CGT CTA GAC	+	Sequencing
GE73R	2951–2970	CAG CCT TAA GTG CCT GCC AC	–	Sequencing
SEQ100	5634–5653	TGT CGC CAA ATG TTG ATG AG	+	Sequencing
SEQ101	6343–6360	TTG CCA CAG GCA CTC AGC	+	Sequencing
SEQ102	6860–6877	GGA AAC ACG TGG TGG TCA	+	Sequencing
SEQ103	7487–7504	AAG TAG AAT GAT TGG TGA	+	Sequencing
SEQ104	8063–8082	GAG TTT GAC AAG ATG AAC AG	+	Sequencing
GSP1	497–520	GCT TCA GAG ATC AGA ATA TCG TTG	–	5′ RACE
GSP2	366–385	GTG GTC AGA GCC TTG GTC AG	–	5′ RACE

*Position is based on the sequence of strain Bari/212/07/ITA (GenBank accession no. JN204722).
†RACE, rapid amplification of CDNA ends.

sequences shared 73.6%–74.8% nt identity with CaCV strain 48. We determined the full-length genomic sequence (8,453 nt) of 1 of the canine vesivirus strains, Bari/212/07/ITA, by using consensus primers and 3′ and 5′ RACE protocols (Invitrogen Ltd, Milan, Italy); the sequence was deposited in GenBank (accession no. JN204722). Primers used for virus detection and sequencing are listed in the Table.

The full-length genomic sequence of strain Bari/212/07/ITA shared 89%–90% nt identity with sequences of viruses identified as CHO cell contaminants; it shared only 71.0% nt identity with the prototype CaCV strain 48. Three open reading frames (ORFs) were predicted by sequence analysis of the nucleotide sequence and by comparing results with the genomic organization and ORFs of other vesiviruses (Figure 1). ORF1 was 5,796 nt in length (nt 12–5807) and encoded a 1,921-aa polyprotein. The ORF1 stop codon was followed by 3 nt and then by the ORF2 start codon. ORF2 was 2,079 nt in length (nt 5811–7889) and encoded a 692-aa capsid protein. ORF3 was 405 nt in length (nt 7886–8290) and encoded a 134-aa protein. ORF2 overlapped with ORF3 by 4 nt (Figure 1). The 163-nt 3′ untranslated region of CHO cell–associated strain 2117 was shorter than the 235-nt region in strain 48. The full-length ORF1-encoded polyprotein of Bari/212/07/ITA shared highest amino acid

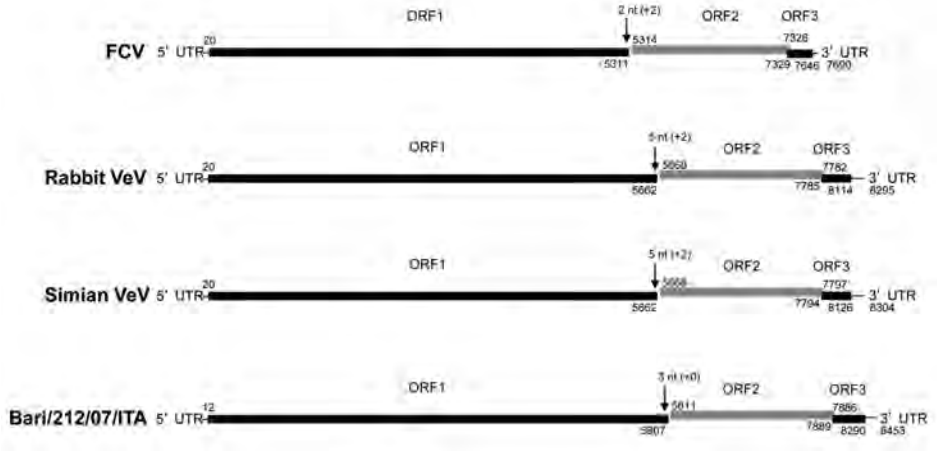

Figure 1. Genome organization of vesiviruses (VeVs). The genomic organization and open reading frame (ORF) usage are shown for representative viruses in the main VeV genetic groups: feline calicivirus (FCV) strain F9 (GenBank accession no. M86379), rabbit VeV (GenBank accession no. AJ866991), simian VeV strain Pan1 (GenBank accession no. AF091736), and canine VeV Bari/212/07/ITA. Numbers above and below the genome bar indicate the nucleotide (nt) position of the ORF initiation and termination, respectively. UTR, untranslated region.

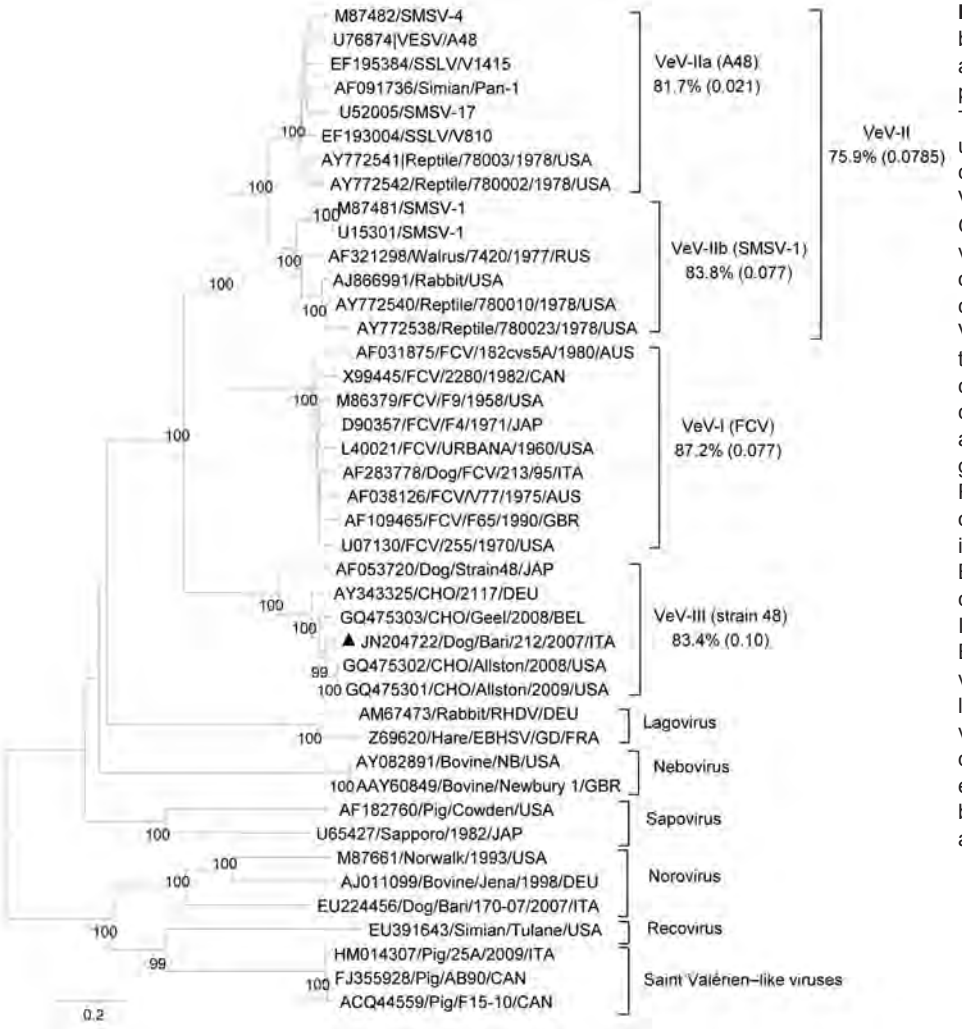

Figure 2. Phylogenetic tree based on the complete amino acid sequence of the capsid protein of vesiviruses (VeVs). The tree was constructed by using a selection of feline calicivirus strains and all of the VeV strains available in the GenBank database. In addition, viruses representative of the other established and candidate calicivirus genera were included. VeV groups were defined on the basis of distance matrix comparison and phylogenetic clustering. The mean identity among strains of the main genetic groups (indicated by Roman numerals and a letter or both) is shown. Numbers in parentheses indicate SDs. Black triangle indicates the canine VeV strain Bari/212/07/ITA. DEU, Germany; EBHSV, European brown hare syndrome virus; SMSV, San Miguel sea lion virus; SSLV, stellar sea lion virus; RHDV, rabbit hemorrhagic disease virus; VESV, vesicular exanthema of swine virus. Scale bar represents the number of amino acid substitutions per site.

identity (98.5%–98.9%) with CHO cell–associated vesiviruses; it shared 80.5% aa identity with the ORF1-encoded polyprotein of canine strain 48 and <56% aa identity with other vesiviruses. Within the ORF2-encoded protein, the capsid cleavage motive FRAES (aa 155–159) was conserved. As observed, in CHO cell–associated strain 2117 and canine strain 48, a 7-aa insertion (KTIKSQV) was present in the conserved region D. The full-length capsid protein of Bari/212/07/ITA shared 92.6%–92.9% aa identity with the CHO cell–associated vesivirus strains Allston/2009/USA and Allston/2008/USA from the United States, 90.0% aa identity with the CHO cell–associated isolate Geel/2008 from Belgium, 86.3% aa identity with the CHO cell–associated isolate 2117 from Germany (*13*), and only 70.3% aa identity with the prototype CaCV strain 48 isolated in Japan in 1990 (*9*). Strain Bari/2012/07/ITA shared ≤35.8% aa identity with other vesiviruses (Figure 2).

In previous investigations in dogs, strain 48–like vesiviruses were detected in only 2 (1.7%) of 119 samples: a fecal specimen from a dog with signs of enteric disease and a tonsillar swab specimen from a dog with respiratory disease (*11*). However, serologic investigations suggested that vesiviruses actively circulate in the canine population (*11*,*12*). In our virologic investigations, vesivirus RNA was detected in 64.8% (57/88) of dogs housed in 4 shelters but in only 1.1% (2/183) and 3.5% (4/114) of symptomatic and asymptomatic household dogs, respectively. These findings demonstrate that canine vesiviruses can be widespread in some settings or populations (e.g., kennels or shelters) where high population densities create favorable epidemiologic situations for circulation of some microorganisms.

Of interest, partial sequence analysis of the RNA-dependent RNA polymerase fragment of several of the dog-derived strains and full-length genomic sequencing of strain Bari/212/07/ITA showed that these canine vesiviruses were more similar to some vesiviruses found as contaminants of CHO cell cultures than to the prototype CaCV strain 48. Contamination of CHO cells by vesiviruses was documented in 2003 in Germany (*13*), 2008 and 2009 in the United States, and 2009 in Belgium. The

2008–2009 contamination in the United States was estimated to cost US $100–300 million in lost revenues to the biotech company because production was interrupted to provide adequate sanitation and maintenance of the bioreactors (*15*). In addition, these contaminations have raised concerns for the potential exposure of humans to these novel viruses because vesiviruses can readily cross the host species barrier (*5*).

Conclusion

Our findings show that genetically heterogeneous vesiviruses are common in dogs in Italy. Further studies are necessary to understand the ecology of this group of vesiviruses; that is, it must be determined whether vesiviruses circulate in humans or other animal species and, above all, whether they are associated with disease in humans, animals, or both.

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Smallpox Vaccination of Laboratory Workers at US Variola Testing Sites

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To evaluate the need to revaccinate laboratory workers against smallpox, we assessed regular revaccination at the US Laboratory Response Network's variola testing sites by examining barriers to revaccination and the potential for persistence of immunity. Our data do not provide evidence to suggest prolonging the recommended interval for revaccination.

The eradication of smallpox (variola) is arguably the greatest public health feat in the history of civilization. Smallpox was an infectious disease that plagued global health from the earliest documented settlements (1500 BCE) through nearly the end of the 20th century (*1*). In 1980, the World Health Organization declared that smallpox was eradicated and paid homage to a large cadre of dedicated and tireless persons who collectively eliminated a disease that had killed one third of its victims for >3,500 years (*2*).

Despite concern about the existence of live smallpox virus housed in government research laboratories in the United States and the Soviet Union (later Russia), not until 2002 did the US government declare the resurgence of smallpox a credible biothreat (*1*). In the United States, the Department of Health and Human Services launched a national campaign to vaccinate volunteers from health care and public health professions. The 2003 National Smallpox Vaccination Program resulted in the vaccination of almost 40,000 volunteers in 9 months in the United States (*2*).

After the National Smallpox Vaccination Program ended in October 2003, experts at the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) and members of the Advisory Committee on Immunization Practices (ACIP) met to determine future steps for response planning. These experts recommended that vaccinated persons from health care and public health professions be revaccinated only if a smallpox event occurred. However, laboratory workers handling orthopoxviruses at the proposed variola testing sites would need to be revaccinated every 3 years to maintain optimal immunity or protection. The ACIP admitted that this recommendation was based on "best available science, historic precedent, and expert opinion" (*3*). Although the ACIP recommendations do not address the frequency with which workers are exposed to orthopoxviruses, they imply that even periodic exposure to these viruses might warrant the protection that only vaccination can provide. Some orthopoxviruses remain extremely dangerous (high rates of illness and death) for humans (*4*).

During 2012, we further evaluated the need for revaccination of laboratory workers by examining barriers to revaccination and the potential for persistence of immunity in laboratory workers at the Laboratory Response Network (LRN) variola testing sites who had received at least 1 smallpox vaccination since 2003. Our intent was to balance the risks for rare exposures to the virus against the risks for severe adverse events from the vaccine.

The Study

We used the LRN as a conduit to maintain the confidentiality and anonymity of the variola testing sites. A convenience sample of 45 laboratory workers completed an online survey developed by researchers at the University of Nebraska Medical Center (Omaha, NE, USA). Nonidentifying demographic information was collected, in addition to any adverse effects after vaccination and perceived barriers to revaccination. To determine a significant difference existed regarding the success (presence or absence of a "take" after vaccination) of the vaccine based on intervals between vaccines, we measured the mean interval (in years) between vaccinations. Finally, respondents were asked whether they worked with orthopoxviruses in their laboratories.

Respondents' mean age was 46 years; they had worked a mean of 20.5 years in the laboratory setting. Eighty-four percent of respondents reported that the only adverse events from vaccination were related to the skin irritation caused by the occlusive dressings worn over the vaccination lesion. Sixty-seven percent listed a medical condition in themselves or a close household contact as the barrier to revaccination. The mean interval from first to second vaccination was 4.8 years for vaccinees who had a successful vaccine and 6.0 for those who did not. Statistical analysis demonstrated no significant difference ($p = 0.149$) between the number of years between first and second vaccinations and the "take" rates. Sixty-two percent of respondents indicated they did not work with non–highly attenuated orthopoxviruses.

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Conclusions

In this study, laboratory workers continued to have successful "takes" (i.e., developed lesions) regardless of number of years since previous vaccination, suggesting that immunity might have waned. Therefore, our data do not provide evidence to suggest that the ACIP recommended interval for revaccination be prolonged. Although most respondents reported having no adverse effects from the vaccine, for some this vaccination caused discomfort. Many reported symptoms related to the occlusive dressing worn as a precautionary measure to ensure that the lesion site was properly covered during work hours. Other measures to ensure the lesion is covered appropriately, such as nonocclusive dressings and long sleeves, may be considered given that laboratory workers do not have direct contact with patients.

Although the LRN asks this small group of laboratory workers to comply with the ACIP recommendations, the question remains whether this requirement should include laboratory workers who do not handle orthopoxviruses. Revaccination of most laboratory workers at variola testing sites every 3 years would be expected to be sufficient to provide an initial immunologic response, whereas laboratory workers who do not handle orthopoxviruses could be vaccinated in the same fashion as other health care and public health workers who have at least 1 recent (since 2003) documented successful vaccination (*5*). This recommendation is based on the same premise as using the vaccine as prophylaxis for documented exposure to a smallpox-infected person. This practice was used regularly during the smallpox eradication program. Because the average incubation period for vaccinia is 3–4 days shorter than the incubation period for smallpox, a person exposed to smallpox would have a 3–4 day window in which to be vaccinated with and immunologically respond to vaccinia, which also confers immunity to smallpox (*6*)

Compromised immune systems or cardiac risk factors that make vaccinees ineligible for vaccination are more likely to develop as they age (*7*). Most barriers to revaccination were related to medical conditions (compromised immunity and/or exfoliative skin disorders) that place vaccinees at high risk for adverse events to the currently licensed smallpox vaccine. The conditions are an added challenge for the aging pool of laboratory workers assigned to national variola testing sites (*8*). Currently unlicensed third-generation smallpox vaccines may be considered (pending licensure) as replacements to ACAM2000 (Sanofi Pasteur Biologics, Lyon, France), the currently licensed vaccinia vaccine, for laboratory workers at national variola testing sites or perhaps an even broader population of laboratory workers throughout the United States. Third-generation vaccines are nonreplicating and safer in populations that might have contraindications to traditional vaccines (*9*–*11*).

The risk to the US population from a release of smallpox has decreased considerably. This reduced risk stems not from a lower threat from terrorism but from the existence of a stockpile of the new ACAM2000 smallpox vaccine, in addition to a cadre of health care and public health professionals who could be revaccinated quickly and mobilized accordingly (*12*).

More research on the immunogenicity of smallpox vaccine is needed but is challenged by the absence of smallpox disease to test the efficacy of vaccination. Researchers now appreciate that the complex mechanism of the immune response to vaccinia and/or smallpox infection might lead to better treatment options for infectious and autoimmune diseases (*7*). Future opportunities may arise to challenge the vaccine with the actual virus to measure vaccine efficacy and provide sound recommendations to protect all public health and health care responders against smallpox (*13*). In the meantime, ensuring that recommendations created to protect some populations are properly interpreted and applied is important to protecting the most vulnerable persons without exposing others to unnecessary harm.

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Enterovirus A71 Meningoencephalitis Outbreak, Rostov-on-Don, Russia, 2013

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Seventy-eight cases of enterovirus infection, including 25 neuroinfections, occurred in Rostov-on-Don, Russia, during May–June 2013. The outbreak was caused by an enterovirus A type 71 (EV-A71) subgenotype C4 lineage that spread to neighboring countries from China ≈3 years earlier. Enterovirus-associated neuroinfection may emerge in areas with a preceding background circulation of EV-A71 with apparently asymptomatic infection.

Enterovirus A type 71 (EV-A71; family *Picornaviridae*, genus *Enterovirus*, species *Enterovirus A*) is the most neuropathogenic nonpolio enterovirus in humans. Over the past 30 years, this virus has caused many outbreaks and epidemics of hand, foot and mouth disease (HFMD) with neurologic complications in the Asia-Pacific region and China (*1*). In Europe, EV-A71 commonly causes asymptomatic infection (*2–4*) and is only occasionally associated with severe neuroinfection (*3*,*5*). The last epidemics of EV-A71 infection in Europe occurred during 1975–1978 (*6*,*7*).

The Study

During 2013, an outbreak of EV-A71 infection occurred in Rostov-on-Don, a city in southern Russia. The first case was diagnosed on May 31 in a 2-year-old boy. A cerebrospinal fluid sample from the boy contained EV-A71 and pneumococcal bacteria, suggesting combined bacterial and viral meningoencephalitis. The boy died of meningoencephalitis on day 5 after disease onset. According to the official State Surveillance System report, 366 children (1–7 years of age) attended the same childcare facility as the boy who died, and over the 3 weeks after the index case, 77 more children were involved in the outbreak (Figure 1). Of the 78 children, 43 were boys and 35 were girls. Fourteen children were 1–2 years of age, 63 were 3–6 years of age, and 1 was 7 years of age. A total of 68 children were hospitalized, of whom 25 (15 boys, 10 girls) had meningitis or meningoencephalitis; 6 of these children were 1–2 years of age, and 19 were 3–6 years of age.

Enterovirus RNA was detected in fecal samples (n = 53), throat swab samples (n = 23), or both (n = 17) from 59 of the 78 patients. EV-A71 was identified by partial viral protein 1 genome region sequencing in the cerebrospinal fluid sample from the first patient and in fecal and throat swab samples from the other patients. The outbreak was officially defined as including only those children from the childcare facility attended by the index patient. Additional cases of HFMD and meningitis that occurred outside that kindergarten were not officially accounted for in outbreak statistics; thus, it is likely that the outbreak size was underestimated. Simovanian et al. (*8*) indicated that a total of 139 EV-A71 infections were reported in Rostov-on-Don during May–June 2013. According to that report, 30.2% of patients had meningitis and 7.2% had meningoencephalitis. Of note, exanthema was observed in only 79.9% of patients.

The ratio of neuroinfection (meningitis and meningoencephalitis) cases relative to mild infection (i.e., HFMD and fever) cases was unusually high in this outbreak. Among the 78 officially reported cases, 25 (32.1%) were meningitis or meningoencephalitis. After the first fatal case, all children in the childcare facility were proactively screened for enterovirus infection symptoms, so it is unlikely that many patients with mild infection were missed. Asymptomatic children were not screened for EV-A71 RNA. However, the ratio of meningitis cases was high even relative to the total number of children attending the childcare facility (6.8% [25/366 children]). This finding differs from those from a 1998 outbreak in Taiwan, in which 405 (0.3%) of 129,106 persons with HFMD had severe disease (i.e., fever >38°C or neurologic manifestations) (*9*). Thus, the proportion of severe disease in a localized setting can be substantially higher than that for an epidemic overall.

Thirteen isolates from the EV-A71–infected children were available for study. Viruses were propagated in cell culture, and the entire viral protein 1 genome region (891 nt) was amplified as described previously (*10*). The outbreak samples differed from each other by ≤3 nt substitutions (0.3%). Furthermore, they shared 97%–98% nt sequence identity with several subgenotype C4 viruses

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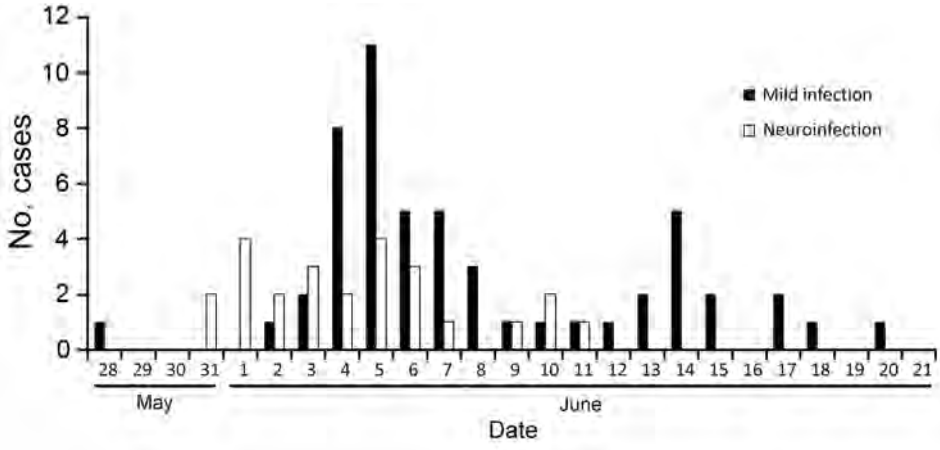

Figure 1. Incidence of neuroinfection during an enterovirus A type 71 infection outbreak among children attending a childcare facility, Rostov-on-Don, Russia, 2013. Of 78 infected children (1–7 years of age), 25 experienced neuroinfection (meningitis or meningoencephalitis) and 53 experienced mild infection (hand, foot and mouth disease or fever).

isolated in China during 2009–2011, indicating that the strains belonged to this subgenotype.

Phylogenetic analysis of the virus spread was performed by using a Bayesian phylogenetic approach implemented in BEAST version 1.7.5 (*11*). The substitution rate in this dataset was 6.8×10^{-3} substitutions per site per year, which is similar to rates reported for EV-A71 subgenogroup C4 in other studies (*12*,*13*). Sequences from Rostov-on-Don and the Rostov region were monophyletic (Figure 2, node A); the time to the most recent common ancestor was 200 days (95% high probability density interval 68–347 days); however, this group was imperfectly supported by a posterior probability value of 0.85. The high number of days to the most recent common ancestor of the outbreak strain is surprising because the outbreak presented as a point-source expansion with a traceable source and transmission chains. A possible explanation for this finding might be that that the substitution rate of EV-A71 might accelerate substantially during an outbreak, or additional substitutions might have been introduced upon isolation in cell culture.

In the phylogenetic tree, isolates from Rostov-on-Don grouped (with a posterior probability of 1) with an additional 6 viruses that were isolated in the neighboring Stavropol region during and up to 2 months after the outbreak (Figure 2, node B); however, no cases of neuroinfection were reported in that region. Isolates from Rostov-on-Don and Stavropol grouped reliably with another 7 viruses isolated in Siberia, the Pacific region of Russia, Kazakhstan, and Kyrgyzstan during 2012–2013 (Figure 2, node C). The time to the most recent common ancestor of these viruses was 921 days (95% high probability density range 665–1,195 days) before the outbreak. Therefore, the virus variant that caused the outbreak was likely introduced to Russia or Middle Asia ≈3 years before the outbreak and circulated extensively without causing notable illness. Of interest, another lineage of EV-A71 subgenogroup C4 representing an independent introduction from China was circulating at the same time in Central Russia and Stavropol region (Figure 2, node D).

Conclusions

Over the past 2 decades, EV-A71 became a prominent emerging virus in Asia. However, the EV-A71 epidemiologic situation remained calm in Russia, Europe, and North America, despite common circulation of the virus, as suggested by surveillance and seroepidemiologic studies (*3*,*4*). According to our previous study (*10*), EV-A71 was circulating in Rostov-on-Don before 2008, but EV-A71 seroprevalence among infants 1–2 years of age (4.5%) was substantially lower than that observed in other regions of Russia (9.9%–22.7%). It is possible that lower herd immunity could have facilitated the outbreak we describe here. Our findings show that an outbreak of EV-A71 neuroinfection may emerge in new areas despite preceding background circulation of EV-A71 with apparently asymptomatic infection.

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Dr. Akhmadishina is a postdoctoral researcher. In 2013 she completed a PhD thesis on molecular epidemiology and seroepidemiology of EV-A71 in Russia.

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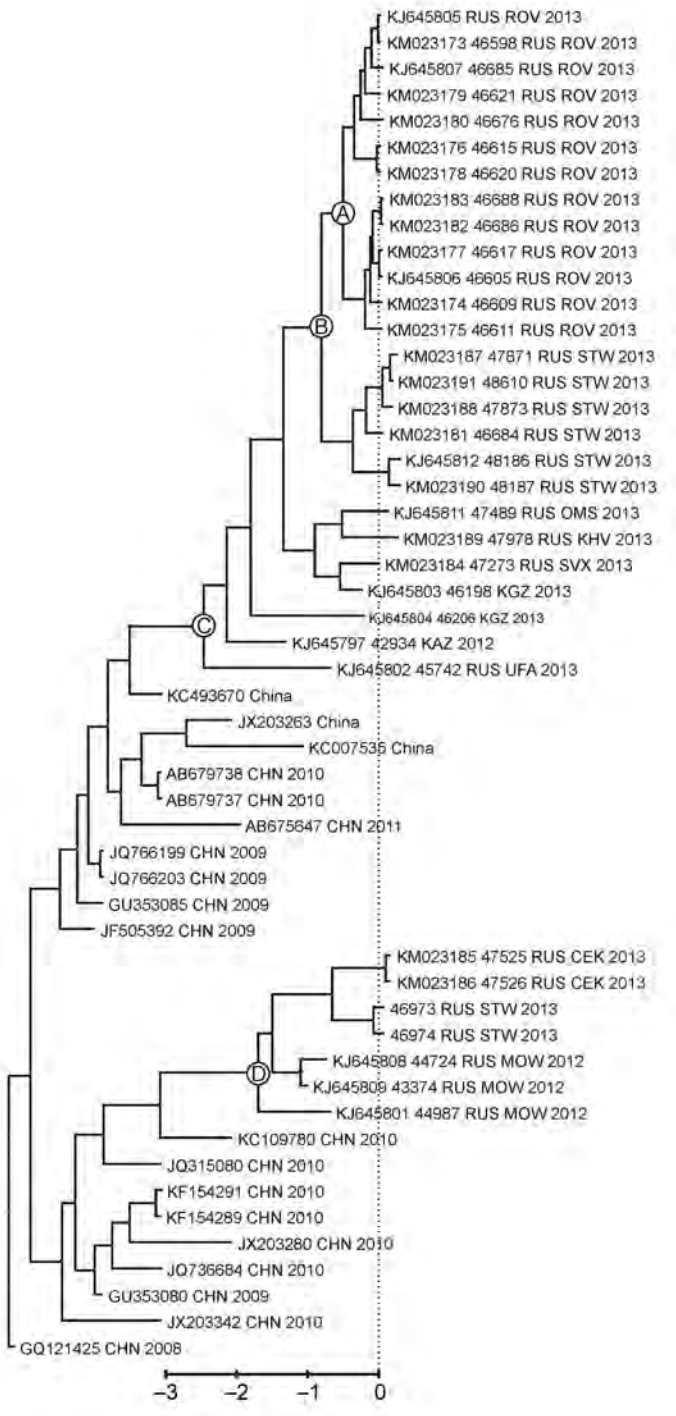

Figure 2. Phylogenetic tree comparing sequences of outbreak and other enterovirus A type 71 (EV-A71) subgenogroup C4 strains isolated in Russia during 2012–2013 with the most closely related sequences in GenBank. Complete viral protein 1 genome regions (891 nt) were compared. The tree was reconstructed by using a coalescent Bayesian algorithm implemented in BEAST 1.7.5 (*11*) with the SRD06 substitution model, relaxed exponential clock, and constant population prior. The dataset included 63 GenBank sequences that were most similar to the outbreak virus, as determined by using BLAST (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi), and 22 EV-A71 subgenogroup C4 sequences obtained elsewhere in Russia, Kazakhstan, and Kyrgyzstan during 2011–2013 (*10*). Only the part of the tree that is relevant to the discussion is shown. Strain names contain the GenBank or internal reference number, country of isolation, city code for Russian isolates, and year of isolation. City codes: CEK, Chelyabinsk; CHN, China; KAZ, Kazakhstan; KGZ, Kyrgyzstan; KHV, Khabarovsk; MOW, Moscow; OMS, Omsk; ROV, Rostov; RUS, Russia; STW, Stavropol; SVX, Yekaterinburg; UFA, Ufa. GenBank accession numbers for previously unpublished viruses (KM023173–KM023191) are shown in the tree. The dotted line shows the outbreak onset on May 31 (some strains were isolated after that date). Circled letters indicate sequences for viruses isolated in Rostov-on-Don and the Rostov Region of Russia (A); Stavropol region of Russia, which neighbors Rostov Region (B); Siberia, the Pacific region of Russia, Kazakhstan, and Kyrgyzstan (C); and Stavropol, central Russia and Ural Region (D). Scale bar indicates years before the 2013 outbreak.

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Extensive Nosocomial Transmission of Measles Originating in Cruise Ship Passenger, Sardinia, Italy, 2014

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We report a measles outbreak in Sardinia, Italy, that originated in a cruise ship passenger. The outbreak showed extensive nosocomial transmission (44 of 80 cases). To minimize nosocomial transmission, health care facilities should ensure that susceptible health care workers are vaccinated against measles and should implement effective infection control procedures.

Measles is a highly infectious acute viral disease that can cause severe complications. It is transmitted by direct contact with large respiratory droplets and by the airborne route. This disease will develop in ≈90% of susceptible close contacts of infectious persons. We report a large outbreak of measles in Sardinia, Italy, that originated from a reported outbreak on a cruise ship (*1*) and showed extensive nosocomial transmission.

The Study

A rash developed in the index case-patient (an unvaccinated woman) on February 23, 2014, nine days after she disembarked the cruise ship on which she had traveled during February 6–14, and returned to Sardinia. While infectious, she was seen in the emergency department (ED) of a local hospital, where she was admitted for 3 days for laboratory-confirmed measles. The index case-patient transmitted the infection to 6 persons: a coworker (onset of rash March 6), 1 adult relative (onset of rash March 10), 1 adult visitor to the ED in which the index case-patient sought medical care (onset of rash March 11), and 3 health care workers (HCWs) in the same hospital (onset of rash March 2, 10, and 11).

Transmission continued in families, work settings, and the hospital setting. As of July 2014, a total of 136 cases from the cruise ship outbreak were reported to the national measles surveillance system in Italy, of which 80 were from the secondary outbreak described in this report (Figure). The remaining 56 cases include 28 primary cases (crew and passengers) and 28 secondary cases; 39 of 56 case-patients were Italian and 17 were of foreign origin. One other secondary outbreak linked to the cruise ship has been reported (*2*).

Classification of measles cases was based on European Union 2012 case definitions (*3*). A probable case-patient was any person meeting clinical criteria for measles and having an epidemiologic link to the outbreak who did not undergo laboratory testing. A confirmed case-patient was any person with an epidemiological link to the outbreak meeting clinical criteria and having laboratory evidence of infection (positive IgM serologic result or identification of measles virus RNA by PCR). A nosocomial case-patient was defined as any person with measles who had contact with a probable or confirmed case-patient in the hospital (including the waiting area of the ED) 7–21 days before rash onset and had no other source identified. An HCW was any hospital staff or other worker who had regular contact with patients, including clinicians, nurses, students in these disciplines, paramedical professionals, social workers, ambulance workers, porters, and other hospital support staff.

Median age of 80 case-patients was 26 years (range 8 months–55 years); 50 (62.5%) were female. Vaccination status was known for 76/80 (95.0%) patients, of whom 74 (97.4%) were unvaccinated and 2 (2.6%). had received only 1 dose of measles vaccine. Forty-one (51.3%) patients reported ≥1 complication, and 19 (23.8%) reported ≥2 complications (Table). Thirty-five (44.9%) of 78 patients were hospitalized, including 2 patients admitted to the intensive care unit for respiratory insufficiency. In addition, 14 patients were seen in the ED.

Thirty-four cases (42.5%) were laboratory confirmed; the remaining 46 cases were classified as probable. Measles virus genotype B3 was identified in 7 cases, and phylogenetic analysis showed that viral sequences were identical to each other and to those obtained from the cruise ship outbreak (*2*,*4*) (measles nucleotide surveillance [MeaNS] database accession nos. 62563, 62564, 67009, 67016, 62565, 67017, and 67020). Nosocomial cases included HCWs (n = 15; 18.8%) and persons infected in the waiting area of the ED or in a hospital ward (n = 29; 36.2%). Six additional case-patients were infected by an HCW in a family setting.

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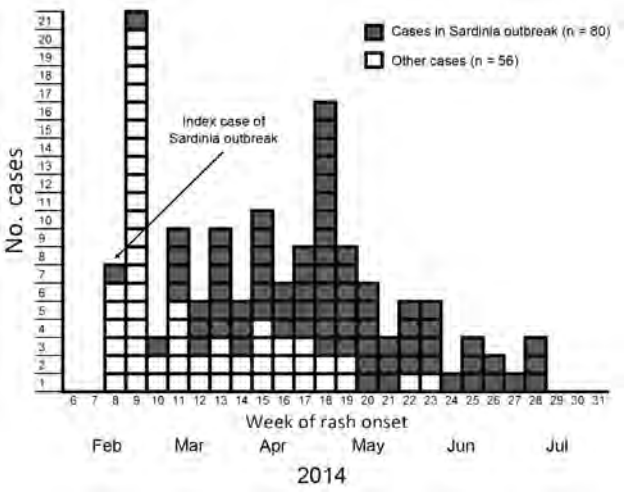

Figure. Number of primary and secondary cases (n = 136), by week of rash onset, during a measles outbreak that originated in a cruise ship passenger, including cases reported in a secondary outbreak, Sardinia, Italy, February–July 2014.

Local health authorities conducted case investigations, including active case finding by regular visits/phone calls to the hospital, and contact tracing and vaccination of susceptible contacts. Hospital directors were contacted by local health authorities to inform them of measles transmission in the hospital. No data were available for vaccination coverage among HCWs because this information is not routinely obtained. An information circular that invited susceptible staff to receive measles vaccine and consent/dissent forms were distributed in the affected wards (ED, intensive care unit, medicine). However, participation has been negligible: only 2 of 114 HCWs agreed to be vaccinated.

Conclusions

Clinical and genotype data suggest that the index case-patient for this outbreak was infected during the cruise, either onboard or in 1 of the cities visited during the cruise (*5*). The index case-patient triggered an outbreak with extensive spread, which highlights the ease with which measles is transmitted in various settings, especially the hospital setting, which accounted for 44 (55%) of the cases.

Nosocomial transmission of measles has been frequently reported and has a major role in the epidemiology of this disease, especially in industrialized countries (*6*,*7*). As described in this outbreak, nosocomial transmission can lead to measles in HCWs, other patients at the hospital, and susceptible hospital visitors. Measles is one of the most contagious viral diseases and patients frequently visit hospitals or health care facilities for diagnosis and management, which might lead to nosocomial transmission if appropriate infection control measures are not immediately instituted (*8*).

The potential for airborne transmission of measles in waiting areas of health care facilities is higher than for other airborne infections, such as tuberculosis and influenza, and persons might become infected after a relatively short exposure time (*9*). Also, measles virus can survive for up to 2 hours in the air or on objects and surfaces, which indicates that a susceptible person can be infected even after an infected person has left the area (*10*,*11*). Infectivity of measles is greatest during the 3 days before the onset of rash, when the disease might not be suspected (*12*).

The consequences of measles transmission in a health care setting are serious because the infection might be transmitted to immunocompromised patients, young children, pregnant women, or other persons at high risk for severe complications. More than 50% of the case-patients in the described outbreak reported complications, and respiratory insufficiency developed in 2 patients. In addition to illness and death attributable to measles, nosocomial transmission also leads to major use of resources for evaluating and containing an outbreak (*11*).

Measles is effectively prevented by a 2-dose vaccination, and a key preventative measure against nosocomial transmission is vaccination of HCWs (*13*). In Italy, measles vaccine is recommended for all susceptible HCWs. However, HCWs are not required to show evidence of measles immunity for employment. Analysis of national measles surveillance data for Italy during October 2010–December 2011 showed that HCWs accounted for 11.6% of reported case-patients for whom information on occupation was recorded (*14*).

In addition to maintaining high coverage of vaccination in the community, ensuring that susceptible HCWs are vaccinated against measles, and maintaining a complete staff immunity database, it is essential that isolation protocols and infection control guidelines be in place in hospitals to minimize nosocomial spread of infection. Infection control measures should include maintaining a high level of awareness among staff, excluding exposed susceptible HCWs from work during the measles incubation period; immediately isolating suspected case-patients who come to an ED or any outpatient waiting area, contacting

Table. Complications in 80 case-patients during a measles outbreak linked to a cruise ship passenger, Sardinia, Italy, February–July 2014

Complication	No. (%) case-patients
Diarrhea	22 (27.5)
Keratoconjunctivitis	9 (11.3)
Hepatitis*	8 (10.0)
Otitis media	7 (8.8)
Pneumonia	7 (8.8)
Stomatitis	7 (8.8)
Laryngotracheobronchitis	3 (3.8)
Respiratory insufficiency	2 (2.5)
Other	2 (2.5)

*Increased levels of aminotransferases but no jaundice.

persons known to be exposed in hospital or outpatient (including ED) settings, offering postexposure vaccination to susceptible persons, and strengthening surveillance for nosocomially acquired cases (*7*,*10*,*11*,*15*). Stronger recommendations and guidelines are needed at the national level and in Europe.

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Transmission Models of Historical Ebola Outbreaks

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To guide the collection of data under emergent epidemic conditions, we reviewed compartmental models of historical Ebola outbreaks to determine their implications and limitations. We identified future modeling directions and propose that the minimal epidemiologic dataset for Ebola model construction comprises duration of incubation period and symptomatic period, distribution of secondary cases by infection setting, and compliance with intervention recommendations.

Mathematical models are used to generate epidemic projections under different scenarios, provide indicators of epidemic potential, and highlight essential needs for data. To aid the interventions in the 2014 Ebola epidemic in West Africa, in September 2014 we reviewed models of historical Ebola virus (EBOV) outbreaks (Table 1) and their estimated parameters (Table 2; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/14-1613-Techapp1.pdf).

The Review

Chowell et al. (*1*) developed a deterministic SEIR (susceptible-exposed-infectious-recovered) compartmental model and a stochastic continuous-time Markov chain version (Figure). A transmission coefficient, β_0, was assumed to be constant before interventions and reduced transmission after intervention at a constant rate, β_1. The model was fit to cases from the 1995 Democratic Republic of Congo (DRC) outbreak and the 2000 Uganda outbreak by using least squares. The final size was sensitive to the timing of control measures. The authors concluded that a 2-week delay in the timing of interventions would have increased the final size of the outbreak by a factor of 2.

Lekone and Finkenstädt (*4*) modified the model of Chowell et al. for discrete-time, stochastic progression. They fit their model to daily incidence and mortality time series from the 1995 DRC outbreak using Markov chain Monte Carlo. R_0 was estimated by using vague and informative prior distributions. This exercise concluded that interventions shortened the epidemic from 950 days to 200 days and reduced total number of cases from 3.5 million to just over 300. Effective reproduction number (R_E) was estimated to decrease to <1 five days after intervention onset.

Legrand et al. (*5*) accounted for transmission in different contexts through a stochastic model with 6 compartments: susceptible, exposed, infectious, hospitalized, dead-but-not-yet-buried, removed (Figure). Three transmission coefficients corresponded to community transmission, nosocomial transmission, and transmission at funerals. Interventions were assumed to be completely efficient from their onset: no transmission occurred at burials and hospitals, and community transmission was reduced by a multiplier estimated by model fitting. Parameters were estimated by fitting the model to incidence data (DRC, 1995; Uganda, 2000), by using approximate maximum likelihood, and an expression for R_0 was derived. After interventions, community transmission was estimated to have been reduced to 88% and 12% of its initial value in the DRC and Uganda outbreaks, respectively, with respective R_E of 0.4 (95% CI 0.3–0.6) and 0.3 (95% CI 0.2–0.4). The authors acknowledged that the 95% CIs around transmission and efficacy estimates were wide and conducted a sensitivity analysis of intervention parameters. This analysis indicated that community transmission was key to epidemic dynamics in Uganda, whereas funerals contributed more to transmission in the DRC. Rapid hospitalization significantly reduced community transmission and barrier nursing practices along with effective isolation of Ebola patients controlled the epidemics (*5*).

These models (*1*,*4*,*5*) shared certain features. They assumed homogeneous mixing of the population, exponentially or geometrically distributed incubation and infectious periods, and a sudden decay in transmission after intervention. None accounted for underreporting. Future exercises should explore the consequences of these assumptions. With ideal data, fitted models would be stress-tested to assess their validity, for instance challenging models to predict out-of-fit data.

Three additional studies estimated incubation period or R_0 by using statistical models. Eichner et al. (*10*) assumed a log–normally distributed incubation period. Ferrari et al. (*11*) used maximum likelihood with a chain binomial distribution and regression to estimate R_0. White and Pagano (*12*) assumed that the number of secondary cases

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[1]These authors contributed equally to this article.

Table 1. Compartmental models of historical Ebola virus outbreaks

Feature	Model		
	Chowell et al. (*1*)	Lekone and Finkenstädt (*4*)	Legrand et al. (*5*)
Outbreak*	DRC 1995, Uganda 2000†	DRC 1995‡	DRC 1995, Uganda 2000§
Assumed			
Homogeneous random mixing	Yes	Yes	Yes
All human-to-human contact	Yes	Yes	Yes
Considered			
Nosocomial transmission	No	No	Yes
Burial transmission	No	No	Yes
No. transmission parameters	2 (preintervention decays to postintervention)	1 (decay to 0)	3 (community, nosocomial, burial)
Distribution	Exponential	Geometric	Exponential
Underreporting accounted for	No	No	No

*The DRC outbreak was caused by the Zaire strain; the Uganda outbreak was caused by the Sudan strain. DRC, Democratic Republic of Congo.
†Data sources: DRC 1995 (*2*), Uganda 2000 (*3*).
‡Data source: DRC 1995 (*2*).
§Data sources: DRC 1995 (*2*,*6*–*8*), Uganda 2000 (*3*,*9*).

produced by a patient followed a Poisson distribution, with expected value R_0.

Collectively, these studies underscore that practical decisions in modeling dictate trade-offs between fitting to limited data and explicit representation of reality, including interventions. A model with a single transmission rate might fit well to data but might not be useful for decision making that evaluates intervention effects in different transmission contexts. A model with 3 transmission rates might represent transmission in community, nosocomial, and funeral contexts (e.g., [5]), but the 3 parameters are unlikely to be uniquely identifiable. Because EBOV typically amplifies during nosocomial transmission, a model with 2 transmission parameters (community transmission, comprising funeral and household transmission in 1 parameter, and nosocomial transmission) might represent the best compromise. Such a model would enable interventions, such as personal protective equipment and efficient hospitalization of persons with community-acquired EBOV infection, to be considered.

Other features are important for understanding the probable paths of small outbreaks. These include nonexponential incubation and infectious periods (*13*) and individual heterogeneity in the generation of secondary infections, including "super-spreaders" (*14*). The models reviewed are approximations to these processes. The extent to which these approximations introduce bias could be understood by developing a range of models, perhaps using versions of the chain binomial model or other generalized contagion processes.

Table 2. Estimated values of parameters as identified in the Ebola modeling articles*

Reference	Outbreak	Model	R_0 estimate	Incubation period, d (SD)†	Infectious period, d (SD)
Chowell et al. (*1*)	DRC 1995	SEIR‡	1.83 (SD 0.06)	5.3 (0.23)	5.61 (0.19)
	Uganda 2000	SEIR‡	1.34 (SD 0.03)	3.35 (0.49)	3.5 (0.67)
Lekone and Finkenstädt (*4*)	DRC 1995	SEIR, MCMC (vague prior)	1.383 (SD 0.127)	9.431 (0.620)	5.712 (0.548)
	DRC 1995	SEIR, MCMC (informative prior)	1.359 (SD 0.128)	10.11 (0.713)	6.523 (0.564)
Legrand et al. (*5*)	DRC 1995	Stochastic compartmental model (SEIHFR)	2.7 (95% CI 1.9–2.8)		
	Uganda 2000	Stochastic compartmental model (SEIHFR)	2.7 (95% CI 2.5–4.1)		
Eichner et al. (*10*)	DRC 1995	Incubation period estimate based on parameterized lognormal distribution function		12.7 (4.31)	
Ferrari et al. (*11*)	DRC 1995	MLE	3.65 (95% CI 3.05–4.33)		
	DRC 1995	Regression	3.07§		
	Uganda 2000	MLE	1.79 (95% CI 1.52–2.30)		
	Uganda 2000	Regression	2.13§		
White and Pagano (*12*)	DRC 1995	MLE	1.93 (95% CI 1.74–2.78)		

*DRC, Democratic Republic of Congo; MCMC: Markov chain Monte Carlo; MLE, maximum-likelihood estimation; SEIR, susceptible-exposed-infectious-removed; SEIHFR, susceptible-exposed-infectious-hospitalized-funeral-removed. Blank cells indicate that no information was provided from the original study.
†The incubation period for Ebola virus is believed to be the same as its latent period, i.e., infected persons become infectious only when symptomatic.
‡Combination differential equation model and Markov chain model.
§Neither CIs nor SDs were provided in the study.

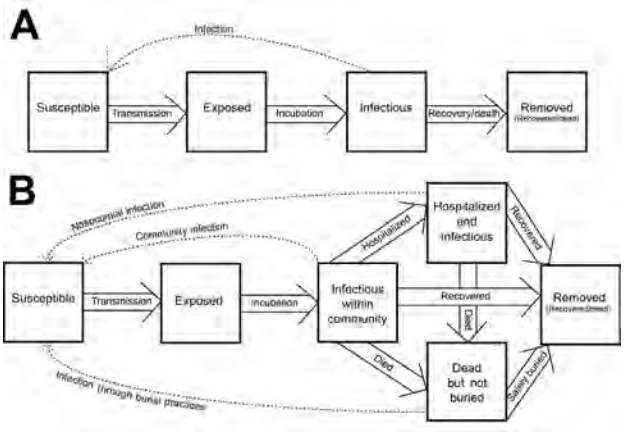

Figure. Conceptual diagrams illustrating Ebola SEIR and SEIHFR models of historical Ebola virus outbreaks. SEIR, susceptible-exposed-infectious-removed; SEIHFR, susceptible-exposed-infectious-hospitalized-funeral-removed.

Another issue that has not been studied is the role of spatial scale. All extensive EBOV outbreaks involved multiple scales of transmission. At the smallest scale, persons most at risk for infection are those caring for an Ebola patient. Understanding these household contacts helps estimate outbreak size. Human settlements constitute a "household of households." Transmission occurs among households in communities, at hospitals, or at funerals. Understanding these between-household contacts is needed to determine the outbreak's extent. Finally, understanding connections between settlements by human movements is needed to determine the paths and speed of large-scale spatial spread and therefore the total infected area and domain for surveillance and monitoring. Although the assumption of population homogeneity can be justified for models of historical EBOV outbreaks, given the limited geographic extent of those outbreaks, models for the 2014 outbreak might need to address heterogeneity in population density and human movements because of the extensive geography involved.

Two issues new to the 2014 EBOV epidemic are underreporting and compliance. To assess underreporting, perhaps comprehensive contact tracing can be performed in a small number of locales and extrapolated. If cases can be identified through 2 independent routes, then case matching can be used to identify the total number of cases (*15*). Concerning compliance, the fraction of patients admitted to hospitals and, of those remaining in the community, the fraction of decedents with safe burials should be identified. Compliance of personal protective equipment among health care workers is central to understanding the role of nosocomial transmission.

Conclusions

Model fitting is craft as well as science. Modeling demands decisions, including what mathematical representations to use, the type and magnitude of variation to be considered, and the values that can be taken as given versus the values still to be estimated. In the face of data scarcity, we suggest that construction of models of the 2014 outbreak would have benefited from a minimal dataset that included 1) the mean and variance of the incubation period and symptomatic period, respectively; 2) the probability distribution of secondary cases by infection setting; and 3) compliance with recommendations. For secondary cases, in addition to the average, the commonness of outliers (super-spreaders), the frequency of zeros, and the variance in the distribution need to be known.

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Multidrug-Resistant Tuberculosis in Patients for Whom First-Line Treatment Failed, Mongolia, 2010–2011

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In Ulaanbaatar, Mongolia, multidrug-resistant tuberculosis (MDR TB) was diagnosed for more than a third of new sputum smear–positive tuberculosis patients for whom treatment had failed. This finding suggests a significant risk for community-acquired MDR TB and a need to make rapid molecular drug susceptibility testing available to more people.

In many resource-limited settings, the high cost and technical complexity of drug susceptibility testing (DST) preclude its routine use for patients in whom sputum smear–positive tuberculosis (TB) has been newly diagnosed. This lack of testing is particularly problematic in settings in which prevalence of multidrug-resistant (MDR) TB (resistant to at least isoniazid and rifampin) is high. Delayed diagnosis and inappropriate treatment prolong the patient's interval of infectiousness and decrease the prospect of treatment success (*1*). Treating MDR TB with inappropriate drug regimens also increases the risk of amplifying drug resistance (*2*–*5*).

In Mongolia, failure of standard first-line TB treatment among patients with diagnosed MDR TB increased from 12% in 2006 to 38% in 2012 (Mongolian National TB Program [NTP], unpub. data; *6*). During the same period, the proportion of new TB patients with MDR TB increased from 0% to 17%. Although these findings partly reflect the implementation of improved MDR TB case-finding strategies, they may also reflect increased MDR TB transmission within the community. In this study, we aimed to determine the prevalence of MDR TB among new sputum smear–positive patients for whom first-line treatment failed and to evaluate factors associated with an increased risk for MDR TB among these patients.

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The Study

Mongolia's Guidelines on Tuberculosis Care and Services recommend DST for patients with newly diagnosed TB when they remain sputum smear-positive after 3 months of TB treatment, when they are in close contact with someone with drug-resistant TB, or when they are co-infected with HIV (Ministry of Health Mongolia, World Health Organization, Global Fund Supported Project on AIDS and TB, unpub. data). We performed a retrospective cohort study of all new sputum smear–positive patients who began directly observed therapy for TB in Ulaanbaatar during 2010 or 2011. HIV-infected patients and those with close contact with MDR TB patients were excluded.

About 45% of the population of Mongolia lives in Ulaanbaatar, the country's capital. TB cases were reported to Mongolia's NTP database from 9 districts of Ulaanbaatar, a prison hospital, and a hospital for the homeless. Cases that were subsequently diagnosed as MDR TB were identified from the NTP MDR TB database. Cases were excluded from the analysis if standard first-line treatment was altered for any reason.

New sputum smear–positive TB cases were defined as cases in patients who had ≥1 acid-fast bacillus in ≥1 sputum sample and who had never received TB treatment before. These patients were given routine first-line treatment, consisting of isoniazid, rifampin, pyrazinamide, and ethambutol during a 2-month intensive phase followed by isoniazid and rifampin during a 4-month continuation phase. MDR TB was diagnosed if the *Mycobacterium tuberculosis* strain isolated was resistant to at least isoniazid and rifampin. Treatment outcomes were defined according to World Health Organization definitions (*6*).

Sputum samples were processed at the Mongolia National Reference TB Laboratory, which used solid cultures (Löwenstein-Jensen medium and Ogawa) and BACTEC Mycobacteria Growth Indicator Tube 960 (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) liquid cultures during the study period. Phenotypic DST was performed by using the BACTEC Mycobacteria Growth Indicator Tube and Löwenstein-Jensen medium (*7*,*8*). Susceptibility to isoniazid, rifampin, ethambutol, and streptomycin was tested. The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) was not available in Mongolia before 2013.

In total, 1,920 new sputum smear–positive patients were identified during the study period; 45 were excluded

Table 1. Characteristics of patients with new sputum smear–positive TB and those for whom first-line treatment failed

Characteristics	Patients with new TB cases, no. (%)	Patients for whom TB treatment failed, no. (%)*	Patients with MDR TB, no. (%)†
All	1,875 (100)	156 (8.3)	54 (34.6)
Sex			
M	1,071 (57.1)	96 (9.0)	27 (28.1)
F	804 (42.9)	60 (7.5)	27 (45.0)
Age, y			
<15	10 (0.5)	2 (20.0)	1 (50.0)
15–34	1,097 (58.5)	82 (7.5)	38 (46.3)
35–54	611 (32.6)	61 (10.0)	14 (23.0)
≥55	155 (8.3)	11 (7.1)	1 (9.1)
Missing	2 (0.1)	0	0
Occupation			
Employed, including self-employed	476 (25.4)	29 (6.1)	16 (55.2)
Unemployed	774 (41.3)	68 (8.8)	19 (27.9)
Retired	123 (6.6)	11 (8.9)	1 (9.1)
Student‡	240 (12.8)	15 (6.3)	11 (73.3)
School-age§	58 (3.1)	6 (10.3)	1 (16.7)
On disability pension	70 (3.7)	8 (11.4)	1 (12.5)
In prison	50 (2.7)	8 (16.0)	1 (12.5)
Homeless	63 (3.4)	10 (15.9)	3 (30.0)
Unknown	21 (1.1)	1 (4.8)	1 (100)
Treatment facility/district			
Bayangol	222 (11.8)	15 (6.8)	10 (66.7)
Bayanzurkh	392 (20.9)	52 (13.3)	14 (26.9)
Songinokhairkhan	426 (22.7)	28 (6.6)	7 (25.0)
Sukhbaatar	203 (10.8)	10 (4.9)	4 (40.0)
Khan-Uul	176 (9.4)	17 (9.7)	9 (52.9)
Chingeltei	261 (13.9)	13 (5.0)	6 (46.2)
Prison hospital	50 (2.7)	8 (16.0)	1 (12.5)
Enerel, hospital for the homeless	63 (3.4)	10 (15.9)	3 (30.0)
Other¶	82 (4.4)	3 (3.7)	0

*Percentage of patients with new sputum smear–positive TB.
†MDR TB, multidrug–resistant tuberculosis (resistant to isoniazid and rifampin). Percentage of patients for whom treatment failed.
‡Student, enrolled in higher education or vocational training.
§School-age, enrolled in primary or secondary school.
¶Districts with <50 new sputum smear–positive patients (Baganuur, Nalaikh, Bagakhangai).

from the analysis because they did not receive standard first-line treatment because of adverse drug effects or drug shortages. Table 1 summarizes the demographic characteristics of all 1,875 patients included in the study. Among these patients, 476 (25%) were employed; 63 (3%) were homeless, and 50 (3%) were prisoners. The median age was 31 years (range 12–97 years).

Successful treatment outcomes fell short of World Health Organization targets. A total of 1,436 (77%) patients were cured, and 102 (5%) completed treatment (*6*), but for 156 (8%) patients, first-line treatment failed. An additional 34 (2%) patients were transferred out (i.e., transferred to a different reporting unit with unknown treatment outcome), 41 (2%) died, and 106 (6%) interrupted treatment for ≥2 consecutive months. Treatment failure rates were highest among adults 35–54 years of age, prisoners, and those who were homeless (Table 1).

Among the 1,875 new sputum smear–positive case-patients, MDR TB was diagnosed for 66 (4%). Of these 66 patients, treatment failure was designated for 54 (82%). Therefore, of the 156 total patients for whom first-line treatment failed, 54 (35%) had MDR TB. Bivariate analysis showed that MDR TB among patients in whom first-line treatment failed was significantly associated with being female (odds ratio [OR] 2.1, 95% CI 1.1–4.1), <35 years of age (OR 3.3, 95% CI 1.6–6.7), and employed or a student (OR 5.1, 95% 2.4–10.8) (Table 2). These associations remained significant after adjusting for sex, age, and occupation (Table 2). For 32 (59%) MDR TB patients for whom first-line treatment failed, complete resistance to all 4 first-line drugs tested (isoniazid, rifampin, ethambutol, and streptomycin) was found (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/21/8/14-1860-Techapp1.pdf).

Conclusions

In Ulaanbaatar, MDR TB was diagnosed for more than a third of sputum smear–positive patients in whom standard first-line TB treatment failed. Resistance against all first-line drugs tested was found for ≈60% of these patients. This finding suggests successful transmission of these highly resistant strains, as has been documented in other MDR TB–endemic areas (*9*). A high level of streptomycin resistance among patients in whom first-line treatment has failed indicates that use of the standard retreatment regimen prolongs the duration of ineffective treatment and should be abandoned. Apart from poor patient outcomes

Table 2. Bivariate and multivariate analysis of factors associated with multidrug-resistant TB among patients for whom first-line tuberculosis treatment failed

Characteristic	MDR TB cases/ treatment failures*	Unadjusted odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)†	p value
Sex					
M	27/96	1.00 (Reference)		1.00 (Reference)	
F	27/60	2.09 (1.06-4.11)	0.032	2.19 (1.01-4.74)	0.047
Age, y					
≥35	15/72	1.00 (Reference)		1.00 (Reference)	
<35	39/84	3.29 (1.62-6.71)	0.001	2.42 (1.11-5.27)	0.026
Occupation					
Unemployed, prisoner, homeless	25/105	1.00 (Reference)		1.00 (Reference)	
Employed, student	27/44	5.08 (2.39-10.81)	<0.001	4.59 (2.04-10.31)	<0.001

*MDR TB, multidrug-resistant tuberculosis.
†Multivariate analysis adjusted for gender, age, and occupation. Persons <18 years of age were excluded when we adjusted for occupation.

and the risk for ongoing TB transmission, continued use of an inadequate treatment regimen encourages the amplification of drug resistance.

Ideally, universal DST would be offered at the time of diagnosis, but in the absence of sufficient resources, increased use of rapid molecular diagnostics should be considered. Despite implementation hurdles, the Xpert MTB/RIF assay rapidly confirms *M. tuberculosis* infection and assesses resistance to rifampin without the need for extensive laboratory infrastructure (*10*). Rapid testing should be considered for all patients in whom MDR TB is suspected, including those for whom sputum smears did not convert to negative after 2–3 months of first-line treatment. Prompt initiation of appropriate therapy should improve patient outcomes and reduce ongoing MDR TB transmission within the community. In this context, the addition of spatial information and accurate mapping of MDR TB hot spots within Mongolia may guide targeted strategies for early detection and treatment for MDR TB.

Our study does have limitations. DST was performed at the discretion of the treating physician, and detection bias may have influenced MDR TB risk factor analyses. Because not everyone was tested, the reported MDR TB rate represents a minimum estimate. Without DST results from specimens collected before treatment initiation, we cannot provide definite proof of transmitted (primary) MDR TB. However, although a patient can acquire MDR TB after 2–3 months of TB treatment, acquisition of MDR TB is unlikely if quality-assured multidrug treatment and directly observed therapy are used. The conclusion that most cases represented primary MDR TB not detected when the patient originally sought treatment is further supported by the high rate of resistance against all first-line drugs. Comparison with previous drug resistance surveys indicates that the proportion of MDR TB cases among new sputum smear–positive patients increased from 1.0% (4/405) during 1998–99 (*11*) to 1.4% (9/650) in 2007 (*12*) to 3.4% (66/1920) in our study. This increase in transmitted drug-resistant TB requires closer scrutiny and concerted global action (*13*).

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Geographic Distribution and Expansion of Human Lyme Disease, United States

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Lyme disease occurs in specific geographic regions of the United States. We present a method for defining high-risk counties based on observed versus expected number of reported human Lyme disease cases. Applying this method to successive periods shows substantial geographic expansion of counties at high risk for Lyme disease.

Lyme disease is a multisystem tickborne zoonosis caused by infection with the spirochete *Borrelia burgdorferi* (*1*,*2*). Since 1991, state and territorial health departments have reported human Lyme disease cases to the Centers for Disease Control and Prevention through the National Notifiable Diseases Surveillance System. Most cases are reported from the northeastern, mid-Atlantic, and north-central states, although the number of jurisdictions that report a high number of cases has increased over time (*3*). To better quantify and track the geographic distribution of human Lyme disease, we developed a simple but robust method for defining counties where residents have a high risk of acquiring this disease.

The Study

Counties with a high incidence of Lyme disease were identified by using SaTScan version 9.1.1 (*4*). Numbers of confirmed Lyme disease cases reported at the county level during 1993–2012 were aggregated into 5-year intervals (1993–1997, 1998–2002, 2003–2007, 2008–2012) to minimize the influence of travel-associated cases and short-term changes in surveillance practices. Incidence was calculated by using each county's average population at risk, which was estimated from US Census data for the midpoint of each period (i.e., 1995, 2000, 2005, and 2010). Identification of high-risk clusters was based on county incidence rates, with a maximum possible cluster size equal to 25% of the US population (minimum size was 1 county). County centroids were used as geographic reference for analyses. During the study period, 3 different surveillance case definitions were used (i.e., those established in 1991, 1996, and 2008) (*5*).

Relative risk (RR) was defined as the observed number of cases divided by the expected number of cases for a specific period and population size, and adjusted for differences in the population at risk across space (*4*). Calculations were based on a discrete Poisson probability distribution. RR was calculated for potential clusters and for individual counties within detected clusters. Statistical significance of possible clusters was determined by using likelihood ratio tests and standard Monte Carlo hypothesis testing (n = 999 replications) (*4*).

Because of the circular shape used in spatial scanning, not all counties within an identified high-risk cluster were necessarily characterized by high Lyme disease incidence. Some may have been included because they share a border with a county having high incidence. Ultimately, counties designated as high incidence were those within a defined, statistically significant high-risk spatial cluster ($\alpha = 0.05$) and with a county-specific RR $\geq$2.0.

In each period, 2 major foci of largely contiguous counties met the high-incidence county designation: 1 in the northeastern United States and 1 in the north-central United States (Figure). During the first 5-year period (1993–1997), 69 counties were characterized as having high incidence of Lyme disease, including 4 isolated counties in the southeastern United States (Table; Figure). During the next period (1998–2002), 130 counties were characterized as having high incidence, and the 4 counties in the southeastern United States ceased to meet the criteria for this designation. During the third and fourth periods (2003–2007 and 2008–2012), 197 and 260 counties, respectively, were characterized as having high incidence (Table; Figure).

Over time, the number of counties in the northeastern states identified as having high incidence of Lyme disease increased >320%: from 43 (1993–1997) to 90 (1998–2002) to 130 (2003–2007) to 182 (2008–2012). In the north-central states for the same periods, the number of counties having high incidence increased ≈250%, from 22 to 40 to 67 to 78. In each of the latter periods, a small number of counties previously identified as having high incidence ceased to meet the criteria; however, most remained above the threshold during all periods assessed (Table).

The county geographic center of each major focus was calculated according to Euclidean distances between county centroids by using ArcGIS 9.3 (Environmental Systems Research Institute, Redlands, CA, USA). The center of the high-incidence focus in the northeastern United States generally moved westward and northward, away from the coast of northern New Jersey and into east-central Pennsylvania.

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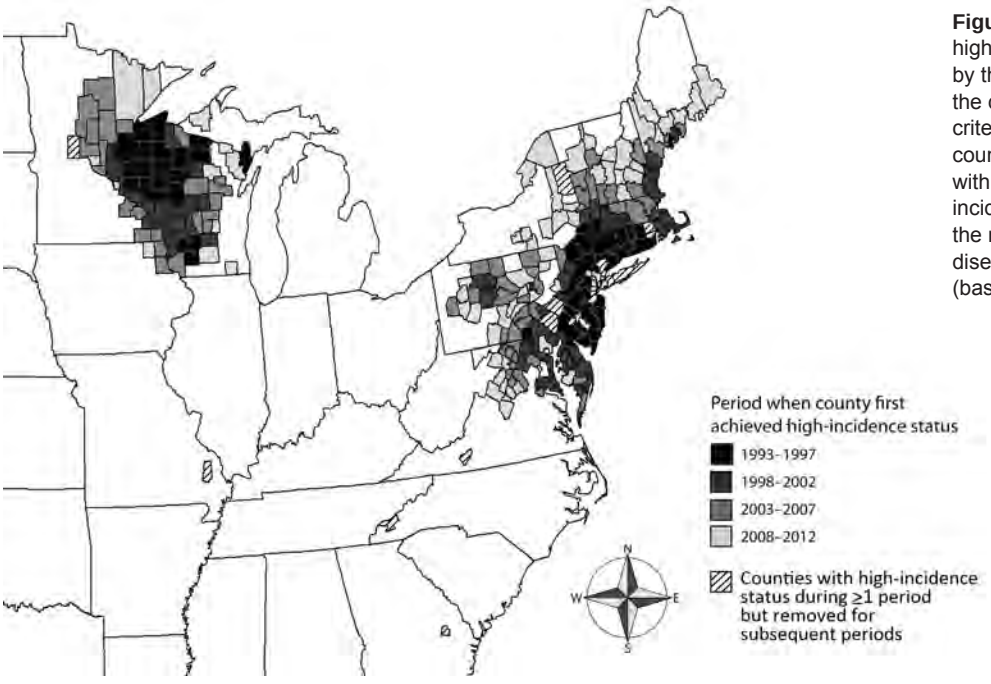

Figure. United States counties with high incidence of Lyme disease by the period when they first met the designated high-incidence criteria, 1993–2012. High-incidence counties were defined as those within a spatial cluster of elevated incidence and those with ≥2 times the number of reported Lyme disease cases as were expected (based on the population at risk).

In the north-central high-incidence focus, the geographic center remained relatively stable in northwestern Wisconsin, moving northward and southward between adjacent counties over time.

Conclusions

We describe a simple measure for objectively defining counties having high incidence of Lyme disease. Systematic application of this method to 4 consecutive periods showed geographic expansion of high-risk areas. Despite the substantial increase in the number of counties with high incidence, the limited movement of the geographic centers suggests relatively constant rates of geographic expansion in all accessible directions.

Although risk maps for Lyme disease have been developed on the basis of entomologic measures such as density of and infection prevalence in nymphal *Ixodes scapularis* vector ticks, these measures do not uniformly predict risk of human Lyme disease (*6*,*7*). Prior analyses of temporal trends in human Lyme disease surveillance have not been explicitly spatial or have been conducted by using data from a single state (*8*–*13*).

Table. Data for United States counties with high incidence of human Lyme disease during four 5-year periods, 1993–2012*

Location, period	No. counties	Relative risk, range†	Average annual incidence, range‡	No. counties added to high-incidence status	No. counties removed from high-incidence status
Overall					
1993–1997	69	2.3–91.1	10.6–402.7	NA	NA
1998–2002	130	2.0–152.6	12.3–912.9	71	10
2003–2007	197	2.0–101.3	15.0–742.8	72	5
2008–2012	260	2.0–48.6	15.9–381.4	72	9
Northeastern focus					
1993–1997	43	2.3–91.1	10.6–402.7	NA	NA
1998–2002	90	2.0–152.6	12.3–912.9	50	3
2003–2007	130	2.0–101.3	15.0–742.8	45	5
2008–2012	182	2.0–48.6	15.9–381.4	60	8
North-central focus					
1993–1997	22	2.6–41.3	12.1–189.6	NA	NA
1998–2002	40	2.0–35.3	12.4–217.3	21	3
2003–2007	67	2.0–29.8	15.0–222.7	27	0
2008–2012	78	2.1–28.1	16.1–220.7	12	1

*In the first period, 4 counties in the southeastern United States met high-incidence criteria but are not included in the geographic focus–specific data. NA, not applicable.
†Relative risk is observed number of cases over a period divided by expected number of cases for the population at risk (*4*). For this analysis, high-incidence counties had a relative risk of ≥2.0.
‡Incidence is reported cases per 100,000 residents per year; population used was average population at risk during a period (US census data for midpoint of each period).

Surveillance data are subject to several limitations, including changing surveillance case definitions, availability of public health resources for surveillance, variations in surveillance practices, and reporting based on county of residence instead of county of exposure. Nevertheless, in accordance with the purpose of public health surveillance, these data provide valuable information about the magnitude and geographic distribution of areas in the United States where residents are at high risk of acquiring Lyme disease (*5*,*14*).

Four counties in the southeastern United States had high incidence of human Lyme disease during the early years of national surveillance but subsequently had low incidence. This circumstance may reflect improved standardization of diagnostic procedures and a recognition that another condition, southern tick-associated rash illness (also known as STARI), occurs in the region. Patients with this illness have rash similar to that of Lyme disease, but the condition is not caused by *B. burgdorferi* bacteria *(15)*. The ability to identify these isolated counties shows that our method is not biased toward detecting only counties near areas with high incidence of Lyme disease.

A true reduction in human risk for Lyme disease or changes in surveillance practices may have influenced the small number of counties meeting high-risk criteria during 1 period but not in subsequent periods. The RR cutoff of 2.0 was arbitrarily chosen to capture counties with not just elevated risk but a substantially higher risk for disease than other counties. The overall pattern of expansion in each period was similar when RR cutoffs of 1.5 and 3.0 were used (data not shown). However, using different RR thresholds to define high incidence changes the number of counties that meet the high-incidence criteria. This variation underscores that risk can be elevated in areas that fail to meet our high-incidence threshold.

Risk for encounters with infected ticks, even within high-incidence counties, is influenced by human behavior and varying landscape characteristics that impact tick abundance and small mammal species composition. Geographic expansion of high-risk areas may occur because of changes in conditions that favor tick survival or because of geographic dispersal of infected ticks by birds and deer to areas where other necessary components already exist to support ongoing transmission. Our results show that geographic expansion of high-risk areas is ongoing, emphasizing the need to identify broadly implementable and effective public health interventions to prevent human Lyme disease.

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Risk Factors for Serogroup C Meningococcal Disease during Outbreak among Men who Have Sex with Men, New York City, New York, USA

Alison Ridpath, Sharon K. Greene, Byron F. Robinson, Don Weiss; and the Meningococcal Investigation Team[1]

Risk factors for illness during a serogroup C meningococcal disease outbreak among men who have sex with men in New York City, New York, USA, in 2012–2013 included methamphetamine and cocaine use and sexually transmitted infections. Outbreak investigations should consider routinely capturing information regarding drug use and sex-related risk factors.

In the United States, meningococcal disease, a nationally reportable bacterial disease caused by *Neisseria meningitidis*, has a case-fatality rate of 10%–15% (*1*). Clusters of serogroup C meningococcal disease have been reported among men who have sex with men (MSM) in Chicago, Illinois, USA; Toronto, Ontario, Canada; and Europe (*2*–*4*). However, case–control studies to evaluate unique risk factors among this population are lacking.

During August 2010–February 2013, New York City (NYC), New York, had a protracted outbreak of serogroup C meningococcal disease among MSM, which is described elsewhere in this issue (*5*). To more fully understand risk factors associated with the outbreak, the NYC Department of Health and Mental Hygiene (DOHMH) conducted a case–control study.

The Study

An outbreak case was defined as an illness clinically compatible with serogroup C meningococcal disease meeting the 2010 Council of State and Territorial Epidemiologists case definition for a confirmed or probable case (*6*) with onset during January 2012–February 2013 in NYC male residents self-identifying as gay or bisexual or reporting sexual contact with another man during the previous year. Meningococcal disease case investigations include interviews with the patient (when possible), health care providers, family members, and close contacts and review of the patient's medical records. Controls were selected from NYC male residents given a diagnosis of infection with *Giardia lamblia* (giardiasis) or *Entamoeba histolytica* (amebiasis) during January 2012–February 2013, who had not been routinely interviewed by DOHMH. Three controls were matched to each case-patient for age at disease diagnosis (± 5 years) and diagnosis date (± 1 month). Controls were ineligible if they were not NYC residents, were non-English speaking, had invasive meningococcal disease during the study, or were non-MSM.

During March–April 2013, case-patients and controls were interviewed by telephone by using a 20-min questionnaire after informed consent was obtained. The questionnaire elicited information regarding demographic features and lifestyle (e.g., alcohol and drug use, smoking, bar and party attendance, and number and ways of meeting sexual contacts). Case-patients and controls were asked about exposures during the 30 days before illness onset. Questionnaires for deceased or unreachable case-patients were completed on the basis of information obtained during initial investigation. If drug use or sexual contact could not be determined, values for these patients were considered unknown and associated data were excluded from analysis. HIV and sexually transmitted infections (STIs) (i.e., chlamydia, gonorrhea, and syphilis) for case-patients and controls were obtained from DOHMH registries on June 5, 2013.

All epidemiologic data were entered into a Microsoft Access 2010 database (Microsoft, Redmond, WA, USA). Statistical analyses were conducted by using SAS version 9.2 (SAS Institute, Cary, NS, USA). Matched odds ratios and 95% CIs were calculated by using conditional logistic regression and the mid-p exact method (*7*). Median unbiased estimates of the odds ratio were reported when warranted. HIV infection, previously reported to be a risk factor for meningococcal disease (*8*), was controlled for in the analysis.

Seventeen outbreak cases occurred during 2012 (n = 13) and 2013 (n = 4). Among 11 surviving serogroup C meningococcal disease case-patients, 10 were re-interviewed and 1 could not be reached. Of 90 possible controls, 51 eligible controls completed interviews. Of the remaining 39 possible controls, 6 were unreachable (after ≥5 call attempts, including evenings or weekends); 11 refused; 21 were ineligible (12 were non-MSM, 6 were non-English speakers, 2 were non-NYC residents, and 1 had a false-positive report); and 1 did not complete the interview. Unmatched characteristics of case-patients and controls are shown in Table 1. Most case-patients were Brooklyn residents, black, and

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Table 1. Characteristics of case-patients with outbreak-related serogroup C meningococcal disease and controls with amebiasis or giardiasis, New York City, New York, USA, 2012–2013*

Characteristic	Case-patients, n = 17	Controls, n = 51
Median age, y (range)	32 (21–59)	30 (22–59)
Borough (1 undomiciled case-patient excluded)		
Brooklyn	9/16 (56.3)	11/51 (21.6)
Manhattan	5/16 (31.3)	30/51 (58.8)
Queens or Bronx	2/16 (12.5)	10/51 (19.6)
Sexual orientation (self-identified)		
Gay	15/17 (88.2)	45/50 (90.0)
Bisexual	2/17 (11.8)	5/50 (10.0)
Race		
Black	10/17 (58.8)	5/50 (10.0)
White	5/17 (29.4)	42/50 (84.0)
Other	2/17 (11.8)	3/50 (6.0)
Hispanic	3/17 (17.6)	9/51 (17.6)
Employed	12/17 (70.6)	41/51 (80.4)
Annual household income		
≤$29,999	9/13 (69.3)	15/46 (32.6)
$30,000–$59,999	3/13 (23.1)	8/46 (17.4)
≥$60,000	1/13 (7.7)	23/46 (50.0)
Health insurance		
Yes	9/14 (64.3)	48/51 (94.1)
No	5/14 (35.7)	3/51 (5.9)
Education		
High school, GED, or less	5/15 (33.3)	5/50 (10.0)
At least some college	10/15 (66.7)	45/50 (90.0)
HIV infected	10/17 (58.8)	13/51 (25.5)

*Values are no. responded/total (%) unless otherwise indicated. Denominators exclude unknown and refused answers. GED, general educational development.

HIV infected, and most controls were Manhattan residents, white, and non–HIV infected. Case-patients appeared to be of lower socioeconomic status, as indicated by income, education, and health insurance status.

Matched odds ratios (crude and adjusted) are shown in Table 2. After we adjusted for HIV infection, exposures that remained independently associated with serogroup C meningococcal disease were black race, methamphetamine or cocaine use during the month before illness onset, and STI during the year before diagnosis. During the month before illness onset, tobacco smoking, sharing drinks, having sex with >1 man, or meeting a sex partner online or at a bar or party, although more common among case-patients, were not major risk factors during this outbreak.

No case-patients had documentation of meningococcal vaccine before illness onset. We were unable to document vaccine receipt in controls, which limited our ability to examine the effect meningococcal vaccination may have had on the risk for meningococcal infection during this outbreak.

Conclusions

Although rates of methamphetamine and cocaine use are not known specifically among MSM in NYC, during 2008–2009, a total of 3% of the general NYC population reported past-year cocaine use (*9*), which was much lower than the 29% reported use among case-patients. Through inhalation, these drugs can damage respiratory mucosa and increase susceptibility to meningococcal disease (*10*). In addition, drug use can be a social activity involving equipment sharing among users, thus increasing respiratory secretion exposure.

Chlamydia, gonorrhea, or syphilis during the year before diagnosis was also a risk factor during this outbreak, despite controls having been selected on the basis of having a disease (giardiasis or amebiasis) that can be transmitted sexually (*11*,*12*). Controls might have been overmatched for sexual behavior (i.e., number of sexual partners or high-risk sexual practices) to case-patients. Whether the source of exposure during MSM sexual contact is through oropharyngeal secretions or represents a novel mechanism (e.g., rectal carriage of *N. meningitidis*) is unknown. More research is needed to better understand the apparent overlap of STIs and meningococcal disease among MSM. This investigation highlights the usefulness for public health practice of collecting and recording information regarding sexual behavior among meningococcal patients, specifically cases among MSM.

Although black race is not a risk factor for meningococcal disease (*1*), black race appeared to be a risk factor during this outbreak and was also common (75%) among patients during a 2005–2006 outbreak in Brooklyn (*13*). Race might be a proxy for a social network or an unidentified cofactor of this outbreak and not a biological risk factor for meningococcal disease. Determining risk factors for infection to target prevention measures during an ongoing outbreak is challenging, and public health authorities often cannot wait for results of epidemiologic studies before

Table 2. Risk factors for meningococcal disease among case-patients with outbreak-related serogroup C meningococcal disease and controls with amebiasis or giardiasis, New York City, New York, USA, 2012–2013*

Variable	Case-patients, no. responded/total (%), n = 17	Controls, no. responded/total (%), n = 51	Crude matched odds ratio (95% CI)	Matched odds ratio adjusted for HIV infection (95% CI)
Black race	10/17 (58.8)	5/50 (10.0)	**12.0 (2.8–81.6)**	**8.0 (1.6–63.7)**
Household with >1 other person	9/16 (56.3)	10/51 (19.6)	**5.6 (1.5–27.0)**	3.7 (1.0–18.0)
Tobacco smoking	6/17 (35.3)	14/50 (28.0)	1.4 (0.4–4.5)	0.9 (0.2–3.3)
Shared a drink	5/11 (45.5)	14/39 (35.9)	1.1 (0.2–4.8)	1.2 (0.3–5.5)
Drug use in month before illness onset				
Marijuana	3/15 (20.0)	13/51 (25.5)	0.7 (0.1–2.9)	0.5 (0.1–2.4)
Methamphetamine	7/17 (41.2)†	0/50 (0)	**28.8 (5.6–∞)‡**	**16.6 (3.1–∞)‡**
Cocaine	4/14 (28.6)	0/51 (0)	**15.9 (2.7–∞)‡**	**11.2 (1.8–∞)‡**
Sexual risk				
Sex with >1 man during month before illness onset	8/13 (61.5)	18/51 (35.3)	2.6 (0.7–10.7)	2.8 (0.7–13.7)
Met a male sex partner during month before illness onset online or at bar or party versus other ways§	7/10 (70.0)	21/40 (52.5)	2.1 (0.5–11.6)	1.8 (0.4–10.6)
Chlamydia, gonorrhea, or syphilis diagnoses during the year before diagnosis date¶	9/17 (52.9)	6/51 (11.8)	**7.2 (2.0–33.8)**	**4.7 (1.2–23.5)**
HIV infected	10/17 (58.8)	13/51 (25.5)	**6.4 (1.5–45.1)**	NA

*Denominators exclude unknown and refused answers. Values in bold indicate mid-p exact p<0.05. NA, not applicable.
†One patient answered don't know, but laboratory testing at hospital indicated methamphetamine use.
‡Median unbiased estimate of the odds ratio.
§Other ways of meeting included work, school, through a friend, gym, sports group, sex party, or other.
¶Diagnosis date refers to the diagnosis date of serogroup C meningococcal disease for case-patients and diagnosis date of amebiasis or giardiasis for controls.

acting. Previously identified risk factors (e.g., smoking, household crowding, or sharing drinks) (*14*,*15*) and behaviors used to target our vaccine recommendations (i.e., meeting men for sex at a bar, party, online, or through digital applications [*5*]) were not associated with this outbreak but were more common among case-patients and might still be associated with meningococcal disease. This study might have had insufficient power to detect such differences.

This case–control study, conducted in the context of a prolonged outbreak of serogroup C meningococcal disease among MSM in NYC, identified 2 key risk factors: having an STI during the year before diagnosis and having used methamphetamine or cocaine during the month before illness onset. We hypothesize these factors might be associated with membership in a common social network in which carriage of serogroup C meningococci is increased (*13*). Consideration can be given to collecting drug use and sexual identity data through routine meningococcal disease surveillance to recognize transmission clusters and to more fully understand if these risk factors are generalizable. Future studies of meningococcal disease outbreaks should consider including assessment of these risk factors.

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Infections with *Candidatus* Neoehrlichia mikurensis and Cytokine Responses in 2 Persons Bitten by Ticks, Sweden

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The prevalence of *Candidatus* Neoehrlichia mikurensis infection was determined in 102 persons bitten by ticks in Sweden. Two infected women had erythematous rashes; 1 was co-infected with a *Borrelia* sp., and the other showed seroconversion for *Anaplasma phagocytophilum*. Both patients had increased levels of *Neoehrlichia* DNA and serum cytokines for several months.

Candidatus Neoehrlichia mikurensis is a tick-borne pathogen found in Europe and Asia (*1*). It causes an infectious disease in immunocompromised persons that is characterized by fever and thromboembolic events (*2*). In contrast, *Candidatus* N. mikurensis infection in immunocompetent hosts has been linked to asymptomatic infection (*3*), systemic inflammation with various symptoms (*4*,*5*), and possibly lethal infection (*6*). Knowledge regarding the capacity of *Candidatus* N. mikurensis to cause disease in immunocompetent persons is still limited. The purpose of this study was to investigate the prevalence, rate of co-infections, clinical picture, and cytokine response to *Candidatus* N. mikurensis infection in immunocompetent patients participating in the Tick-Borne Diseases Study (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/15-0060-Techapp1.pdf).

The Study

The study was approved by the Ethics Committees of Linköping University (M132-06), and Åland Health Care (2008-05-23). DNA was robot-extracted (MagNA Pure Compact Extraction Robot; Roche, Basel, Switzerland) from 400 μL of EDTA-plasma (Nucleic Acid Isolation Kit I; Roche) and analyzed by using a real-time PCR specific for a 169-bp segment of the *groEL* gene of *Candidatus* N. mikurensis. Amplifications were performed in a 20-μL reaction mixture containing 1× FastStart Taqman Probe Master (Roche), 1 μmol/L of each primer (5′-CGG AAA TAA CAA AAG ATG GA-3′; 5′- ACC TCC TCG ATT ACT TTA G-3′), 100 nmol/L of probe (5′-6FAM-TTG GTG ATG GAA CTA CA-MGB-3′), and 4 μL of DNA template. Real-time PCR was performed by using Rotorgene 6000 (QIAGEN, Hilden, Germany). Reaction conditions were 95°C for 10 min, followed by 45 cycles at 95°C for 15 s, and a final cycle at 54°C for 1 min. A synthetic plasmid containing the 169-bp sequence cloned into a pUC57 vector (Genscript, Piscataway, NJ, USA) was used to estimate bacterial gene copy numbers. Positive samples were verified by using a pan-bacterial PCR specific for the 16S rRNA gene (online Technical Appendix). All PCR products were sequenced after electrophoresis on 2% agarose gels and analyzed by using an ABI PRISM 3130 Genetic Analyzer (Life Technologies Europe BV, Bleiswijk, the Netherlands). Obtained DNA sequences were edited and further analyzed by using the GenBank BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Ripseq mixed software (Isentio, Palo Alto, CA, USA).

Patient serum samples were analyzed for antibodies against *Borrelia burgdorferi* sensu lato by using the RecomBead *Borrelia* IgM and IgG Kit (Mikrogen Diagnostik, Neuried, Germany). Samples were analyzed for IgG against *Anaplasma phagocytophilum* by using the *A. phagocytophilum* IFA IgG Kit (Focus Diagnostics, Cypress, CA, USA) and for 20 cytokines by using the Bio-Plex 200 System (Bio-Rad, Hercules, CA, USA).

A total of 102/3,248 study participants sought medical care during the 3-month study period and were further investigated. Their median age was 63 years (range 28–79 years) and 73 (72%) were women. All but 3 participants were immunocompetent (2 had cancer; 1 of them used methotrexate). *Candidatus* N. mikurensis DNA was detected in 2 (2.0%) of 102 patients, which is consistent with prevalences of 1.1% in China (*5*) and 1.6% in Poland (*3*).

Patient 1 was a healthy 68-year-old woman who lived on the island of Tjurkö, southeast of Sweden. She sought medical care on day 77 of the study because of a rash on her right breast. She reported being bitten by a tick in the same

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Table 1. Evolution of *Candidatus* Neoehrlichia mikurensis gene copy numbers and antibody levels to *Borrelia burgdorferi* sensu lato complex and *Anaplasma phagocytophilum* for patient 1, Sweden

	Days after inclusion in study		
Characteristic	0	77	169
Clinical manifestations	None	Rash on right breast	None
Candidatus N. mikurensis DNA in plasma, gene copies/mL	0	2,200	2,000
Serum *B. burgdorferi* sensu lato IgM (points)*	Negative (4)	Positive (12)	Positive (8)
B. burgdorferi sensu lato antigens			
Positive reactivity	None	OspC, p100	OspC
Borderline reactivity	None	None	None
Serum *B. burgdorferi* sensu lato IgG (points)†	Positive (13)	Positive (16)	Positive (16)
B. burgdorferi sensu lato antigens			
Positive reactivity	P100, VlsE, p58	P100, VlsE, p58, OspC	P100, VlsE, p58, OspC
Borderline reactivity	OspC	None	None
Serum *A. phagocytophilum* IgG (1:64)‡	++	++	++
Serum *A. phagocytophilum* IgG (1:256)§	+	+	+

*Reactivity to either outer surface protein C (OspC) alone or to 2 antigens was required for a positive IgM response.
†Reactivity to ≥2 antigens was required for a positive IgG response. Full reactivity to an antigen is indicated by 4 points.
‡Every serum sample was tested at a dilution of 1/64. ++, strongly positive; +, positive.
§Samples that showed a positive (+) result were further tested at dilutions of 1:128 and 1:256.

location 2 months earlier. The patient was given a diagnosis of erythema migrans, received phenoxymethylpenicillin (1 g, 3×/d for 10 days), and the rash disappeared.

Patient 2 was a 57-year-old woman who lived in Kalmar, Sweden. She had a history of allergy and was regularly taking aspirin. She had received treatment for Lyme borreliosis 8 years earlier. On day 65 of the study, she sought medical care because of a rash on her left breast. She reported being bitten by a tick in the same location 1.5 months earlier. The patient was also given a diagnosis of erythema migrans and received phenoxymethylpenicillin (1 g, 3×/d for 10 days).

Patient 1 had IgM against *Borrelia* outer surface protein C and pre-existing *Borrelia*-specific IgG titers that increased during the study (Table 1). Patient 2 was seronegative for *Borrelia* antigens throughout the study (Table 2). The rash of patient 1 may have been caused by co-infection with a *Borrelia* spp. Although there was no evidence of a *Borrelia* infection in patient 2, only 50% of *Borrelia* culture-positive patients with erythema migrans show development of specific antibodies (*7*). Moreover, early treatment for erythema migrans might abrogate the IgG response (*8*), although not always (*9*). Nevertheless, 20% of patients with erythema migrans show negative results for *Borrelia* DNA in the skin, which indicates that these rashes might be caused by other infectious agents (*10*). Our study indicates that an erythematous rash in persons bitten by ticks might not be caused by *Borrelia* spp. and might require treatment with doxycycline instead of penicillin.

Patient 1 had pre-existing IgG against *A. phagocytophilum* that remained unchanged (Table 1). Patient 2 had borderline levels of IgG against *A. phagocytophilum* on day 0, which increased successively on days 65 and 98 (Table 2). This seroconversion may have resulted from cross-reactivity with *Candidatus* N. mikurensis, which was previously reported for an immunocompetent patient from Switzerland (*4*). Relatively high rates of seropositivity to *A. phagocytophilum* in Sweden (*11*,*12*) might be caused by cross-reactive antibodies because *Candidatus* N. mikurensis is common in ticks in Sweden, in contrast to *A. phagocytophilum* (*13*).

Both patients showed increased serum levels of cytokines, which appeared to mirror the numbers of *Candidatus* N. mikurensis gene copies (Figures 1, 2; online Technical

Table 2. Evolution of *Candidatus* Neoehrlichia mikurensis gene copy numbers and antibody levels to *Borrelia burgdorferi* sensu lato complex and *Anaplasma phagocytophilum* for patient 2, Sweden

	Days after inclusion in study		
Characteristic	0	65	98
Clinical manifestations	None	Rash on left breast	None
Candidatus N. mikurensis DNA in plasma, gene copies/mL	0	260	1300
Serum *B. burgdorferi* sensu lato IgM (points)*	Negative (0)	Negative (2)	Negative (1)
B. burgdorferi sensu lato antigens			
Positive reactivity	None	None	None
Borderline reactivity	None	p39	p39
Serum *B. burgdorferi* sensu lato IgG (points)†	Negative (0)	Negative (4)	Negative (4)
B. burgdorferi sensu lato antigens			
Positive reactivity	None	VlsE	VlsE
Borderline reactivity	None	None	None
Serum *A. phagocytophilum* IgG (1:64)‡	±	+	++

*Reactivity to either outer surface protein C alone or to 2 antigens was required for a positive IgM response.
†Reactivity to ≥2 antigens was required for a positive IgG response.
‡Every serum sample was tested at a dilution of 1/64. ±, weakly positive; +, positive; ++, strongly positive.

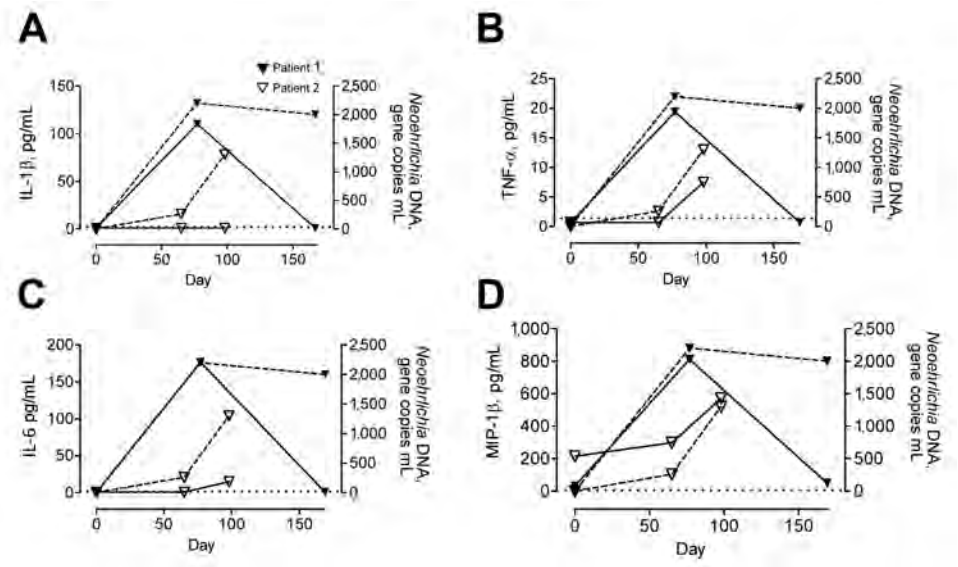

Figure 1. Proinflammatory cytokines in 2 patients infected with *Candidatus* Neoehrlichia mikurensis, Sweden. Concentrations of cytokines A) interleukin-1β (IL-1β), B) tumor necrosis factor-α (TNF-α), C) interleukin-6 (IL-6), and D) macrophage inflammatory protein-1β (MIP-1β) were measured in serum of patient 1 on days 0, 77, and 169 and in serum of patient 2 on days 0, 65, and 98. A rash developed in patient 1 on day 77 and in patient 2 on day 65. Dashed lines indicate levels of *Neoehrlichia* DNA in plasma for both patients. Dotted lines indicate detection limit for each cytokine.

Appendix Figure). Cytokine levels for patient 1 were maximum on day 77 and returned to reference levels on day 167. All cytokines, except for interferon-γ–induced protein 10, reached maximum levels on day 98 for patient 2. The cytokines were selected because systemic inflammation (Figure 1) with neutrophilia (online Technical Appendix) is typical of neoehrlichiosis in immunocompromised patients (*2*). In addition, a Th1-like immune response (Figure 2) is presumably required to eliminate an intracellular pathogen, such as *Candidatus* N. mikurensis. However, the cytokine response of patient 1 may in part have been caused by *Borrelia* spp. (*14*).

Conclusions

Candidatus N. mikurensis DNA was detected in the blood of both patients for ≥1 and 3 months, respectively. Similarly, a healthy person in Poland showed a positive result for *Candidatus* N. mikurensis twice in a 4-month period (*3*). This finding suggests that *Candidatus* N. mikurensis infections persist for a long time or that frequent reinfections occur. Prolonged carriage seems more probable in view of the common occurrence of neoehrlichiosis during winter among immunocompromised patients (*2*); immunosuppressive therapy might reactivate such infections. An analogous finding was reported in a dog, which was believed to have been a chronic carrier of *Candidatus* N. mikurensis; infection became symptomatic when immune defenses were compromised by surgery (*15*).

In conclusion, an erythematous rash in a person bitten by a tick can be caused by *Candidatus* N. mikurensis, rather than by *Borrelia* spp. Moreover, immunocompetent persons may be infected by *Candidatus* N. mikurensis for

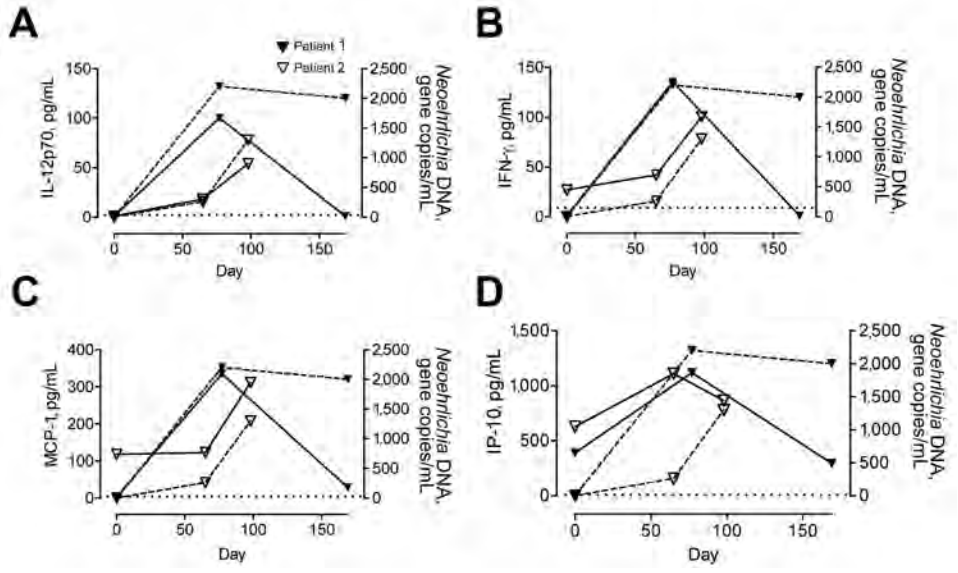

Figure 2. Th1 cytokines in 2 patients infected with *Candidatus* Neoehrlichia mikurensis, Sweden. Concentrations of cytokines A) interleukin-12p70 (IL-12p70), B) interferon-Regular (IFN-γ), C) monocyte chemoattractant protein-1 (MCP-1) (C), and D) IFN-γ–induced protein 10 (IP-10) were measured in serum of patient 1 on days 0, 77, and 169 and in serum of patient 2 on days 0, 65, and 98. Dashed lines indicate levels of *Neoehrlichia* DNA in plasma for both patients. Dotted lines indicate detection limit for each cytokine.

unexpectedly long periods, even after symptoms have disappeared. Patients scheduled to receive immunosuppressive treatment, and who live in *Candidatus* N. mikurensis–endemic areas should be screened for this pathogen before beginning therapy.

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Ribavirin for Chronic Hepatitis Prevention among Patients with Hematologic Malignancies

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Findings among a cohort of 26 patients who had hematologic malignancies and hepatitis E virus (HEV) infection support that HEV can induce chronic hepatitis. However, a 3-month course of ribavirin can induce a rapid viral clearance, reducing the risk for chronic hepatitis and enabling continuation of cytotoxic treatments for underlying malignancies.

Hepatitis E virus (HEV) is a nonenveloped RNA virus transmitted by consumption of contaminated water, undercooked infected pork or wild boar, deer, or rabbit meats (*1*), or by blood transfusion or solid organ transplant (SOT) (*2*). In addition to acute hepatitis, HEV causes chronic hepatitis and cirrhosis in immunocompromised patients (*3*,*4*). Knowledge of hepatitis E in patients with hematologic cancers is mostly derived from case reports or small series (*5*–*10*). We previously described 6 patients who had various hematological malignancies and HEV infection and reported chronic hepatitis in 3 of them (*6*). Management of HEV infection in the context of allogeneic stem cell transplantation (SCT) has been a matter of controversy (*5*,*6*). Ribavirin is the treatment of choice in patients infected during SOT (*11*). In hematologic patients infected with HEV, data concerning efficacy and safety of ribavirin are scarce (*12*).

The Study

During 2003–2009, patients at Institut Universitaire du Cancer, Toulouse, were randomly tested for HEV infection; patients who had elevated liver enzymes were routinely assessed for the virus. Patients were generally counseled to avoid undercooked foods or products that increased risk for HEV transmission according to guidelines from the French Ministry of Health. All patients with a diagnosis of HEV infection were counseled. HEV RNA was detected and quantified by PCR testing, and HEV IgM and HEV IgG were assessed with commercially available kits: the EIAgen HEV IgG and IgM kits (Adaltis, Casalecchio Di Reno, Italy) before 2012, and the Wantai HEV IgG and IgM enzyme-linked immunosorbent assay (Beijing Wantai Biologic Pharmacy Enterprise Co., Ltd, Beijing, China) since 2012. Diagnosis of hepatitis E required liver enzyme abnormalities and detectable HEV RNA in serum or fecal samples. Chronic HEV infection was defined by HEV viremia lasting >3 months (*1*).

Ribavirin monotherapy was proposed for infected immunocompromised patients beginning in May 2010, after publication of preliminary data reporting its efficacy in such patients (*12*). Assessment for administering ribavirin treatment was made on a case-by-case basis according to clinical and therapeutic context. Although there was no formal protocol, the general consensus was to consider ribavirin with the intent to complete treatment for the underlying disease rather than biologic criteria such as alanine aminotransferase levels or HEV viral loads. This study was approved by our institutional review board.

During 2003–2014, we identified 26 patients whose laboratory test results showed HEV replication. Clinical characteristics and treatments of patients with hematologic malignancies before or within the 6 months after the diagnosis of hepatitis E are summarized in Table 1 and in the online Technical Appendix Table (http://wwwnc.cdc.gov/EID/article/21/8/15-0199-Techapp1.pdf).

The diagnosis of HEV infection occurred a median of 10 (range 0–227) months after identification of the hematologic disorder. No persons in the study had traveled outside France during the year before hepatitis was diagnosed. Primary signs and symptoms were fever, diffuse or abdominal pain, vomiting, or asthenia; 16 patients were asymptomatic. Liver enzyme levels ranged from 1.5–100´ the upper limit of normal (Figure). No patient had fulminant hepatitis. HEV RNA was detectable in the serum of all patients and in the fecal samples of 88% of patients (HEV strain genotype 3f in 73%, 3c in 23%, and 4b in 4%). Of 23 tested patients, 9 (36%) had HEV IgG and 13 (61%) had HEV IgM. This finding emphasizes the need to rely on HEV RNA detection rather than serologic results for HEV diagnosis in immunocompromised patients. HEV was diagnosed concomitantly to the onset (n = 2) or at relapse (n = 2) of the hematologic malignancy, which raises the hypothesis of an association between HEV infection and carcinogenesis (*13*).

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Table 1. Clinical characteristics of 26 patients subsequently diagnosed with hepatitis E and hematologic malignancy, University Hospital of Toulouse, France, 2003*

Characteristic	Value	Ribavirin		p value
		Yes, n = 12	No, n = 14	
Sex, M/F	17/9 (65/35)	7/5	10/4	0.40
Age, median y (range)†	59 (21–86)	55 (21–86)	63 (33–84)	0.55
Hematological malignancy				
Acute leukemia	9 (34.6)	3 (25)	6 (42.8%)	NA
Indolent NHL‡	8 (26.9)	4 (33.5)	4 (28.6%)	NA
Aggressive NHL§	3 (15.3)	2 (1)	1 (7.2%)	NA
Multiple myeloma	3 (11.6)	1 (8.5)	2 (14.3%)	NA
Others¶	3 (11.6)	2 (17)	1 (7.2%)	NA
SCT before or concomitant to hepatitis E				
Allogenetic SCT	1/3	0/2	1/1	NA
Hepatitis E				
Serology, n = 23				
IgM+ IgG+	6 (26.1)	2 (16.7)	4 (28.6%)	NA
IgM– IgG+	3 (13)	1 (8.4)	2 (14.3%)	NA
IgM+ IgG–	7 (30.5)	3 (25)	4 (28.6%)	NA
IgM– IgG–	7 (30.5)	4 (33.5)	3 (21.5%)	NA
At onset				
AST, IU/L (range)	150 (17–2,309)	92 (17–1,688)	179 (20–2,309)	0.30
ALT, IU/L (range)	293 (24–4,273)	297 (24–2,189)	278 (47–4,273)	0.74
γGT, IU/L (range)	202 (18–1,665)	220 (50–1,665)	181 (18–492)	0.70
Bilirubin, μmol/L (range)	13 (6.3–107)	16 (6.3–107)	13 (7.7–88)	0.43
At month 6				
AST, IU/L (range)	51 (14–1,127)	19.5 (14–325)	86 (38–1,127)	0.03
ALT, IU/L (range)	50 (10–1,523)	14 (10–446)	133 (39–1,523)	0.01
γGT, IU/L (range)	101 (14–1,375)	28 (14–1,375)	135 (34–671)	0.07
Bilirubin, mmol/L (range)	18 (6.1–2,61)	18 (7.4–261)	12 (6.1–37)	0.41

*Values are no. (%) patients except as indicated. NA, not applicable; NHL, non-Hodgkin lymphoma; SCT, stem cell transplant; AST, aspartate aminotransferase; ALT,alanine aminotransferase; γGT, gamma-glutamyl transferase.
†Age at diagnosis of HEV infection.
‡Indolent B cell lymphoma: follicular lymphoma (n = 2).
§Aggressive non-Hodgkin lymphoma: Burkitt-like B cell lymphoma (n = 1), anaplastic T-cell lymphoma (n = 1), Diffuse large B cell lymphoma (n = 2).
¶Others: myeloproliferative neoplasm (n = 1); granulocytic sarcoma (n = 1); myeloid variant of hypereosinophilia with FIP1L1-PDGFRα rearrangement. p value refers to comparison between patients who received ribavirin and patients who did not. Data from 6 patients of this cohort were previously reported in a preliminary study (*6*).

The outcomes of both HEV infection and hematologic malignancies are summarized in Table 2. Twelve patients were treated with ribavirin after a median delay of 1 month (range 0.5–12 months) after the onset of hepatitis. Among the 14 patients with acute hepatitis E who did not receive ribavirin, 9 showed spontaneous clearance of HEV within the 2 months after diagnosis. The remaining 5 patients had prolonged HEV replication lasting >3 months, including 1 patient who had superimposed nonalcoholic steatohepatitis and had developed overt cirrhosis and refractory ascites. Immunosuppression withdrawal in 1 patient, who received allogeneic-SCT and in whom chronic hepatitis E developed, resulted in a severe transient incident of elevated liver enzymes, followed by spontaneous clearance of the virus.

Oral ribavirin was administered to 12 patients at a median dose of 800 mg/d (range 600–1,000 mg/d); 1 patient had chronic hepatitis. In all patients, HEV RNA became undetectable after 30 (range 10–60) days, and liver blood tests were normalized after 2.5 (range 1–12) months. Ribavirin was given for a median time of 3 (range 0.5–10) months. The heterogeneity of timing and duration of treatment likely

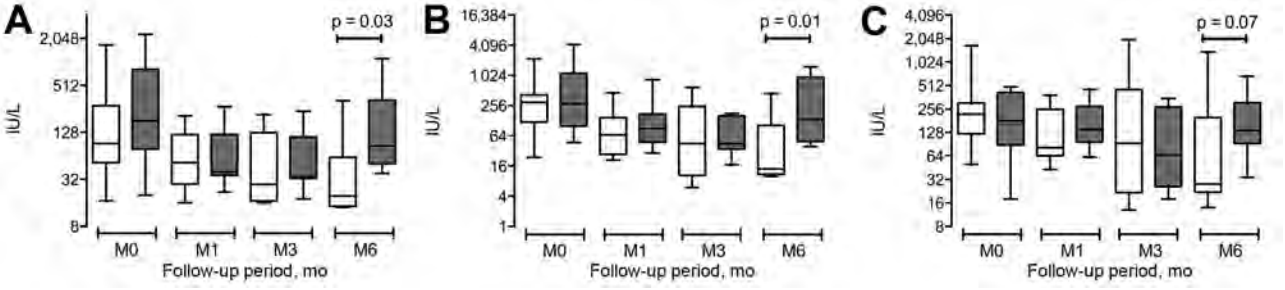

Figure. Liver test results for 26 patients at diagnosis of hepatitis E and at months 1, 3, and 6, Toulouse, France, 2003–2014. A) Aspartate aminotransferase ST; B) alanine aminotransferase; C) Gamma-glutamyl transferase.

Table 2. Hepatitis E and hematologic malignancies outcomes of 26 patients observed at the University Hospital of Toulouse, France, 2003–2014*

Characteristic	Acute leukemia, n = 9	Indolent NHL, n = 8	Aggressive NHL, n = 3	Myeloma, n = 3	Others, n = 3
Chronic hepatitis	2 (22)	2 (25)	1 (33.5)	1 (33.5)	0
Ribavirin	3 (33.3)	4 (50)	2 (66.5)	1 (33.5)	2 (66.5)
HEV clearance	8 (89)	7 (87.5)	3 (100)	3 (100)	3 (100)
Postponed chemo/SCT	3 (33.3)	2 (25)	0	0	0
Hematologic complete response	5 (55.5)	7 (87.5)	2 (66.5)	0	1 (33.5)
Follow-up period, mo (range)	4.2 (0.9–51.7)	23.1 (5.6–105)	13.1 (3–29.1)	27 (12.8–83)	8.4 (1.5–10)
Deaths	4 (44.5)	1 (12.5)	1 (33.3)	1 (33.3)	1 (33.3)

*Values are no. (%) patients except as indicated. NHL, non-Hodgkin lymphoma; SCT, stem cell transplantation.

reflected the progressive introduction of ribavirin into current practice and the specific context of several patients. In general, 2 consecutive negative PCRs during 1 month were required to discontinue ribavirin. Three patients had abnormal liver test results and HEV recurred within the month after ribavirin treatment was withdrawn, including 2 who received ribavirin for 1 month only. Definitive virologic response was obtained after ribavirin retreatment. Thus, the combination of a 3-month intake of ribavirin, 2 negative HEV blood RNAs at month 2 and 3, and negative RNA levels in stools at month 3 seems to be an appropriate approach.

Overall, aspartate aminotransferase and alanine aminotransferase serum levels were significantly higher at 6 months in patients who did not receive ribavirin compared with patients who did ($p = 0.03$ and $p = 0.01$, respectively; online Technical Appendix Table). Ribavirin was well tolerated among 10 of the 12 recipients and did not require specific red blood cells or erythropoietin support. One patient, 84 years of age, became anemic while receiving 1,000 mg of ribavarin per day, but treatment was continued. Treatment was discontinued for another patient, 41 years of age, when pruritus developed after 2 weeks of therapy.

Conclusions

After SOT, chronic hepatitis develops in 60% of untreated HEV-infected patients (*3*). Here, we show that patients with hematologic malignancies are also at risk for chronic HEV infection. SOT patients showed a high response rate to ribavirin treatment (*11*). Because most hematologic malignancies required intensive immune treatments that should not be postponed, HEV infection could notably alter the therapeutic schedule of some patients (*5*,*6*). Cytotoxic treatments or SCT were postponed while awaiting HEV clearance for 5 patients, of whom 4 reached complete hematologic response. Among the 12 ribavirin-treated patients, 9 were able to complete the planned treatment that included SCT. Thus, although spontaneous but unpredictable HEV clearance could occur in some patients, therapeutic intervention is often mandatory to fully complete the therapeutic schedule of the underlying disease and to prevent chronic hepatitis. Confirming previous reports (*11*,*12*), a 3-month course of ribavirin induced complete and sustained HEV eradication in most if not all patients. Moreover, ribavirin may also decrease the risk for nosocomial HEV transmission among patients (*14*).

Our study has limitations. The retrospective nature prevented accurate assessment of the incidence of HEV infection among patients who have hematologic malignancies. Some obvious bias, including occurrence of HEV viremia without concomitant liver tests abnormalities or inability to ask patients about their exposure risk to HEV, also need further considerations.

By inducing a rapid clearance of HEV, ribavirin can prevent chronic hepatitis in patients with hematologic malignancies. Hepatitis E can induce chronic hepatitis and cirrhosis in patients with hematologic cancers; patients with liver enzyme abnormalities should be screened for HEV infection and a 3-month course of ribavirin proposed to avoid postponement in the treatment of patients who have the underlying disease as well as chronic hepatitis or cirrhosis.

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Macrolide-Resistant *Mycoplasma pneumoniae*, United States[1]

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Macrolide-resistant *Mycoplasma pneumoniae* (MRMP) is highly prevalent in Asia and is now being reported from Europe. Few data on MRMP are available in the United States. Using genotypic and phenotypic methods, we detected high-level MRMP in 13.2% of 91 *M. pneumoniae*–positive specimens from 6 US locations.

Macrolides are the preferred treatment for infections caused by *Mycoplasma pneumoniae* in children (*1*). Since 2000, macrolide resistance has developed in Asia and has now been reported from many parts of the world (*2*). This resistance is of a high level and has been associated with longer duration of fever, cough, and hospital stay and the need to switch to alternative antimicrobial agents (*3*–*5*). Scant information is available about the prevalence of macrolide-resistant *M. pneumoniae* (MRMP) in the United States, and no organized ongoing surveillance exists.

The Study

During August 2012–April 2014, respiratory specimens that tested positive for *M. pneumoniae* by molecular methods were collected from 6 medical centers throughout the United States. These sites were located in Chicago, Illinois; Kansas City, Missouri; Hackensack, New Jersey; New York, New York; Seattle, Washington; and Birmingham, Alabama. Five medical centers tested for *M. pneumoniae* DNA using the FilmArray respiratory pathogen panel (Biofire Diagnostics, Salt Lake City, UT, USA). The University of Alabama at Birmingham used a laboratory-developed real-time PCR targeting *RepMp1*.

Testing for 23S rRNA mutations conferring macrolide resistance was performed on original specimens by real-time PCR melt curve analysis (*6*) and confirmed by DNA sequencing at the Lurie Children's Hospital (Chicago). Subculture and phenotypic antimicrobial susceptibility testing was performed by using a broth microdilution method approved by the Clinical and Laboratory Standards Institute at the University of Alabama at Birmingham. The study comprised 91 *M. pneumoniae*–positive specimens. Patients' ages ranged from 10 months to 66 years; 83 (91.2%) samples were from patients ≤18 years of age. Specimen types were nasopharyngeal or nasal swabs (72 samples), nasal aspirate/washes (12 samples), bronchoalveolar lavage (5 samples), and throat swab and tracheal aspirate samples (1 sample each).

We found a 23S rRNA point mutation A2063G known to confer macrolide resistance in 10 (10.9%) of 91 specimens by direct real-time PCR with melting curve analysis. All mutations were confirmed by DNA sequencing. *M. pneumoniae* grew in subculture from 62 (68.1%) of these specimens. PCR and sequencing on those subcultures confirmed macrolide resistance in 6 specimens that had been previously identified by direct real-time PCR and in 2 additional specimens that had not previously been detected by PCR. The 2 specimens most likely tested negative for mutations by direct PCR because of a low number of organisms in the original sample. Macrolide resistance was not significantly correlated with patient age or specimen type (data not shown). MRMP was detected in a total of 12 (13.2%) samples (Table).

We conducted phenotypic antimicrobial susceptibility testing on all 62 *M. pneumoniae* isolates obtained by subculture. All 54 macrolide-susceptible isolates showed very low erythromycin MICs (≤0.008 μg/mL), whereas the 8 isolates that had the A2063G mutation showed uniformly high erythromycin MICs (≥256 μg/mL). All 62 isolates were susceptible to tetracycline (MIC 0.031–1 μg/mL) and levofloxacin (MIC 0.031–1 μg/mL).

The FilmArray respiratory pathogen panel detects multiple respiratory pathogens. Of the 80 respiratory specimens for which co-infection data were available, 26 (32.5%) had a viral pathogen detected along with

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Table. Macrolide-resistant *Mycoplasma pneumoniae*–positive respiratory specimens, selected US sites, August 2012–April 2014

Collection site	Specimens	
	No. tested	No. (%) macrolide resistant
Chicago, IL	23	4 (17.4)
Kansas City, MO	40	3 (7.5)
Hackensack, NJ	2	1 (50.0)
New York, NY	5	2 (40.0)
Seattle, WA	15	1 (6.7)
Birmingham, AL	6	1 (16.7)
All	91	12 (13.2)

M. pneumoniae. These viruses were 15 (18.8%) rhinovirus/enterovirus group viruses, 3 (3.8%) respiratory syncytial viruses, 4 (5%) parainfluenza viruses, 1 (1.3%) human metapneumoviruses, and 3 (3.8%) coronaviruses.

Conclusions

MRMP prevalence has been reported to range from 2% to 26% in European countries (*7*,*8*) and is 30% in Israel (*9*). The prevalence is much higher in Asia, where MRMP first emerged in 2000, and now exceeds 90% in some areas of China and Japan (*10*,*11*).

Macrolide resistance has been documented in North America since 2008 (*6*,*12*). The Centers for Disease Control and Prevention recently published results from 199 specimens obtained from case-patients, small clusters, and outbreaks that occurred during 2006–2013 but did not specify geographic locations from which specimens were derived. An overall 10% rate of macrolide resistance was reported (*13*). Yamada et al. tested 49 *M. pneumoniae*–positive specimens collected from children in St. Louis, Missouri, USA, during 2007–2010 and found 4 (8.2%) that contained the A2063G mutation (*14*). Eshaghi et al. detected MRMP in 12.1% specimens collected in Ontario, Canada, during 2010–2011 (*15*).

Our finding of high-level macrolide resistance in 13.2% of specimens from all 6 centers throughout a broad geographic area in the United States proves this problem has emerged in all regions of the nation and might increase over time, as it has in other countries. The design of our epidemiologic study provides the most accurate estimate of the point prevalence of MRMP available thus far. Previous studies reported from the United States were limited primarily to individual case reports, clusters, outbreaks, or single geographic locations.

The mechanism for macrolide resistance in *M. pneumoniae* is point mutations in a few positions of domain V of the peptidyl transferase loop of 23S rRNA, the location of macrolide binding to the 50S bacterial ribosome subunit (*3*). The A2063G transition mutation has been the most common one detected in most studies and was the only mutation found in our study. Although less common, other mutations conferring macrolide resistance, but not found in our study, include A2063T or C, A2064G, A2067G, and C2617A or G (*3*,*10*,*15*).

Unlike previous studies, our study used both molecular and phenotypic techniques to detect macrolide resistance. Thus, we were able to correlate erythromycin MICs with the presence of 23S rRNA mutations. Our results showed striking differences in erythromycin MICs between the susceptible and resistant isolates and confirmed high-level resistance to erythromycin in every isolate. Erythromycin is usually used for testing because mutations in A2063 and A2064 consistently have been shown to cause high resistance to both erythromycin and azithromycin (*3*,*5*,*10*). A limitation of the study is the nature of anonymous specimen collection. Inclusion of patient information, especially antimicrobial therapy, will enhance data interpretation in prospectively conducted future studies.

One notable observation from our study is the co-infection of *M. pneumoniae* with various viral pathogens. A viral co-infection rate >30% supports the use of multiplex testing for viral and bacterial pathogens in children with respiratory infections of uncertain etiology.

Although our study has confirmed MRMP in 6 geographically diverse US states, macrolides should remain the drugs of choice in children with *M. pneumoniae* respiratory infections. Clinicians should be vigilant for macrolide treatment failures and consider using alternative drugs if necessary. Countries such as Japan and China that have a very high macrolide resistance rate for *M. pneumoniae* and other respiratory pathogens often have high consumption of macrolides. Okada et al. (*5*) reported that macrolides account for 30% of all oral antibacterial drugs in Japan and concluded that the increase in macrolide-resistant bacteria during the past several years in that country was closely related to selective pressure resulting from widespread antimicrobial use. Given the common use of macrolides in the United States to treat pediatric respiratory infections, judicious use of antimicrobial drugs should be emphasized. Reevaluation of existing classes and investigation of new classes of antimicrobial agents may be prudent to have additional treatment alternatives for MRMP infections beyond tetracyclines and fluoroquinolones. Surveillance for this resistance in the United States will help monitor the trend.

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Rabies Postexposure Prophylaxis for Travelers Injured by Nonhuman Primates, Marseille, France, 2001–2014

Agathe Blaise, Philippe Parola, Philippe Brouqui, Philippe Gautret

Most exposures of residents of Marseille to nonhuman primates occurred among unvaccinated adult travelers to Southeast Asia within the first 10 days of their arrival at 2 major tourist locations in Thailand and 1 in Indonesia. A small proportion of travelers received rabies immunoglobulin in the country of exposure.

Rabies is estimated to cause >60,000 human deaths worldwide annually on the basis of a probability decision-tree approach; primarily resulting from dog bites, the disease is of public health concern in most countries in Asia and Africa (*1*). Nonhuman primates (NHPs) are not primary reservoirs of rabies; nevertheless, 159 reports of rabies in NHPs, of which 134 were laboratory-confirmed cases, have been retrieved from various sources in South America, Africa, and Asia (*2*). This total is probably underestimated because weak rules pertaining to rabies surveillance in some countries, such as not requiring reports of rabidity in all species, are likely to result in underreporting of rabid NHPs. Cases of rabies in humans after injury by NHPs were also reported in 25 persons, mostly in Brazil (*2*). Although rarely reported, documented cases of rabies infections in NHPs and subsequent transmission to humans do occur, warranting the need for rabies postexposure prophylaxis (PEP) after NHP exposure in countries to which rabies is endemic. The epidemiology of NHP-related injuries has been described in case reports or small cohorts of patients only (*3*,*4*). Here, we describe the epidemiology of 135 cases of NHP-related injuries in persons seen in the Marseille Rabies Treatment Centre in Marseille, France.

The Study

During 2001–2014, epidemiologic data on NHP–related injuries and associated rabies PEP treatment were prospectively collected for patients of the Marseille Rabies Treatment Centre. Demographics, place of exposure, travel characteristics, clinical data, and rabies PEP were documented.

A total of 135 cases of persons injured by NHPs reported during a 14-year period were included, representing an average 10 cases/year (range 4–16 cases/year), with a tendency to increase over time (Figure, panel A). Exposures were more frequently reported during July–October (Figure, panel B). The F/M sex ratio of patients was 1.1, and the mean age was 30 years (median 27 years; range 3–70 years); 20.7% of patients were <15 years of age (Table 1). Of the 135 patients, 2 had complete and 1 incomplete preexposure vaccination against rabies. Most exposures (n = 120, 88.9%) occurred outside of France, notably in Southeast Asia (n = 82); most cases occurred in Thailand (n = 48) and Indonesia (n = 25). Most exposures in Thailand were on Monkey Beach on Koh Phi Phi Island and in the Lopburi Monkey Temple; most exposures in Indonesia were in the Ubud Monkey Forest in Bali (Table 2). Most persons injured abroad were tourists with a mean travel duration of 21 days (median 17 days; range 3–180 days). The

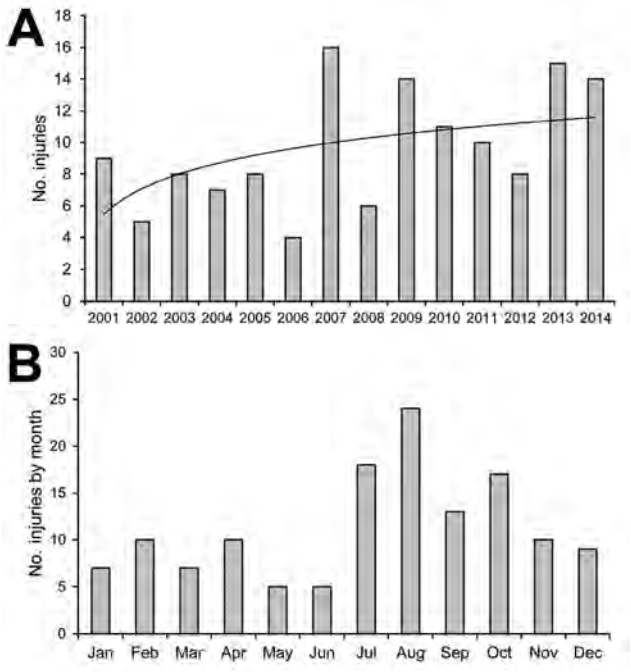

Figure. Number of injuries to humans by nonhuman primates requiring rabies postexposure prophylaxis, Marseille Rabies Treatment Centre, Marseille, France, 2001–2014. A) Logarithmic regression was used to calculate a line of best fit of y = 2,3191ln(x) + 5.4699 (black line). B) Cumulative occurrence by month.

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Table 1. Demographic, vaccination status, place of exposure, and travel characteristics of 135 persons injured by nonhuman primates requiring rabies postexposure prophylaxis, Marseilles, France, 2001–2014

Characteristics	No. (%)
Female sex	70 (51.9)
Age group, years	
0–15	28 (20.7)
16–39	71 (52.6)
40–59	27 (20.0)
60 and over	9 (6.7)
Preexposure vaccination against rabies	2 (1.5)
Place of exposure	
France	15 (11.1)
Thailand	48 (35.7)
Indonesia	25 (18.5)
Other countries in Asia*	14 (10.4)
North Africa†	13 (9.6)
Sub-Saharan Africa‡	11 (8.1)
Central and South America and Caribbean§	6 (4.4)
Spain/Rock of Gibraltar	3 (2.2)
Reason for travel, n = 120	
Tourism	99 (82.5)
Other¶	6 (5.0)
Not documented	15 (12.5)
Days of travel duration, n = 119#	
0–7	6 (5.0)
8–14	21(17.6)
15–21	43 (36.2)
21–28	19 (16.0)
≥29	14 (11.8)
Not documented	16 (13.4)
Days between first date of travel and exposure, n = 119#	
0–7	41 (34.5)
8–14	33 (27.6)
15–21	17 (14.3)
≥22 d	4 (3.4)
Not documented	24 (20.2)

*Vietnam (5), India (4) Cambodia (3), Myanmar (1), Sri-Lanka (1).
†Morocco (10), Algeria (3).
‡Madagascar (4), Kenya (4), Botswana (1), Cameroon (1), Central African Republic (1).
§Brazil (2), Anguilla (1), Dominican Republic (1), Mexico (1), Peru (1).
¶Persons, or their descendants, who have immigrated to Marseille from another country who were exposed while visiting friends and relatives in their country of origin (3), expatriate (1), humanitarian worker (1), and student (1).
#Expatriate traveler was excluded.

mean time between the first date of travel and exposure was 10 days (median 8 days; range 1–50 days).

Injuries most commonly occurred on the upper limbs (57.8%); subsequent occurrences of injury were on lower limbs (28.9%), head (5.9%), trunk (3.7%), or multiple sites (2.2%). Details of 2 cases were not documented. Most injuries (66.7%) were severe transdermal type 3 injuries according to the WHO classification, resulting from severe bites (1); the remaining (33.3%) were type 2 injuries (minor abrasions and scratches without bleeding). According to WHO guidelines (1), rabies immune globulin (RIG) was indicated for 65.2% of patients who had type 3 injuries and no previous vaccination against rabies. Among patients injured abroad, 58 (48.3%) started rabies PEP abroad; the mean time between injury and treatment was 1 day (median 0 days; range 0–21 days). Most (77.6%) underwent the Essen protocol and 17.2% underwent the Zagreb protocol (*1*); and 5.2% underwent the 7-dose mouse brain vaccine protocol (local subcutaneous and intradermal protocol used in North Africa: seven 1.0-mL subcutaneous injections from day 0 through day 6, followed by 2-site 0.1-mL intradermal injections on days 10, 14, 29, and 90). A total of 35 patients (25.9%) had an indication for RIG, of whom 8 (22.9%) received the RIG abroad. Among the 27 remaining patients, the mean time between first injection of vaccine abroad and the first consultation in France was 12 days (median 12 days; range 3–31 days). An additional 6 patients (17.1%) received RIG in France, and the remaining 21 patients (60.0%) did not. Of those 21 patients, 20 sought follow-up medical consultation in France >7 days after the first vaccine was provided abroad; the initiation of RIG is contraindicated if >7 days have passed since vaccine initiation (5). A total of 62 patients started their treatment in France with a mean time between injury and treatment of 15 days (median 11 days; range 2–123 days). Most underwent the Essen protocol (71.0%), followed by the Zagreb protocol (27.4%). One patient who received complete preventive

Table 2. Detailed place of exposure of 73 travelers injured by nonhuman primates in Thailand and Indonesia requiring rabies postexposure prophylaxis, Marseille, France, 2001–2014

Location	No.
Thailand, total	48
Koh Phi Phi	
Monkey Beach	15
Lopburi	
Lopburi Monkey Temple	8
Bangkok	
Park	2
Zoo	1
Temple	1
Koh Samui	
Monkey show	2
Koh Phangan	
Undocumented	2
Phuket	
Patong Beach	2
Other	
Temple, Chiang Mai	1
Street, Korat	1
Temple, Singburi	1
Temple, Sukhothai	1
Not documented	11
Indonesia, total	49
Bali	24
Ubud sacred monkey forest sanctuary	1 7
Temple, Uluwatu	2
Temple, undocumented	2
Undocumented	3
Not documented	1

vaccination received 2 booster doses at days 0 and 3. Of 44 patients for whom RIG was indicated, 39 (88.6%) received RIG. No cases of rabies were observed among the 135 persons injured by NHPs.

Conclusions

NHP bites among travelers are not infrequent. An estimated 31% of injuries requiring rabies PEP among international travelers are caused by NHPs, which rank second after dogs in most studies and first in studies conducted among travelers returning from Southeast Asia (*2*). In a recent GeoSentinel survey involving 2,697 travelers requiring rabies PEP, NHPs accounted for 66% of animal bites occurring in Southeast Asia, and tourists made up a disproportionately large portion of the NHP exposures (92%) (*6*).

Bites by NHPs can transmit other potentially life-threatening zoonoses, such as the herpes B virus (*Cercopithecine herpesvirus* 1). The human herpes B virus infections that have been described have all occurred after contact with macaques in a biomedical research setting (*7*). However, samples of >80% of macaques at the Monkey Forest in Bali tested positive for antibodies against the herpes B virus, a naturally occurring infectious agent that is endemic among macaque monkeys from Asia (*8*). Appropriate B virus prophylaxis may be considered for travelers injured by Asian macaques, although this treatment is not applicable to those injured by New World NHPs (*3*,*4*).

In this study, we observed that most exposures occurred among adult tourists to Southeast Asia during the summer months, which probably reflects travel volume. Furthermore, we identified 2 major tourist destinations in Thailand and 1 in Indonesia as locations where monkeys were most likely to bite tourists, reflecting the regional distribution of both monkey and tourist populations. Most patients were taking relatively short trips and were bitten within the first 10 days after their arrival. Indeed, monkey bites are relatively frequent at these tourist places. An estimated 6% of visitors to Bali monkey temples are bitten by macaque monkeys (*9*). No detailed information is available about documented cases of rabies among monkeys at these tourist destinations; however, confirmed cases have been reported in monkeys as well as in gibbons and langurs in Thailand (Panichabhongse P., The epidemiology of rabies in Thailand [thesis]. 2001 [cited 2015 Jun 1] (http://mro.massey.ac.nz/bitstream/handle/10179/4545/02_whole.pdf?sequence=1&isAllowed=y). We show that most of the cases involved persons who had not received preexposure immunization and that ≈33% of patients for whom RIG was indicated did not receive RIG. Only a small proportion of travelers received RIG in the country of exposure, which reflects the limited supply of this biological agent in many countries.

Unvaccinated Western travelers who are unaware of the risk for rabies regularly engage in contact with animals during their trips, often resulting in expensive PEP. To decrease the need for rabies PEP after animal bites, it is crucial that travelers to countries in which rabies is endemic be fully informed of this specific risk, which can be easily minimized by avoiding contact with animals, avoiding feeding them, avoiding smiling at them (showing teeth is a sign of aggression), avoiding dropping something that a monkey has grabbed, and avoiding showing fear. Our findings indicate that tourists should be given strong warnings before travel to visit Koh Phi Phi, Lopburi, or Ubud.

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Dr. Blaise is a physician who specializes in infectious diseases. Her research interests include rabies and other zoonoses.

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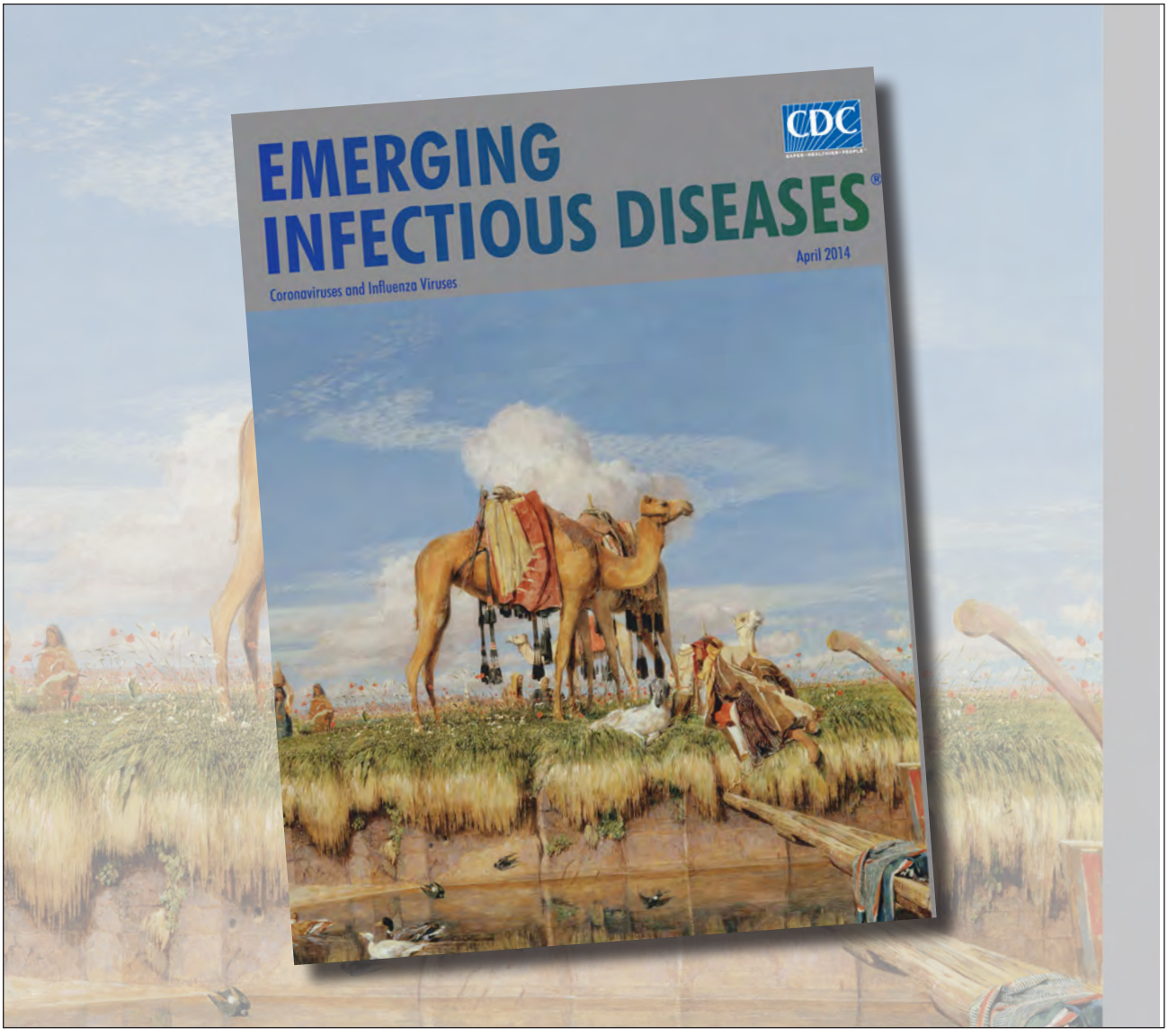

Genome of Emerging Norovirus GII.17, United States, 2014

Gabriel I. Parra, Kim Y. Green

To determine whether the norovirus strain GII.17 recently detected in Maryland, USA, (Hu/GII.17/Gaithersburg/2014/US) is spreading globally, we characterized the genome. High similarity with the norovirus GII.17 that caused recent outbreaks in Asia indicates that the same strain was present in the United States during the 2014–15 norovirus season (winter).

Noroviruses are major pathogens associated with acute gastroenteritis among persons in all age groups. In developing countries, noroviruses are responsible for an estimated 200,000 deaths per year among children <5 years of age (*1*). These viruses characteristically cause outbreaks in partially enclosed settings such as schools, childcare centers, nursing homes, military facilities, and cruise ships (*2*).

Noroviruses possess a genome of single-stranded positive-sense RNA that is organized into 3 open reading frames (ORFs). ORF1 encodes the nonstructural proteins required for replication, including the RNA-dependent RNA polymerase (RdRp); ORF2 encodes the major capsid protein (viral protein [VP] 1); and ORF3 encodes the minor capsid protein (VP2) (*2*). Noroviruses are genetically diverse and are divided into 6 genogroups (GI–GVI) and ≈30 genotypes according to comparison of VP1 sequences. The fact that patients have been sequentially infected with distinct strains suggests a lack of cross-protection among genotypes (*3*–*5*). The current norovirus classification system uses a dual nomenclature based on differences in the RdRp (polymerase or P genotype) and the VP1 region (capsid or G genotype) (*6*). Because noroviruses are prone to recombine within the ORF1/ORF2 junction, strains with different combinations of P and G genotypes can be detected in nature (*7*).

Although several norovirus strains circulate, for ≈2 decades, GII.4 has been the predominant genotype infecting humans. It has been proposed that GII.4 strains successfully persist and infect humans by the periodic emergence of new GII.4 variants that escape from herd immunity developed against previous variants (*8*). Recently, increased detection of GII.17 as the predominant outbreak strain in China has been reported (*9*). In this study, we characterized the genome of a norovirus GII.17 strain recently detected in Maryland, USA, to determine whether the same GII.17 virus is spreading globally.

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The Study

On November 25, 2014, acute gastroenteritis developed in a 3-year-old child in Gaithersburg, Maryland, USA. The child's parent gave informed consent for the child's enrollment in National Institutes of Health (NIH) clinical study NCT01306084. No other family member became ill, and no one in the family had a history of recent travel. A fecal sample was collected from the child and tested for norovirus by reverse transcription PCR (RT-PCR) by using generic primers that annealed to the polymerase region. Results were confirmed by nucleotide sequencing, and the P genotype was assigned by using the Norovirus Genotyping Tool (http://www.rivm.nl/mpf/norovirus/typingtool) (*10*). The capsid region of the sample was assigned to GII.17, and the polymerase region was classified as "unknown genotype." To further assess the identity of the virus, we amplified the complete genome by using RT-PCR and primers selective for the conserved 3′ and 5′ end regions; we sequenced it by using an Ion Torrent platform (Life Technologies, Carlsbad, CA, USA). The sequence of the virus (designated Hu/GII.17/Gaithersburg/2014/US) was deposited into GenBank under accession no. KR083017.

A BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) identified 9 strains (all recently detected in countries from Asia) as having the highest similarity to Hu/GII.17/Gaithersburg/2014/US. A phylogenetic analysis of all GII.17 strains with full-length (or nearly full-length) capsid regions deposited in the GenBank database revealed the presence of 3 distinct clusters (A–C); the Hu/GII.17/Gaithersburg/2014/US strain clustered with strains recently detected in Taiwan, Hong Kong, and Japan and not with the GII.17 strain detected before 2009 (Figure 1). The 3 clusters differed by ≥36 aa substitutions; the greatest divergence was found for cluster C (Figure 1). Sequence analysis of the VP1 of strains representing each cluster showed a number of amino acid substitutions unique for each cluster (62/543). Three deletions (residues 295, 296, and 384) and 1 insertion (Asp344) were present only in the strains from cluster C. Of note, the Hu/GII.17/Gaithersburg/2014/US strain presented 25 aa substitutions, compared with the other cluster C strains, and contained 2 unique insertions (Asp380 and Asp396) only present in 2 GII.7 strains from cluster C (Hu/GII.17/CUHK-NS-463/2014/HK and Hu/GII.17/Kawasaki308/2015/JP). Molecular modeling of the GII.17 capsid showed that most of the substitutions (43/62) mapped onto the surface of the VP1. The 2 deletions (295–296) of the novel GII.17 (cluster C) mapped into 1 of the major epitopes (epitope A) described for GII.4 norovirus,

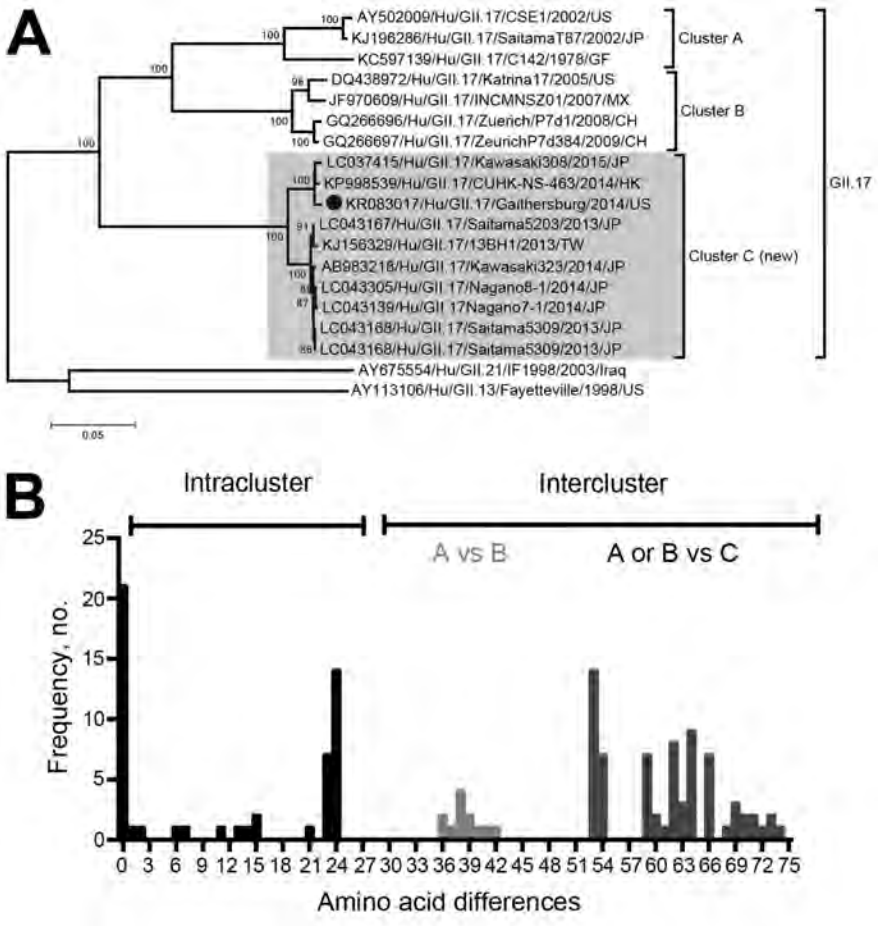

Figure 1. Relationship of major capsid protein (viral protein [VP] 1) of norovirus strain Hu/GII.17/Gaithersburg/2014/US with other GII.17 noroviruses. A) Phylogenetic tree of the Hu/GII.17/Gaithersburg/2014/US VP1 region showing comparison with those of GII.17 norovirus strains available in public genetic databases. Phylogenetic analyses were conducted by using MEGA version 6 (*11*), neighbor-joining as the algorithm for reconstruction, and Tamura-Nei as the model of substitution. Bootstrap (500 replicates) analysis was used for the statistical support of the tree; values >70% are shown. The Hu/GII.17/Gaithersburg/2014/US strain is indicated by a black circle. For each strain, the GenBank accession number/name/year of detection/country is shown. Gray shading indicates GII.17 cluster C strains. Scale bar indicates nucleotide substitutions per site. B) Amino acid differences among the GII.17 strains.

and the remaining insertions and deletions also mapped near residues involved in major epitopes (*8*) (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/8/15-0652-Techapp1.pdf). These modifications in the capsid of the new GII.17 cluster C strains might confer new antigenic or biological characteristics that would provide evolutionary advantages for infection, rapid spread, or both.

Analysis of the RdRp region showed that the Hu/GII.17/Gaithersburg/2014/US strain clustered between GII.3 and GII.13 strains and had genetic distances of ≈0.102 and ≈0.103, respectively (Figure 2). Although the RdRp from the Hu/GII.17/Gaithersburg/2014/US strain clustered with that of GII.3 strains, neither bootstrap values nor genetic distances reached values (>70% or ≥0.143, respectively) that enabled them to be classified within any known P genotype (*6*,*12*). The other GII.17 cluster C strains grouped with the Hu/GII.17/Gaithersburg/2014/US strain in the polymerase region and displayed only 1 amino acid substitution, confirming their similar evolutionary origins (Figures 1, 2). Of note, certain GII.17 viruses (probably cluster B) with the GII.P13 genotype in their polymerase circulated during 2004–2009 in various countries around the world (online Technical Appendix). Full-length sequences of these GII.P13/GII.17 strains will be needed for determination of their evolutionary relationship with the new GII.17 cluster C viruses.

Conclusions

Surveillance in China has shown that norovirus GII.17 predominated over GII.4 Sydney as the cause of outbreaks during the 2014–15 season (*9*). The sequences reported in that study showed a high similarity with the Hu/GII.17/Gaithersburg/2014/US strain and clustered within GII.17 cluster C. Thus, we conclude that the same GII.17 virus that caused outbreaks in Asia was present in the United States near the beginning of the 2014–15 season. It is possible that these new GII.17 viruses bear substitutions in VP1 that define new antigenic sites, which, coupled with new characteristics in ORF1, provide an adaptive advantage for rapid spread. Continued monitoring of emerging norovirus strains is needed for a better understanding of their evolution and epidemiology.

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Dr. Parra is a research fellow at the National Institute of Allergy and Infectious Diseases, NIH. His research interests include

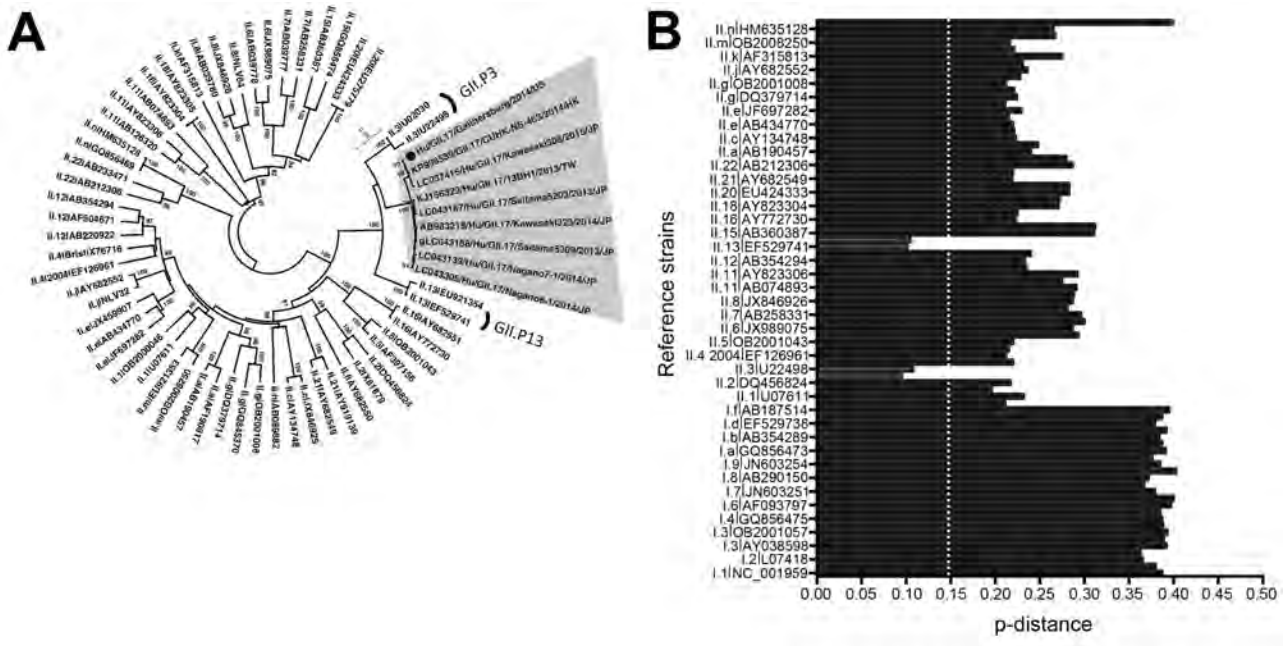

Figure 2. Relationship of the RNA-dependent RNA polymerase (RdRp) region of norovirus strain Hu/GII.17/Gaithersburg/2014/US to that of other noroviruses. A) Phylogenetic tree of the RdRp region (nt 4526–5116) from novel GII.17 strains (cluster C) and reference strains from each genotype described (*6*). Phylogenetic analyses were conducted by using MEGA version 6 (*11*), neighbor-joining as the algorithm for reconstruction, and Tamura-Nei as the model of substitution. Bootstrap (500 replicates) analysis was used for the statistical support of the tree. Gray shading indicates GII.17 cluster C strains, and Hu/GII.17/Gaithersburg/2014/US is indicated by a black circle. Strains from genotypes GII.P3 and GII.P13 are indicated by brackets. B) Distances (p-distances) among the Hu/GII.17/Gaithersburg/2014/US strain and reference strains. Values compared with reference strains from genotypes GII.P3 and GII.P13 are shaded. Values considered cut off for genotype designation are indicated with a dotted line (*6*,*12*).

epidemiology, genomics, immunology, and development of vaccines against gastrointestinal viral infections.

Dr. Green is chief of the Caliciviruses Section of the National Institute of Allergy and Infectious Diseases, NIH. Her research is directed toward the prevention and control of acute gastrointestinal disease caused by noroviruses.

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Measles Vaccination Coverage and Cases among Vaccinated Persons

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To the Editor: In December 2014, a measles outbreak that had started at Disneyland Park in Anaheim, California, USA, and subsequently spread to numerous states garnered substantial media attention in the United States. In 2014, the US Centers for Disease Control and Prevention reported the highest number of measles cases (644) since the disease had been declared eliminated from the United States in 2000 (*1*). This number is still relatively lower than the numbers reported from 30 countries of the European Union and the European Economic Area; the highest numbers of measles cases in 2013 were from the Netherlands (2,499 cases), Italy (2,216), the United Kingdom (1,900), and Germany (1,772) (*2*). There is widespread concern that increasing hesitancy to vaccinate in the United States might lead to outbreaks as large as the ones in Europe.

Measles vaccine is highly effective, and analyses of a large measles outbreak at a school in Germany have shown that receipt of ≥1 doses of vaccine can prevent infection in up to 99% of persons (*3*,*4*). One might therefore be tempted to think that the proportion of measles case-patients who had been vaccinated must be very small. However, when vaccination rates are high, most persons exposed to an infected person will have received ≥1 doses of vaccine. As a consequence, the expected proportion of persons who had received ≥1 doses of vaccine among reported measles case-patients will be substantially higher than 1%.

One can derive a simple quantitative relationship between vaccination coverage and the proportion of case-patients who had been vaccinated. Assuming vaccination coverage of v and vaccine effectiveness of α, the proportion of the population who are susceptible to measles infection is $1 - \alpha v$. If all susceptible persons are at the same risk of getting infected, the proportion of vaccinated persons among all case-patients will be $v(1 - \alpha)/(1 - \alpha v)$. This equation is similar to the screening method that has been used to calculate vaccine effectiveness on the basis of the proportion of case-patients who were vaccinated and vaccination coverage (*5*). Perhaps somewhat counterintuitive at first, the proportion of vaccinated measles case-patients increases with vaccination coverage (Figure).

We hypothesized that the observed proportion of measles case-patients who had been vaccinated can be used to infer the vaccination coverage in a population at risk (Figure). To this end, we assume a vaccine effectiveness of 99% among persons who had received ≥1 doses (*3*,*4*). In 2013, countries in the European Union/European Economic Area reported 9,708 measles case-patients for whom vaccination status was known (*2*). Of those, 11.8% had received ≥1 doses of measles vaccine. On the basis of the relationship derived above, this proportion corresponds to an expected vaccination coverage of 93.1% who had received ≥1 doses, which is consistent with reported numbers. Switzerland reported 3,850 measles case-patients with known vaccination status from August 2006 through June 2009; of these, 7.0% had been vaccinated with ≥1 doses (*8*). The inferred vaccination coverage of 88.3% is very close to the reported national level of 87.0% for receipt of ≥1 doses at 2 years of age (*8*). In contrast, the most recent numbers from the United States suggest that vaccination coverage for receipt of ≥1 doses is still well over 90%.

Various complexities might affect the relationship between vaccination coverage in a community and the proportion of case-patients who had been vaccinated. First, we assume a vaccine effectiveness of 99% among persons who received ≥1 doses. Other estimates indicate that

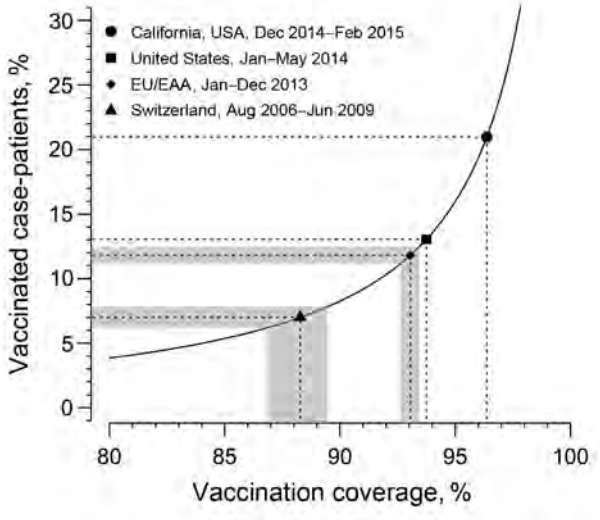

Figure. Relationship between vaccination coverage with ≥1 doses and the proportion of measles case-patients who had been vaccinated. The observed numbers of vaccinated case-patients can be used to infer the vaccination coverage for different populations. Of 62 (21.0%) measles case-patients with known vaccination status in California, USA, 13 had received ≥1 doses (*6*). Of 230 (13.0%) case-patients with known vaccination status in the United States during January–May 2014, a total of 30 had received ≥1 doses (*7*). Vaccine effectiveness is assumed to be 99% (*3*,*4*). The shaded areas for the countries of the European Union (EU) and European Economic Area (EEA), and Switzerland correspond to the 95% CIs. 95% CIs are omitted for California and the United States because of the small sample sizes.

vaccine effectiveness is 92% for persons who received 1 dose and 95% for those who received 2 doses (*9*). Assuming that vaccine effectiveness is lower shifts the curve (Figure) to the left and would result in a lower estimate of vaccination coverage. Second, different numbers of persons who received 1 and 2 doses complicate the identification of overall vaccine effectiveness. Third, vaccination status is unknown for some measles case-patients. The proportion of nonvaccinated persons among those case-patients might be higher than that among those known to be vaccinated, also leading to a lower estimate of vaccination coverage. Finally, nonvaccinated persons might be clustered together, and their risk for infection could be higher than that for the general population (*10*). This scenario would imply that the estimated vaccination coverage does not reflect the general population but instead corresponds to a clustered subpopulation among whom vaccination rates are lower. The effects of these complexities warrant further investigation. However, as the examples demonstrate, a model ignoring those effects is in good agreement with empirical data.

Our analysis suggests that the number of vaccinated measles case-patients should be closely followed through surveillance programs. A continuous decrease in the proportion of measles case-patients who had been vaccinated over the years could indicate a decrease in vaccination rates. Conversely, an increase in the proportion of measles case-patients who had been vaccinated would demonstrate the effectiveness of ongoing efforts to increase vaccination rates and could serve as a benchmark toward measles elimination.

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Lassa Virus in Multimammate Rats, Côte d'Ivoire, 2013

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To the Editor: Lassa fever is a zoonosis caused by Lassa virus (LASV; family Arenaviridae, genus Lassavirus). The primary reservoir of LASV is the multimammate rat (Mastomys natalensis), which is found throughout sub-Saharan Africa. LASV outbreaks among humans occur only in West Africa in 2 noncontiguous areas: 1 in Guinea, Liberia, and Sierra Leone; and 1 in Nigeria. Rare cases and evidence of exposure of humans have been documented in neighboring countries (i.e., Benin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, and Togo) (*1*). LASV RNA has been detected in only 4 patients: 1 in Germany who had traveled in Burkina Faso, Côte d'Ivoire, and Ghana (*2*); 1 in the United Kingdom who had returned from Mali (*3*); and 2 in

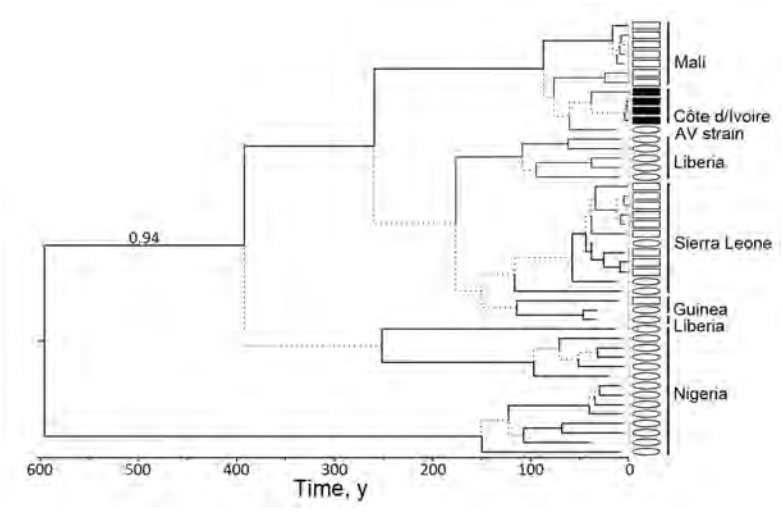

Figure. Bayesian chronogram of Lassa virus (LASV) sequences determined on the basis of a fragment of the large genomic segment. Branches receiving posterior probability values <0.95 and bootstrap values <50 (poorly supported) are dashed. LASV sequences of human origin are indicated by ovals, and those of multimammate rats are indicated by squares. Sequences reported in this study are indicated by black squares. This tree was built under the assumption of a molecular clock and is therefore rooted. The numerical value on the tree's most basal branch is the root posterior probability of this branch; it supports the notion that LASV sequences from Nigeria and other countries are not reciprocally monophyletic. GenBank accession nos. of sequences used for phylogenetic analyses are shown in online Technical Appendix Table 2 (http://wwwnc.cdc.gov/EID/article/21/8/15-0312-Techapp.pdf). AV strain indicates the strain from a German patient.

Ghana, for whom no viral sequence was available because detection was performed by reverse transcription PCR only (*4*). In the region in Mali where the patient from the United Kingdom was infected, identical LASV sequences were found in multimammate rats (*5*). The sequence of the strain identified from the patient in Germany, who was designated AV, is the closest known relative of the clade formed by sequences from Mali (*5*). However, LASV was not found in its natural host in any of the countries visited by patient AV (*6*,*7*).

For a study investigating zoonotic pathogens in rural habitats, we caught small mammals in 3 ecologic zones of Côte d'Ivoire: 1) dry bushland in northern Côte d'Ivoire, around Korhogo (*2*); semiarid bushland in central Côte d'Ivoire, around Bouake; and rainforest in southwestern Côte d'Ivoire, near the Taï National Park (*3*) (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/21/8/15-0312-Techapp.pdf). Traps were installed within and around 15 villages and enabled the capture of 27 eulipotyphlans and 254 rodents during August–October 2013. Animals were assigned at the genus level in the field on the basis of morphology. For 88% of them, assignment could later be refined to the species level by sequencing a fragment of the mitochondrial cytochrome *b* gene. A total of 14 animal species representing 8 genera were detected. All host sequences were deposited in Dryad (http://www.datadryad.org/; online Technical Appendix Table 1). Multimammate rats were the dominant commensals at all sampling locations, comprising 64.5% of the overall sample (online Technical Appendix Figure).

Tissue samples were collected from all animals according to standard protocols. Total nucleic acids were extracted from lung samples and tested for the presence of LASV RNA by using a real-time PCR system amplifying a 400-bp fragment of the large genomic segment (*8*) (online Technical Appendix). LASV RNA was detected in 4 of 18 specimens of *M. natalensis* captured in Gbalôhô, near Korhogo (online Technical Appendix Figure). This site is much farther north in Côte d'Ivoire than previously examined sites (*6*). The 4 PCR-positive animals were 3 males and 1 female that were all captured indoors, 3 in the same house. PCR products were sequenced according to the Sanger method (GenBank accession nos. LN823982–LN823985). According to phylogenetic analyses performed in maximum likelihood and Bayesian frameworks (online Technical Appendix), LASV sequences identified in multimammate rats from Côte d'Ivoire formed a robust clade with sequences from the human AV strain and the LASV infecting multimammate rats in southern Mali (bootstrap 97, posterior probability 1.00; Figure). This phylogenetic placement opens up the possibility that patient AV was infected during her travel through Côte d'Ivoire, possibly in or near the city of Korhogo. Tip date calibration of Bayesian analyses showed that the most recent common ancestor of all LASV sequences from Côte d'Ivoire and Mali circulated ≈90 years ago (Figure; online Technical Appendix Table 2).

Further studies will be needed to investigate the geographic distribution of LASV in Côte d'Ivoire and the frequency of human infections. The current lack of diagnosed cases in the area may be caused by underreporting. Sensitization campaigns are needed to increase awareness of the risk for LASV infection among the local population and to improve detection of cases by health workers.

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Rickettsia felis Infection among Humans, Bangladesh, 2012–2013

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To the Editor: *Rickettsia felis*, which belongs to the spotted fever group of rickettsiae, causes febrile illness in humans. The main vector of this bacterium is the cat flea (*Ctenocephalides felis*). Since publication of reports of *R. felis* as a putative pathogen of humans in the United States in 1994, *R. felis* infection in humans worldwide has been increasingly described, especially in the Americas, Europe, Africa, and eastern Asia (*1*,*2*). *R. felis* infection is common among febrile patients (≈15%) in tropical Africa (*3*) and among apparently healthy persons in eastern coastal provinces of China (*4*). However, little is known about prevalence of *R. felis* infection of humans in southern Asia, although 3 serologically diagnosed cases in Sri Lanka have been described (*5*) and *R. felis* has been detected in rodent fleas in Afghanistan (*6*). Hence, we conducted a cross-sectional study in Bangladesh to explore the presence of rickettsial pathogens among patients with fever of unknown origin.

Study participants were 150 patients at Mymensingh Medical College (MMC) hospital in Mymensingh, north-central Bangladesh, from July 2012 through January 2014, and 30 healthy control participants from the staff at the same college. Selected patients met the following criteria: 1) fever (axillary temperature >37.5°C) for >15 days that did not respond to common antimicrobial drug therapy; 2) any additional clinical features including headache, rash, lymphadenopathy, myalgia, and eschars on skin; and 3) titer according to the Weil-Felix test (antibodies against any of 3 *Proteus* antigens) of >1:80. Patients with evident cause of fever (e.g., malaria diagnosed by blood smear or immunochromatography) were excluded from the study. This research was approved by the college institutional review board, and informed consent was obtained from patients (or guardians) and healthy controls before their entry into the study.

Venous blood samples were aseptically collected from the patients, and DNA was extracted by conventional method by using proteinase K and sodium dodecyl sulfate. Nested PCR selective for the 17-kDa antigen gene was used to screen for rickettsiae according to the method described previously (*7*); ≈100 ng of DNA in a 50-mL reaction mixture was used. For each PCR, a negative control (water) was included and utmost care was taken to avoid contamination. Among the 150 samples tested, results were positive with a 232-bp amplified product for 69 (46%) and negative for all controls.

PCR products from 20 samples were randomly selected for sequence analysis. All nucleotide sequences from

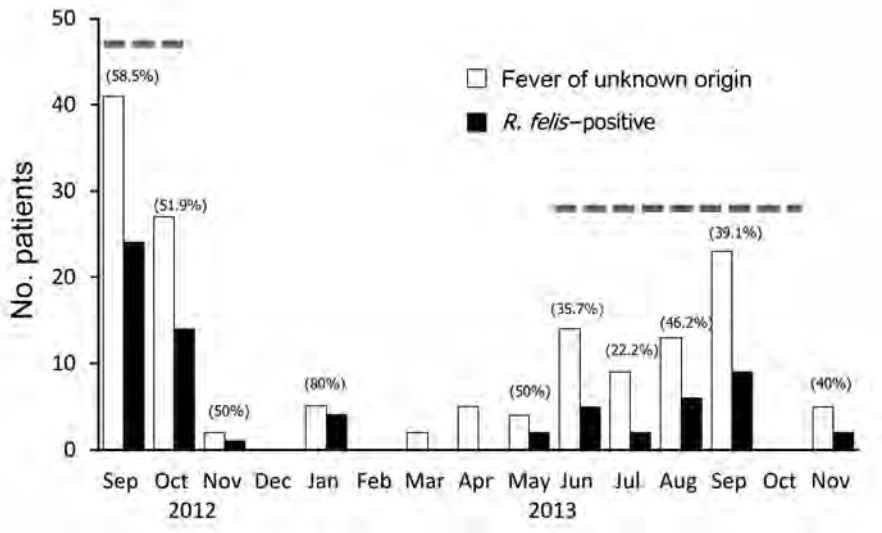

Figure. Number of patients with fever of unknown origin and *Rickettsia* felis–positive cases in the Mymensingh Medical College hospital, Bangladesh, 2012–2013. Numbers in parentheses indicate rates of *R. felis* positivity for each month; dashed lines indicate monsoon season (June–October).

the 17-kDa antigen gene (186-bp) were identical to that of reference strain *R. felis* URRWXCa12 (GenBank accession no. CP000053). Among all 17-kDa–positive samples, positivity was further confirmed by PCR detection of the *R. felis* 16S rRNA gene and *gltA* in 95% and 75% of samples, respectively. Partial 16S rRNA gene sequences (305-bp) from 12 samples were 100% or 99% (10 and 2 samples, respectively) identical to that of *R. felis* URRWXCa12. The complete open reading frames of *ompA* (1773-bp), partial *ompB* (413-bp), and *gltA* (611-bp) sequences determined for 3, 3, and 5 samples, respectively, were also identical to those of *R. felis* URRWXCa12. The 5 gene sequences were determined for samples from 3 patients (2-year-old girl, 8-year-old boy, 17-year-old boy). The 5 gene sequences from the 2-year-old girl (strain Ric-MMC7) and 2 partial sequences of 16S rRNA (Ric-MMC71 and Ric-MMC133) were deposited in GenBank under accession nos. KP318088–KP318094.

According to PCR, the positivity rate for the *R. felis* 17-kDa antigen gene was higher among male (54%, 40/74) than among female (38%, 29/76) patients and higher among patients in young and old age groups (0–15 years, 57%; 45–60 years, 62%) than among patients in other age groups (15–30 years, 41%; 30–45 years, 44%). During the study period, rates of *R. felis* positivity were highest during the late rainy season of 2012 (September [59%] and October [52%]) and lowest (0%) from December 2012 through April 2013 (Figure). The rate was significantly higher among farmers (76%, 13/17) than among persons of other occupations (e.g., housewives, teachers, students) (42%, 56/133); p = 0.016. Among the 69 rickettsiae-positive patients, headache and myalgia were reported by 29 (42%) and 17 (25%), respectively, whereas rash was detected in only 2 (3%) patients, both of whom were female.

This study demonstrated *R. felis* infection in patients in Bangladesh with unidentified febrile illness. The high prevalence (46%) of *R. felis* infection suggests that this infection is endemic to the north-central area of this country and might be associated with contact between humans of low socioeconomic status and the large number of stray cats and dogs. In contrast, the number of genetically confirmed cases of *R. felis* infection in humans reported to date in China, Taiwan, Thailand, and Laos have been very few (*1*,*2*,*4*,*8*–*10*), although widespread presence of this bacterium in cat fleas has been documented. For further confirmation of spread of this infectious disease, the prevalence of *R. felis* infections among humans, vectors, and reservoirs in other areas in Bangladesh and in other countries in southern Asia should be investigated.

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Malaria Prophylaxis Failure with Doxycycline, Central African Republic, 2014

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To the Editor: Doxycycline is an effective antimalarial prophylactic drug when administered as a monotherapy 1 day before, daily during, and for 4 weeks after travel to an area where malaria is endemic (*1*). Doxycycline is currently a recommended chemoprophylactic regimen for travelers visiting areas where malaria is endemic and has a high prevalence of chloroquine or multidrug resistance (*2*). The World Health Organization also recommends doxycycline in combination with quinine or artesunate as the second-line treatment for uncomplicated *Plasmodium falciparum* malaria (*3*).

Prophylactic and clinical failures of doxycycline against *P. falciparum* have been associated with both inadequate doses (*4*) and poor patient compliance (*5*). However, resistance can also explain failures of prophylaxis. Cycline resistance in *Plasmodium* spp. has been documented as a consequence of selective drug pressure in a *P. berghei* murine malaria model (*6*). The administration of increasing doses of minocycline to mice infected with 1×10^7 parasites for 86 successive passages over 600 days made it possible to obtain a resistant *P. berghei* strain with a median drug inhibitory concentration (IC_{50}) of 600 mg/kg/d, which is 6-fold higher than that of the susceptible starting strain (100 mg/kg/d) (*6*). A Bayesian mixture modeling approach identified 3 different phenotypes (low, medium, and high doxycycline IC_{50} phenotypic groups) among *P. falciparum* clinical isolates (*7*,*8*). Using 90 isolates from 14 countries, we demonstrated that increases in copy numbers of *P. falciparum* metabolite drug transporter gene (*Pfmdt*, PFE0825w) and *P. falciparum* GTPase TetQ gene (*PfTetQ*, PFL1710c) are associated with reduced susceptibility to doxycycline (*9*); this association was later confirmed (*7*). In addition, isolates with *PfTetQ* KYNNNN motif repeats are associated with in vitro reduced susceptibility to doxycycline and with a significantly higher probability of having an IC_{50} above the doxycycline resistance threshold of 35 mM (*9*,*10*).

We report a case of documented malaria prophylactic failure with doxycycline in a 26-year-old soldier from France who was infected during a 6-week peacekeeping mission in the Central African Republic in 2014. According to his colleagues and the collective prophylaxis intake, the patient had been compliant with doxycycline prophylaxis. On admission to a hospital in Bangui, Central African Republic, the patient had fever (temperature 40°C), alteration of consciousness, and hypotension. The diagnosis of severe *P. falciparum* malaria was made on the basis of a rapid diagnostic test confirmed by a blood smear test (parasitemia 8% on day 0). Intravenous artesunate was immediately started, in accordance with World Health Organization recommendations (*3*). The patient's clinical condition worsened, and kidney failure developed. Twenty-four hours later (day 1), he was transported by airplane to Bégin Military Teaching Hospital (Saint-Mandé, France). On admission, he had

cerebral edema and a *P. falciparum* parasitemia level of 0.7%. The patient died 1 day later (day 2).

A blood sample obtained from the patient on day 1 in France showed a doxycycline concentration of 195 μg/mL plasma. This concentration, which was determined by liquid chromatography coupled with tandem mass spectrometry, was compatible with a last doxycycline uptake 1 day before diagnosis (day –1). The finding of the expected doxycycline plasma concentration, together with assurances (colleague's statements and collective intake of doxycycline) that the patient had followed the drug regimen, was sufficient to suggest prophylaxis failure in a treatment-compliant patient.

The *P. falciparum* sample obtained from the patient on arrival in France was evaluated for in vitro susceptibility to doxycycline, but the evaluation was unsuccessful. The number of copies of *PfTetQ* and *Pfmdt* genes were evaluated relative to the single-copy *P. falciparum b-tubulin* gene (*pfβtubulin*), as previously described (*7*,*8*). The sample was assayed in triplicate. The $2^{-\Delta\Delta Ct}$ method (where C_t indicates cycle threshold) of relative quantification was used and adapted to estimate the number of copies of *Pfmdt* and *PfTetQ* by using the formula $DDC_t = (C_{t\ (PfTetQ\ \text{or}\ Pfmdt)} - C_{t\ (Pf\beta tubulin)})_{\text{Sample}} - (C_{t\ (PfTetQ\ \text{or}\ Pfmdt)} - C_{t\ (Pf\beta tubulin)})_{\text{Calibrator}}$. Genomic DNA extracted from 3D7 *P. falciparum*, which has a single copy of each gene, was used for calibrator sample; *Pfβtubulin* served as the control housekeeping gene. The experiment was assayed twice. The sample had 2 copies of *PfTetQ* and *Pfmdt* genes, which suggested decreased in vitro susceptibility of the sample to doxycycline (*8*,*9*). The genotyping of *PfTetQ* sequence polymorphisms was done by using conventional methods with the primers *PfTetQ* forward (5'-TCACGACAAATGTGCTAGATAC-3') and *PfTetQ* reverse (5'-ATCATCATTTGTGGTGGATAT-3'), as previously described (*10*). Two *PfTetQ* KYNNNN motif repeats were found in the sample; <3 KYNNNN motif repeats are predictive of in vitro *P. falciparum*–resistant parasites with an IC_{50} of >35 mM (odds ratio 15) (*10*). The 2 copies of *Pfmdt* and the 2 KYNNNN motif repeats have been shown to be associated with parasites with in vitro resistance to doxycycline (*9*,*10*). The association of doxycycline resistance (prophylactic failure with statement of correct intake and the presence of an expected concentration) with increased *Pfmdt* copies and decreased *PfTetQ* KYNNNN motif repeats suggest that these molecular markers are predictive markers of doxycycline resistance that can be used for resistance surveillance.

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Avian Gyrovirus 2 DNA in Fowl from Live Poultry Markets and in Healthy Humans, China

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[1]These authors contributed equally to this article.

To the Editor: In 2011, a chicken anemia virus (CAV)–related sequence, designated avian gyrovirus 2 (AGV2), was first identified in serum samples from diseased chickens in Brazil (*1*). During the same year, a human gyrovirus (HGyV) sequence that had high identity to AGV2 was detected in the skin of humans in France (*2*). As with CAV, 3 open reading frames (ORFs) for encoding viral proteins (VP) 1–3 (*2*) overlapped in genome of AGV2. Recently, HGyV/AGV2 has been detected in Hong Kong in chicken meat for consumption by humans, in human blood samples from donors in France, and in HIV-positive persons and organ transplant recipients in Italy and the United States (*3*–*5*). However, the epidemiology, host range, transmission route, and pathogenesis of AGV2 remain poorly understood. Bullenkamp et al. found that AGV2 VP3 protein, like CAV VP3, can induce apoptosis of tumor cells (*6*). Also, Abolnik et al. reported the detection in Southern Africa of AGV2 in brain tissue of chickens that showed severe neurologic signs (*7*). These findings highlight the potential pathogenesis of AGV2.

So far, little is known about AGV2 in mainland China among chickens and humans. Because live poultry market (LPMs) play a critical role in the transmission of poultry pathogens to humans, we used PCR to investigate the presence of AGV2 in chickens (54 feather shaft samples) from 4 LPMs in Yangzhou and in 178 human blood samples from healthy persons living in Yangzhou. The DNA from the feather shafts and human blood were extracted as previously described (*8*). PCR was performed by using the following 2 primers: AGV2_F 5′−CGTGTCCGCCAGCAGAAACGAC-′3 and AGV2_R 5′-GGTAGAAGCCAAAGCGTCCACGA-′3. The PCR targets partial VP2 and VP3 genes that have an expected size of 346 bp. The parameters of the PCR were as follows: 1 cycle at 95°C for 5 min; then 30 cycles at 94°C for 30 s, 64°C for 30 s, and 72°C for 30 s; and 1 cycle at 72°C for 10 min. PCR showed that a band with the size of ≈346 bp could be amplified in 10 of 54 chicken feather samples and in 2 of 178 human blood samples.

We confirmed the AGV2 specificity of these PCR–amplified bands by direct sequencing using the Sanger method. The sequence assay showed that the 12 sequences identified here had 98.3%–100% homology to each other and 92.2%–99.1% aa identity to AGV2 samples previously deposited in GenBank (see Figure legend for accession numbers). The positive rates for samples from the 4 LPMs tested were 25%, 12.5%, 15.8%, and 20%; the positive rate for the 178 human blood samples was 1.1%. The low positive frequency of AGV2 in human blood detected in this study is consistent with that found by investigation in other countries (*3*,*4*). Because the limit of detection of PCR in this study was estimated to be 2.7 copies of AGV2 DNA using dilutions of a plasmid with partial AGV2 sequence, we determined that the copy number of AGV2 in the 2 positive human blood samples was 2.7×10^3 copies/mL plasma.

We also constructed a phylogenetic tree using the neighbor-joining method (1,000 bootstrap replications) with MEGA6 (*9*). The tree analysis revealed that the 12 AGV2 isolates we identified and 7 AGV2 isolates from GenBank clustered into 2 subgroups on the basis of the PCR amplified fragment (Figure). The 12 AGV2 sequences we identified clustered together with gyrovirus sequences detected in ferret and human samples in subgroup I, and the prototype sequence Ave3 was located in subgroup II. The 12 AGV2 showed ≈92.2%–93% aa identity to Ave3, and <99.1% homology with isolates CL33, G13, and 915F06007 detected in ferret and human samples. The 12

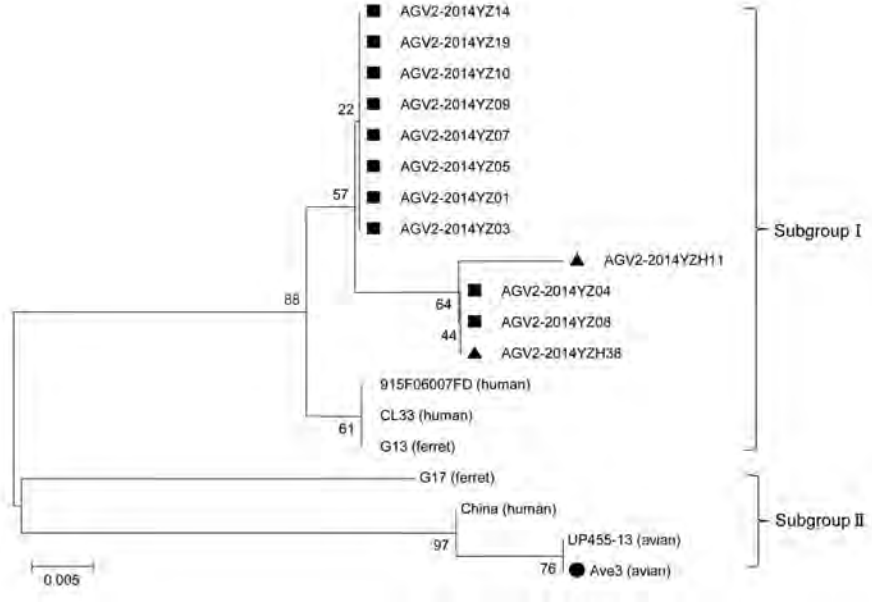

Figure. Phylogenetic analysis of AGV2. The phylogenetic tree was constructed by using the neighbor-joining method (1,000 bootstraps) with MEGA6 (9). Black squares indicate the 10 AGV2 identified from live poultry markets; black triangles indicate the 2 AGV2 identifed from human blood; black dot indicates the prototype AGV2 sequence. Sequences and GenBank accession nos.: AGV2–2014YZ01, KP993124; AGV2–2014YZ03, KP993125; AGV2–2014YZ04, KP993126; AGV2–2014YZ05, KP993127; AGV2–2014YZ07, KP993128; AGV2–2014YZ08, KP993129; AGV2–2014YZ09, KP993130; AGV2–2014YZ10, KP993131; AGV2–2014YZ14, KP993132; AGV2–2014YZ19, KP993133; AGV2–2014YZH11, KP993134; AGV2–2014YZH38, KP993135; 915 F 06 007 FD, FR823283; CL33, JQ308212; G13, KJ452214; G17, KJ452213; China, JQ690763; UP455–13, KF436510; Ave3, HM590588. Scale bar indicates amino acid substitutions per site.

AGV2 sequences also showed ≈93%–93.9% identities to ACV2 sequence that was previously identified in human fecal samples from mainland China (GenBank accession no. JQ690763). The China sequence also clustered with Ave3 in subgroup II. These findings indicate that ≥2 subgroups of AGV2 are circulating in mainland China.

Our results demonstrate the presence of AGV2 in LPMs and human blood in mainland China. The amplification and analysis of partial AGV2 sequences was the major limitation in our method. The high homology between sequences identified in LPMs and human blood indicates the LPMs are a potential source for AGV2 in humans. Unlike our 12 conserved AGV2, AGV2 identified by Santos et al. in southern Brazil varied <15.8%, and these variants of AGV2 were mainly detected in diseased chickens (*8*). However, little is known about the molecular epidemiology of these AGV2 variants in other countries. More recently, Varela et al. reported the detection of AGV2 in poultry vaccines, indicating the potential role of contaminated vaccines in the spread of AGV2 (*10*). Future studies should investigate the large geographic distribution of AGV2 and monitor the variants, the host range, and the associated diseases.

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Mapping Disease Transmission Risk: Enriching Models Using Biogeography and Ecology

A. Townsend Peterson

Johns Hopkins University Press, Baltimore, Maryland, USA, 2014

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Global human population density is increasing, as are our abilities to assemble large ecologic datasets and perform surveillance for and respond to diseases as they emerge. Consequently, multidimensional ecologic data may help us improve public health locally and globally. This engaging book empowers disease modelers and public health policy makers by introducing them to ecologic niche models as predictors of disease transmission risk.

Part I describes distributional ecology, contrasting the ecologic approach that takes into account multiple layers of distributional data with an approach that only plots disease cases or absences. Part II elaborates on the kinds of data necessary to develop ecologic models rather than arbitrarily complex "black box" models. Part III critiques poor study design and data assembly and demonstrates how not to construct a dataset. Part IV summarizes approaches to calibrating, processing, and evaluating models and the production of risk maps, warning readers about the complex factors that are associated with human society.

Peterson presents examples where models calibrated for one dataset are used to transfer rules to another dataset to assess risk. By contrasting these models with models that incorporate only disease cases, Peterson shows how to define the niche of vectors of disease where occurrence data are rich, then evaluate the potential presence of the niche in novel locales or across changing environments, yielding the risk of emergence.

In this book, Peterson has put together an easy read that demonstrates his expertise and persuasively frames disease transmission risk in terms of niche models. A reader already convinced that understanding the geography of ecologic interactions is essential to public health disease modeling may want to pick up a more technical book that addresses ecologic niche modeling in detail. For readers interested in mechanistic models, Mapping Disease Transmission Risk is not the right book. Peterson could have handled some of the issues about the relative value and weighting of presence and absence data by using appropriate likelihood models of the observation process itself. Bayesian analyses could obviate many of the issues of uncertainty associated with low counts and zero-observation cells. However, for readers who would like to move into the geographic mapping of disease emergence and aren't sure where to start, this book provides many dos and don'ts and references that could jump-start a project.

Peterson concludes by noting the historical link between public health and geographic mapping. As we begin to view and quantify every foot of the Earth we depend on, it becomes increasingly possible and necessary to incorporate many layers of knowledge to guide policy for human—and ecological—health. To quote Martin Luther King, Jr., "It really boils down to this: that all life is interrelated. We are all caught in an inescapable network of mutuality, tied together into a single garment of destiny. Whatever affects one directly, affects all indirectly" (*1*).

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Correction: Vol. 21, No. 4

An incorrect version of the Technical Appendix was provided online for the article Population Structure and Antimicrobial Resistance of Invasive Serotype IV Group B Streptococcus, Toronto, Ontario, Canada (S. Teatero et al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/21/4/14-0759_article).

Cornelius Norbertus Gijsbrechts (ca 1630−after 1683) *Trompe l'oeil with Studio Wall and Vanitas Still Life*, 1668. Oil on canvas. 59.84 x 46.46 in / 152 x 118 cm. Digital image from the public domain collection, Statens Museum for Kunst, Copenhagen.

Beyond First Impressions

Byron Breedlove and Jared Friedberg

Few details endure about the life and family circumstances of Flemish painter Cornelius Norbertus Gijsbrechts. We know that he was born in Antwerp, Belgium, and his talent earned him a position of court painter with two Danish kings, Frederick III (1609–1675) and Christian V (1646–1699). In 1660, Gijsbrechts was accorded membership in the prominent, influential Guild of St. Luke. Over his career, the artist worked in various locations, including Antwerp, Regensburg, Hamburg, and Copenhagen, and it was in the latter city where he created his signature series of trompe l'oeil (deception of the eye) paintings during 1668–1672.

Trompe l'oeil, a subgenre of still life painting, embraces use of realistic imagery to create an optical illusion of depth in works that can be perceived as real, three-dimensional objects rather than flat paintings. It flourished from the Renaissance onward, though murals of trompe l'oeil art are found among the ruins of Pompeii and Herculaneum and remain popular today. According to the National Gallery of Art, "The discovery of perspective in fifteenth-century Italy and advancements in the science of optics in the seventeenth-century Netherlands enabled artists to render objects and spaces with eye-fooling exactitude."

This month's cover painting *Trompe l'oeil with Studio Wall and Vanitas Still Life* is a prime example of this inventive subterfuge and also of a vanitas painting, a meditation on mortality. Gijsbrechts makes a skull the focal point of the painting, ensuring no one misses his allusion to the temporal nature of life. Other symbols supports the theme of transience: smoke trailing from an extinguished candle and an hourglass tipped on its side symbolize death; a violin, tankard, clay pipe, and tobacco represent fleeting pleasures. The unity of these symbols represent the ebb and flow of birth, death, and resurrection, encouraging both a wistful examination of life's purpose and a solemn acceptance of death's finality.

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Gijsbrechts pulls the scene back and reveals the vanitas on a temporary canvas hung on a wooden wall, surrounded by the tools of his craft, including brushes and a palette featuring the colors he used in his construction of the painting. A miniature self-portrait affixed to the frame reminds viewers that art endures beyond the life of the artist. Those who fall for the deception and perceive the vanitas as the sole painting miss the message that Gijsbrechts intends: nothing is what it seems without context.

In trompe l'oeil, the background imagery surrounding the painting's center provides perspective and clues that engage viewers and contribute to understanding the overall work. Disease surveillance for emerging infections relies on a somewhat similar, albeit more complicated process. To gain perspective, one must assess a complex but incomplete set of intertwined, dynamic factors, which may include antimicrobial drug resistance, climate change, food production practices, global mobility, the route(s) of transmission of the etiologic agents, and ecology of the pathogens.

By their nature, surveillance data are representative of disease in populations, and they are always incomplete and sometimes inaccurate. It takes a critical, well-prepared mind to properly interpret and analyze the meaning and significance of surveillance data or unravel clues about how an emerging infectious disease spreads. Gijsbrechts' portrait displays the tools and medium that he used to construct his illusion, but not everyone who views the painting will come to this realization. The experience of viewing a trompe l'oeil reminds us that seeing beyond our initial assumptions—whether we are studying art, investigating the outbreak of an emerging infection, or analyzing reams of data—requires that we connect background information, delve beyond the surface, and recognize patterns and aberrations.

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- Improving Accuracy of Influenza-Associated Hospitalization Rate Estimates
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- Laboratory Testing for Middle East Respiratory Syndrome Coronavirus, California, USA, 2013–2014
- Follow-up of Contacts of Middle East Respiratory Syndrome Coronavirus–Infected Returning Travelers, the Netherlands, 2014
- Acute Respiratory Infections in Travelers Returning from MERS-CoV–Affected Areas

Complete list of articles in the September issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

August 24–26, 2015
ICEID
International Conference on Emerging Infectious Diseases
Atlanta, GA, USA
http://www.iceid.org/

August 29–September 2, 2015
IDBR
20th Annual Infectious Disease Board Review Course
McLean, VA, USA
http://smhs.gwu.edu/cehp/activities/courses/idbr

September 17–21, 2015
ICAAC
Interscience Conference on Antimicrobial Agents and Chemotherapy
San Diego, CA, USA

October 25–29, 2015
ASTMH
American Society of Tropical Medicine and Hygiene
64th Annual Meeting
Philadelphia, Pennsylvania
info@astmh.org

December 6–9, 2015
2015 National HIV Prevention Conference
Atlanta, GA
http://www.cdc.gov/nhpc/index.html

February 8–10, 2016
ASM Biodefense and Emerging Diseases Research Meeting
Arlington, VA, USA
biodefense@asmusa.org

March 2–5, 2016
ISID
17th International Congress on Infectious Diseases
Hyderabad, India
activities/courses/idbr

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Article Title:

Escherichia coli O157 Outbreaks in the United States, 2003–2012

CME Questions

1. You recently treated a 28-year-old man with a 3-day history of bloody diarrhea. His stool culture returns with positive results for *Escherichia coli* O157. In the current study by Heiman and colleagues, what was the most common source of outbreaks of *E. coli* O157?

A. Foodborne
B. Person-to-person
C. Animal contact
D. Other/unknown

2. Which of the following specific foods was associated with the majority of foodborne cases of *E. coli* O157 in the current study?

A. Beef
B. Dairy
C. Leafy vegetables
D. Poultry

3. You call the patient with the laboratory result, and he is feeling somewhat better since starting antibiotics. Which of the following statements regarding the severity of illness with *E. coli* O157 in the current study is most accurate?

A. Person-to-person outbreaks accounted for the majority of cases of mortality
B. Person-to-person outbreaks were associated with the highest rates of hospitalization
C. Hemolytic uremic syndrome was most associated with outbreaks from animal contact
D. Outbreaks related to beef were associated with the highest rates of hospitalization

4. What else should you consider regarding the epidemiology of *E. coli* O157 outbreaks in the current study?

A. Nearly 80% of patients were men
B. Most cases occurred in the winter
C. Outbreaks were more common in Southern vs Northern states
D. More than 80% of waterborne outbreaks were reported in states that border the Mississippi River

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

Community-Based Outbreak of *Neisseria meningitidis* Serogroup C Infection in Men who Have Sex with Men, New York City, New York, USA, 2010–2013

CME Questions

1. Which of the following statements regarding invasive meningococcal disease in general and its prevention is most accurate?

A. Approximately half of cases are fatal
B. The rate of serious long-term complications exceeds 10%
C. Young adults carry the highest risk
D. No booster for the meningococcal vaccine is required if it is delivered by age 11 years

2. Which of the following statements regarding the epidemiology and outcomes of the serogroup C invasive meningococcal disease (MenC) outbreak described in the current study is most accurate?

A. Approximately half of individuals affected in the outbreak were men who have sex with men (MSM)
B. Most cases were fatal
C. Nearly all patients were white
D. More than half of participants with data available had used electronic tools to find sexual partners

3. Which of the following statements regarding the vaccine campaign against meningitis in the current study is most accurate?

A. Less than 1,500 vaccine doses were distributed during the outbreak
B. Free vaccine events at bars and clubs were highly successful
C. Vaccine events hosted by clinicians occurred solely in medical offices
D. Vaccine events hosted by clinicians were successful overall

4. Which of the following elements of the meningococcal vaccination outreach campaign described in the current study was most effective?

A. Email
B. Banner advertisements on websites
C. Articles in The New York Times
D. Pop-up advertisements on mobile applications

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

Visit www.cdc.gov/vaccines/parents. Learn about the vaccines your baby needs from a reliable source. The CDC's website explains the 14 diseases vaccines prevent, CDC's recommended schedule, possible side effects, how to comfort your baby during vaccine visits and more. Talk to your child's doctor, and visit our website to get the facts about vaccines.

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Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. 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 editable figures as separate files (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .tif or .jpeg files. Do not embed figures in the manuscript file. Use Arial 10 pt. or 12 pt. font for lettering so that figures, symbols, lettering, and numbering can remain legible when reduced to print size. Place figure keys within the figure. Figure legends should be placed 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.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references (not to exceed 15) but no abstract, figures, or tables. Include biographical sketch.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities (500–1,000 words) are published online only. They should be submitted no later than 6 months after the conference and focus on content rather than process. Provide illustrations, references, and links to full reports of conference activities.

Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Photo Quiz. The photo quiz (1,200 words) highlights a person who made notable contributions to public health and medicine. Provide a photo of the subject, a brief clue to the person's identity, and five possible answers, followed by an essay describing the person's life and his or her significance to public health, science, and infectious disease.

Etymologia. Etymologia (100 words, 5 references). We welcome thoroughly researched derivations of emerging disease terms. Historical and other context could be included.

Announcements. We welcome brief announcements of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.

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