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Crockett Johnson (1906–1975), *Mystic Hexagon (Pascal)* **(1965)** (detail). House paint on Masonite, 49 in x 25 in / 24.5 cm x 63.5 cm. National Museum of American History, Kenneth E. Behring Center, Constitution Avenue NW, between 12th and 14th Streets, Washington, DC, USA.

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A Framework for Modeling Emerging Diseases to Inform Management

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The rapid emergence and reemergence of zoonotic diseases requires the ability to rapidly evaluate and implement optimal management decisions. Actions to control or mitigate the effects of emerging pathogens are commonly delayed because of uncertainty in the estimates and the predicted outcomes of the control tactics. The development of models that describe the best-known information regarding the disease system at the early stages of disease emergence is an essential step for optimal decision-making. Models can predict the potential effects of the pathogen, provide guidance for assessing the likelihood of success of different proposed management actions, quantify the uncertainty surrounding the choice of the optimal decision, and highlight critical areas for immediate research. We demonstrate how to develop models that can be used as a part of a decision-making framework to determine the likelihood of success of different management actions given current knowledge.

Despite continued calls to improve the response to emerging infectious zoonotic diseases (*1,2*), universal guidelines for determining the best course of action when a new disease emerges are unavailable. Increasing ease of global travel (*3*), continued encroachment of human populations into wildlife-occupied areas, climate change (*4*), and increasing rates of microbial evolution and antimicrobial drug resistance (*5*) have increased the likelihood that wildlife pathogens will be introduced into novel areas or naive populations and spill over into human populations (*1*). This accelerating rate of disease emergence leaves decision makers with a short time frame to determine and implement an appropriate course of action. A framework that quickly, rigorously, and effectively synthesizes relevant information about a wildlife pathogen in the early stages of emergence is essential for informing management at critical stages and ultimately reducing the potential effects of the disease on humans, livestock, and other wildlife populations.

Decision theoretic approaches provide formal guidelines for transparent, repeatable, and defensible decision-making that addresses specific management objectives, uncertainty of consequences, and potential trade-offs (*6*). Using approaches such as structured decision-making to frame decisions, modelers are provided a mechanism for including multiple and potentially competing objectives and evaluating the importance of uncertainties to a decision (*7*). An essential component for applying decision theory to emerging diseases is the development of predictive models that can be used to evaluate trade-offs between different management actions and disease consequences (*8*). The role of predictive models in informing management decisions is to estimate the consequences of alternative control strategies and help determine which strategies are optimal. Models can be used to assist decision makers with assessing the probability of a successful management outcome versus the risk of an unacceptable outcome (including non-ecologic consequences), avoid unintentional consequences that might be exacerbated by delaying management interventions (*9*), and accommodate different goals and values of the decision maker and stakeholders (*5,8*). However, researchers are often reluctant to develop a model for forecasting the potential effects of emerging pathogens and the potential consequences of management actions because of uncertainty regarding the structure of the system (i.e., which parameters should be included in the model) and model parameter estimates (*10*).

Uncertainty often limits the ability to choose effective management strategies; therefore, it is vital to discriminate between uncertainties that are irreducible (i.e., environmental or demographic stochasticity, which might not be resolved with more information but must be considered regardless in making forecasts) and uncertainties that are reducible through research, monitoring, and surveillance. Reducible uncertainties might include the choice of model (i.e., structural uncertainty) that best describes system dynamics, the effects of system drivers (i.e., parametric uncertainty), and variation in system states across the landscape (i.e., spatial variability). Structural uncertainty can be resolved by testing different models and observing which

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model(s) best predict the system in future years. Parametric uncertainty and spatial variability can likewise be reduced with monitoring data or by conducting research.

In this article, we outline 3 components essential to building a predictive modeling framework that researchers and managers should consider early in the emergence of a wildlife pathogen: 1) which modeling frame is most appropriate, 2) which parameters or factors are critical to making preliminary predictions, and 3) how to collate existing data to parameterize the initial models. We describe 4 commonly used models for disease systems, identify 5 key characteristics of disease systems that represent minimally sufficient information needed to parameterize models, and identify 3 ways to parameterize models when reliable data are lacking. Using this 4-5-3 framework, researchers can work with managers to rapidly develop useful predictions with uncertainty and prioritize information gathering to improve the management of emerging diseases (Figure).

Choosing the Modeling Framework

Many disease modeling frameworks are available to select from (11,12) (online Technical Appendix Table 1, https://wwwnc.cdc.gov/EID/article/23/1/16-1452-Techapp1.pdf). By considering the objectives of the modeling, the assumptions of the different model frameworks, and the type of data that is either available or being collected, the list of modeling options can be narrowed down. Four generally useful classes of models are commonly used either on their own or in tandem with other model types to predict the spread and dynamics of wildlife pathogens: occupancy or patch dynamic models (13,14); compartmental (e.g., susceptible-infected-resistant) models (15); ecologic diffusion models (16); and agent-based (or individual-based) models (17).

Occupancy modeling focuses on patch dynamics, colonization, and extinction rates and is appropriate for hosts that live in discrete habitats, such as in wetlands, in forest or prairie remnants, or on mountain tops, where subpopulations are discrete and connected by occasional dispersal (18). The disease status (detected or not detected, percentage of hosts with disease) and the detection or nondetection of the host species in the patch is considered in these models, and the observed data can be corrected for nondetection bias. These models are appropriate for understanding landscape-level occurrence (number of patches occupied by disease) and extinction dynamics of an emerging disease (19). These models work best for disease systems in which the effects of the disease are severe and likely to result in patch extinction rather than sublethal effects that result in small declines in abundance. Alternatively, occupancy models have been used to model the dynamics of chytrid fungus for studies in which individual hosts within a patch are assessed for disease, and prevalence is estimated as the

proportion of infected hosts (inferred via PCR detection of a pathogen) in a patch (20).

Compartmental models can capture the subtleties of sublethal effects on populations; these models require longitudinal information on individual hosts, although a sample of the population during 1 time period across multiple age groups can substitute for temporal information under certain assumptions (21). Traditional susceptible-infected-resistant models assume the population is homogeneous with little spatial structure. This type of model works well for host populations in which individual disease states can be observed through time (e.g., the host-disease system of brucellosis in bison, in which species are well-connected in space and can be captured and recaptured over time) (22).

Diffusion models can be used to model the spread of diseases and can be useful for predicting new areas of disease emergence. Information needed for these models includes host movement characteristics, contact rates between host species, and transmission pathways of the disease. Observations of new disease locations over time can also be used to estimate the rate of spread of the disease. Diffusion models have been used successfully to estimate the rates of spread of rabies in foxes (23) and foot-and-mouth disease in feral pigs (24).

Agent-based models (also known as individual-based models) can be used to assess the overall population dynamics of the host and the spread of the disease (25). These models can be particularly useful when it is necessary to model the disease system in a spatially-explicit fashion or when host behavior is complex (e.g., when hosts learn). Agent-based models have been used to assess the spatial patterns of parasite transmission in red colobus (*Procolobus rufomitratus*) monkeys, in which each host has a spatial memory of the value of patches, and each host weighs the benefits of being in a group for safety versus the costs of food competition (25). Only agent-based models are capable of capturing this complex behavior. By modeling what is known about individual host behavior and pathogen characteristics, systems-level patterns can be revealed by performing simulations. Agent-based models lend themselves to scenario development in which different patterns of host behavior can be modeled and the effects on the model outcome examined. These models, however, can be extremely data intensive, which impedes the modeling of systems with limited information (25,26).

After selecting the framework among the different classes of models, model development usually progresses in a similar fashion. A common first step in model development is identifying the key characteristics of disease systems that are necessary to estimate the potential effects on the host population and identifying key points where management options will be most effective.

Identifying Key Parameters

In general, 5 characteristics of a disease system are needed for predictive modeling: pathogenicity, environmental niche, taxonomic breadth of the hosts, transmission pathways between host and pathogen, and social behavior and movement patterns of the host species (Figure; Table; online Technical Appendix Table 2). Knowledge of each of these characteristics can be used in each of the 4 model frameworks, but the specific parameters used depends on the model chosen.

Pathogens can affect host species in a variety of ways, and management decisions should take into account the estimated long-term impacts on the population. Knowledge of the pathogenicity of the disease agent is essential for estimating long-term and population-scale effects. For example, diseases such as plague (27) might result in rapid die off of hosts, which might reduce the risk for pathogen spread beyond the local infected population. Some pathogens cause long-term sublethal effects, such as reduced fecundity or growth, and greater vulnerability to predators and other stressors (28), or they result in infected hosts that are long-lived and capable of infecting numerous other potential hosts (e.g., chronic wasting disease) (29).

The environmental niche of the disease agent or vector is also needed for developing models to predict the potential geographic extent of the disease (30). This information can help inform whether a disease might affect a species throughout its geographic range or whether environmental refuges might be expected (31). In addition, the taxonomic breadth of the hosts can indicate the potential for the pathogen to spread across multiple taxa, including humans. Multihost pathogens able to infect hosts across multiple taxonomic groups are more likely to cause emerging infectious diseases in humans or livestock (32).

Transmission pathways determine the rate at which the pathogen spreads and ultimately the spatial distribution of the disease (33). Knowledge of the transmission pathways is key to assessing the potential for the pathogen to have long-term and widespread effects, as well as evaluating the effectiveness of potential management actions. Mosquitoborne diseases, for example, have spread patterns very different from those for parasitic infections (e.g., toxoplasmosis, brain worm), which rely on specific hosts to complete their lifecycles; these differences lead to different predictions of spread (34,35).

Finally, the social behavior (which might be explicitly characterized by a contact network) of the host population can affect transmission rates by influencing the frequency and number of contacts (36–38). Panmictic populations (i.e., species that have interconnected populations mixing uniformly across their distribution) will be more likely to facilitate the rapid spread of disease compared with hosts that reside in small groups with low interpopulation

connectivity. Similarly, hosts that commonly move long distances (such as bats or migratory birds) are more likely to facilitate rapid pathogen spread at large spatial scales. For example, the spread of white-nose syndrome among bats (https://www.whitenosesyndrome.org/resources/map) occurred over a relatively short period of time. Host species with large continuous spatial distributions (such as deer) also have an increased potential for spreading disease among populations on a continental scale, even when they might not individually travel long distances; however, their rate of geographic spread is generally slower (http://www.nwhc.usgs.gov/disease_information/chronic_wasting_disease/). Network theory has provided recent advances in the estimation and depiction of contact networks for disease transmission (36).

Parameterization of the Model

When little information is available regarding the true parameter estimates and variance, several options can be used for parameterization, including empirical observation (39), borrowing information from similar diseases (40), and expert elicitation (41). Typically, model parameterization will likely include a combination of sources and scientific experts depending on the emerging disease of interest and model frame selected.

Empirical observations of initial patterns and dynamics of pathogen spread can be used to estimate parameters, which can be updated as the pathogen is monitored through the initial introduction (42). Alternatively, observations from other areas where the pathogen previously emerged can be used to make initial predictions about introduction, spread, and establishment (40). Direct evidence of a disease agent's potential for infection, transmission, and illness severity or death can be determined by laboratory trials and can identify which species might be most vulnerable to immediate population declines (43). Uncertainty primarily involves whether initial observations are characteristic of later infections on the basis of variations in disease
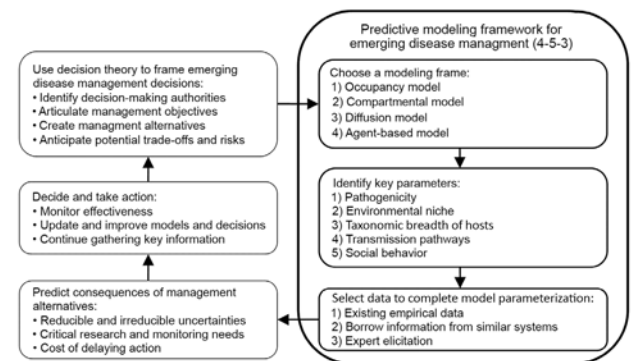
Figure. Predictive 4-5-3 modeling framework for emergency disease management.

Table. Key information needs for management of emerging diseases of wildlife

Pathogen characteristics	Description
Pathogenicity	What is the severity, lethality, and rapidity (rate of mortality) of effects on hosts?
Environmental niche	What environmental conditions (temperature, humidity) restrict persistence?
Taxonomic breadth of host	Is there evidence that the agent type can affect hosts across multiple taxa? Which taxa?
Host characteristics	
Contact networks	Spatial structure: What is the spatial structure of host populations: panmictic, metapopulations, or isolated? Does this vary across the landscape?
	Movement patterns: What is the average and maximum distance an infected host might travel?
	Social behavior: What is the social behavior of individual members in the host populations? What is the rate of contact between species?
Transmission pathways	How is the pathogen transmitted between hosts? How many different transmission pathways are there?

processes and environmental conditions and whether ecologic niches are consistent among areas where the disease has and has not emerged.

A hallmark of emerging pathogens is that little empirical data exists, especially in the initial stages of emergence (44). The time required to obtain empirical data on a disease agent might be costly in terms of windows for effective action and should be explicitly evaluated in initial research efforts. However, borrowing information from more thoroughly described pathogens that cause similar diseases and expert elicitation might include additional uncertainty that can only be resolved through observation of the disease of interest. Despite these uncertainties, delaying management actions while information is collected might reduce effectiveness of the management strategy, limit available actions, and result in unacceptable population declines. Instead of waiting for results from empirical studies, information from other related diseases can be used for parameterization of a novel disease model. This borrowing-of-information method used to estimate parameters can include both the uncertainty in the estimates from the original disease (i.e., variance), and the uncertainty in the relatedness between the novel and the original pathogen (which can be deduced by phylogenetic distances, origin, or environmental niche differences, if these are known or can be estimated).

In combination with empirical observation and borrowed information, modelers can use expert elicitation methods to formally query experts for parameter estimates (online Technical Appendix) (45). A variety of methods exist to reduce biases associated with acquiring subjective information from experts, but all of these methods involve identifying explicitly the parameters for which expert opinion is needed; preparing experts to normalize beliefs and experience (e.g., providing experts with common literature and explaining to them the uncertain parameters); summarizing and discussing the rationale; and quantifying individual and group uncertainty. A strength of expert elicitation during early stages of disease emergence is that it permits rapid evaluation of management alternatives (e.g., control, eradication) under system and parameter uncertainty.

Uncertainty

After initial parameterization of a given model, an analysis of the sensitivity and uncertainty associated with the model should be conducted. In general, sensitivity analyses examine the contribution of each predictor variable to the uncertainty in the response variable, while uncertainty analyses describe the examination of the range of outcomes possible given the uncertainty in the input variables (46). Multiple methods are available for assessing the extent of the uncertainty associated with various parameters, including variance-based methods, global uncertainty and sensitivity analyses, and Bayesian belief networks, which can help identify the uncertainties that are most likely to affect the management decision (47). These uncertainties can then become the focus of future research and monitoring efforts (48,49). Decision models that can evaluate trade-offs among multiple objectives (such as multicriteria decision analysis and portfolio decision analysis) (49) under uncertainty and evaluate different optimal policies over time (stochastic dynamic programming and Markov decision process models) can be integrated with probabilistic disease predictive models to provide insights about optimal disease management strategies under deep uncertainty.

Conclusions

Identifying robust management strategies in the early stages of disease emergence, when more control options are available, is limited by numerous uncertainties. Predictive models can be useful in evaluating control options, forecasting spread, and calculating risk (the potential for an outcome to occur and the uncertainty surrounding the outcome), but parameterization of such models for emerging wildlife diseases is challenging. By outlining 4 common models, 5 key parameters, and 3 methods for obtaining data, we outline a process for developing useful predictive models within a decision analysis framework (Figure). Ultimately, the development of models that capture key aspects of pathogen transmission and the severity of its effects can be used to evaluate the utility of different management decisions, to determine where to focus limited resources, and to identify and justify immediate research needs (50). As

a burgeoning human population continues to encroach on wildlife habitats, encounters between humans and wildlife will likely become more common. Identifying diseases that have the potential to profoundly impact human, livestock, and ecosystem health, and responding in a rapid and logical manner is a priority. Control and mitigation of emerging diseases will benefit from the early development and application of predictive modeling frameworks.

Dr. Russell is a research statistician at the U.S. Geological Survey National Wildlife Health Center. Her research interests are in the application and development of quantitative methods to improve the management of wildlife populations.

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Release date: December 14, 2016; Expiration date: December 14, 2017

Learning Objectives

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

1. Identify rates of invasive candidiasis (IC)–associated hospitalizations in the United States from 2002–2012, based on an analysis of inpatient hospitalization records from the Healthcare Cost and Utilization Project
2. Determine risk factors for IC-associated hospitalizations in the United States from 2002–2012
3. Assess healthcare utilization and costs of IC-associated hospitalizations in the United States from 2002–2012.

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Invasive candidiasis is a major nosocomial fungal disease in the United States associated with high rates of illness and death. We analyzed inpatient hospitalization records from the Healthcare Cost and Utilization Project to estimate incidence of invasive candidiasis–associated hospitalizations in the United States. We extracted data for 33 states for 2002–2012 by using codes from the International Classification of Diseases, 9th Revision, Clinical Modification, for invasive candidiasis; we excluded neonatal cases. The overall age-adjusted average annual rate was 5.3 hospitalizations/100,000 population. Highest risk was for adults ≥65 years of age, particularly men. Median length of hospitalization was 21 days; 22% of patients died during hospitalization. Median unadjusted associated cost for inpatient care was $46,684. Age-adjusted annual rates decreased during 2005–2012 for men (annual change −3.9%) and women (annual change −4.5%) and across nearly all age groups. We report a high mortality rate and decreasing incidence of hospitalizations for this disease.

Opportunistic fungi are a major cause of invasive nosocomial infections, particularly among patients with long-term stays in intensive care units; central venous catheters; recent surgery; and immunosuppression, such as those with hematopoietic stem cell transplantation and hematologic malignancies (1–4). *Candida* species are associated with invasive fungal infections among at-risk groups and have been ranked seventh as a cause of nosocomial bloodstream infection in the United States and elsewhere (4–6). These fungi are common gastrointestinal flora that cause a wide range of severe manifestations when disseminated into the bloodstream. Although candidemia has been described as the most common manifestation of invasive candidiasis, deep-seated infections of organs or other sites, such as the liver, spleen, heart valves, or eye, might occur after a bloodstream infection and persist after clearance of fungi from the bloodstream (1,7).

Candidemia is associated with high rates of illness and death and has an attributable mortality rate >30%–40% in the United States (8). However, unadjusted mortality rates vary widely in the literature, ranging from 29% to 76% (3,8–13). Increased hospital costs and prolonged length of stay associated with invasive candidiasis contribute to a major financial burden, which is believed to exceed 2 billion dollars in the United States per year (14).

A population-based study of candidemia in the United States with active laboratory surveillance data for 2 cities (Atlanta, Georgia, and Baltimore, Maryland) reported incidences in these areas and a major decrease during 2008–2013 (13,15). However, current nationally representative data with state-specific estimates for the United States are lacking. To provide a more complete and current picture of the epidemiology of invasive candidiasis, including state-specific prevalence of hospitalizations, geographic patterns, and cost, we analyzed nationally representative hospital discharge data for this disease.

Methods

Data Source and Study Population

We extracted data from the State Inpatient Databases maintained by the US Agency for Healthcare Research and Quality (AHRQ) through the Healthcare Cost and Utilization Project (16). This project was conducted through an active collaboration between the National Institutes of Health (Bethesda, MD, USA) and the AHRQ Healthcare Cost and Utilization Project. As of 2014, the SID included 48 participating states and encompassed 97% of all US community hospital discharges.

Inpatient hospital discharge records were extracted by using codes from the International Classification of Diseases, 9th revision, Clinical Modification (ICD-9-CM), for invasive candidiasis, specifically those records with disseminated candidiasis (code 112.5), candidal endocarditis (code 112.81), and candidal meningitis (code 112.83) listed anywhere in primary or secondary diagnostic fields. All secondary diagnostic fields are those other than primary fields and have 1–29 additional diagnostic codes. We excluded records with ICD-9-CM codes for localized *Candida* species infections. In addition, to avoid misclassification of noninvasive neonatal candidiasis as invasive candidiasis, we excluded records with codes for neonatal candidiasis (code 771.7), and records for infants <1 month (28 days) of age.

Our analysis covered 33 states that had complete demographic data and continuous participation during 2002–2012; these states contain ≈81% of the US population. Variables collected for each discharge record included year of admission, state of hospitalization, age at admission, sex, length of hospitalization, ICD-9 code (primary discharge diagnosis and up to 29 secondary codes), in-hospital deaths, and hospitalization cost.

Data Analysis

We used US Census Bureau age-, sex-, and race-specific state population data as denominators for all hospitalization rate calculations. For national and state estimates, age-adjusted hospitalization rates were calculated by using the US Census 2010 population as the reference population. Primary and secondary discharge codes among all records with an invasive candidiasis–associated hospitalization were analyzed to identify relevant concurrent conditions or procedures. Of the abstracted hospitalization records, 93% had 9 diagnostic codes. AHRQ Clinical Classification Software was used to collapse ICD-9-CM codes into a smaller number of clinically meaningful categories for analyzing concurrent conditions (17).

To estimate economic burden, we used total hospital costs for 15 states with publically available cost-to-charge data during 2002–2012, which represent 36% of the US population. Total cost of hospital stay was converted from hospitalization charge by using AHRQ cost-to-charge ratio files specific for hospital groups (*18*). Total hospital costs approximate the cost of providing the inpatient service, excluding physician services, and have been shown to better represent economic effect than inpatient charges (*19*). Medical Care Consumer Price Index data from the Bureau of Labor Statistics were used to adjust nominal estimated costs to reflect constant 2015 US dollars (*20*). All costs are presented in US dollars.

We analyzed a subset of 23 states with continuous race reporting during 2002–2012, which were representative of 61% of the US population, to describe hospitalizations by race. For trend analysis, we estimated the average annual percent change (APC) from Poisson regression models and used prevalence as the dependent variable and time (year) as the independent variable. Separate models were also fit for each age stratum. A p value <0.05 was considered statistically significant. SEs were scaled by using the Pearson χ^2 statistic to account for overdispersion. All analysis was conducted by using SAS version 9.3 (SAS Institute, Cary, NC, USA).

Results

During 2002–2012, we identified 138,433 invasive candidiasis–associated hospital discharges (average annual age-adjusted hospitalization rate 5.3 hospitalizations/100,000 population). Overall, 97% (134,225/138,433) of invasive

candidiasis–associated hospitalizations were coded as disseminated candidiasis, 3% (4,253) as candidal endocarditis, and 1% (1,321) as candidal meningitis. Over the 11-year period, 1% (1,366 discharges) of hospitalization records were coded for disseminated candidiasis and candidal endocarditis or candidal meningitis; 16% (22,151 discharges) had an invasive candidiasis code as the primary diagnosis. State-specific, age-adjusted, average annual hospitalizations per 100,000 population ranged from a low of 2.0 in Vermont to 7.1 in Maryland (Figure 1). Temporal trends were similar across states, and no clear regional patterns among states were observed.

During 2002–2012, the annual age-adjusted hospitalization rate ranged from 4.3 to 5.8 hospitalizations/100,000 persons. To better describe the annual rates, we fitted a Poisson model for the period beginning in 2005 when rates appeared to be stable or decreasing. During 2005–2012, hospitalization rates decreased and showed an average APC of 4.5% for women and 3.9% for men. With the exception of persons 18–34 years of age, invasive candidiasis decreased in all other age groups during 2005–2012. The most marked decrease occurred for patients ≥1 month to <1 year of age; this group had an average annual decrease of 16.9% during 2005–2012 (Figure 2).

Overall, 67,432 (49%) of hospital discharges were for men, and 99,738 (72%) were for persons >50 years of age. The highest average annual invasive candidiasis–associated hospitalization rate was for persons ≥65 years of age (20/100,000 population), and within this group, men were at highest risk (Figure 3). For persons >34 years of age, rates appeared to double within successive age groups up to
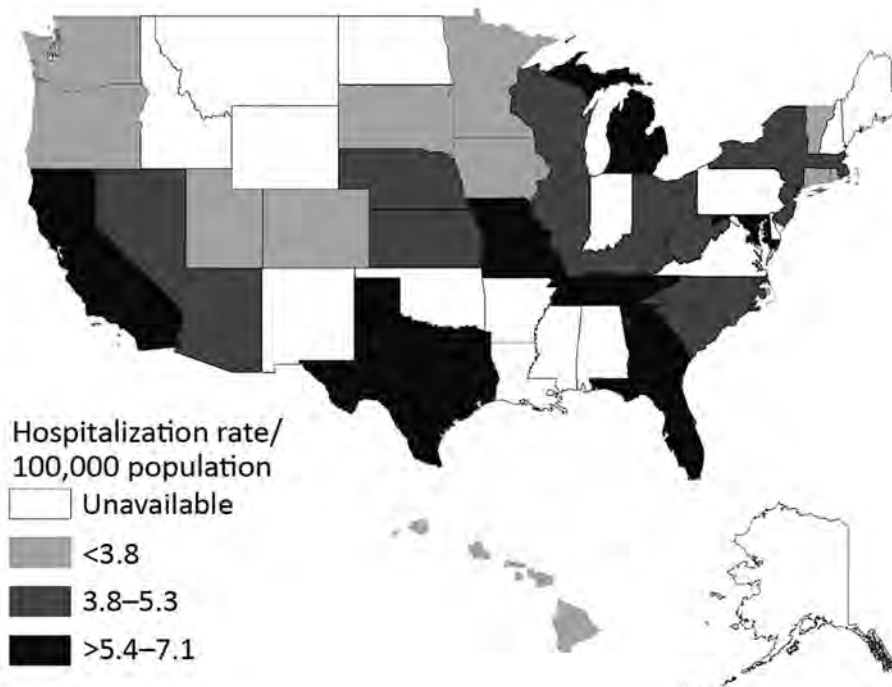
Figure 1. Average annual invasive candidiasis-associated hospitalizations, United States, 2002–2012. Data were provided by State Inpatient Databases through the Healthcare Cost and Utilization Project maintained by the US Agency for Healthcare Research and Quality. Diagnoses were classified by using Agency for Healthcare Research and Quality clinical classification software (*17*) and multiple codes and ranges from the International Classification of Diseases, 9th Revision, Clinical Modification.

Hospitalization rate/
100,000 population
☐ Unavailable
<3.8
3.8–5.3
>5.4–7.1
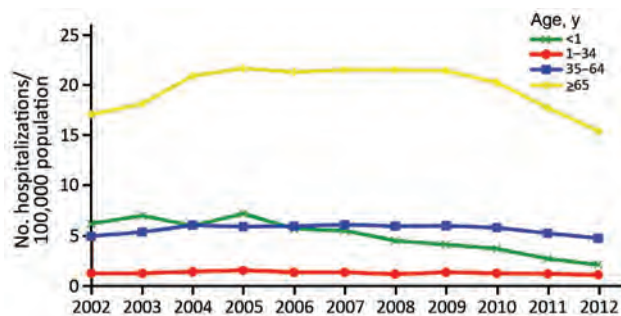
Figure 2. Annual rate of invasive candidiasis–associated hospitalizations by age, United States, 2002–2012. Neonates (<1 mo of age) were excluded from <1 population. Data were provided by State Inpatient Databases through the Healthcare Cost and Utilization Project maintained by the US Agency for Healthcare Research and Quality. Diagnoses were classified by using Agency for Healthcare Research and Quality clinical classification software (*17*) and multiple codes and ranges from the International Classification of Diseases, 9th Revision, Clinical Modification.

those 80 years of age. The rate for persons 50–64 years of age was 2.2-fold greater than that for persons 35–49 years of age, and overall rates for those 65–79 years of age were 2.2-fold greater than that for persons 50–64 years of age.

To clarify racial disparities for rates, we analyzed hospitalization rates by racial/ethnic groups in age groups where incidence was highest. For persons >50 years of age, the rate for black men was 25/100,000 population, which was 2.2 times higher than that for white men. For black women, the rate was similar (23/100,000 population), which was 2.1 times higher than that for white women. Rates for Asian and Hispanic racial/ethnic groups were similar to those for whites (Figure 4). We did not find any differences in patterns of concurrent conditions by racial/ethnic group.

The most frequent underlying conditions, as a primary or secondary diagnosis, were gastrointestinal disorders or conditions (46%), hypertension (39%), diabetes mellitus (26%), and kidney disease (25%) (Table). Overall, 72% (99,360) of invasive candidiasis discharges had an ICD-9-CM code for septicemia. A total of 45% (62,092) were associated with complications of a device, implant, or graft, and 28% (38,940) were associated with complications of surgical procedures or medical care.

The overall median length of hospital stay was 21 days. However, the median length of stay decreased from 22 days in 2002 to 17 days in 2012, and APC decreased 1.9%. The overall in-hospital mortality rate for invasive candidiasis was 22%, although a major decrease for the in-hospital mortality rate was observed during this period (average decrease of 3.7%/year). The in-hospital mortality rate was 2-fold higher for patients ≥50 years of age than for those <50 years of age (25.8 vs. 13.4 deaths/100

hospitalizations for invasive candidiasis). The in-hospital mortality rate was 22% for blacks and whites.

The median cost for inpatient care in 15 states was $46,684 (range $48–$1,802,688). The median cost varied little by sex (men $48,796, range $56–$1,579,163; women $45,032, range $48–$1,802,688), but varied greatly by survival status (survived $41,096, range $48–$1,480,386; deceased $72,182, range $48–$1,802,688). The highest median costs were estimated for nonneonatal infants ($58,850) and persons 50–64 years of age ($51,447).

Discussion

We found that state-specific rates for invasive candidiasis varied little across the United States and that hospitalizations for this disease have continued to decrease. Our overall age-adjusted hospitalization rate of 5.3/100,000 population was somewhat lower than those found previously through active population-based laboratory surveillance of candidemia during an overlapping period (2008–2011), which estimated an average annual crude incidence per 100,000 person-years of 13.3 in Atlanta and 26.2 in Baltimore (*13*). We found rates of 5.9 in Georgia and 7.1 in Maryland. The lower rates in our study are expected given that active surveillance limited to an urban area would probably detect more cases.

Our study might have underestimated true rates for invasive candidiasis, given the limitations of administrative data, including undercoding of candidemia because of low sensitivity of blood cultures, poor provider documentation of invasive candidiasis, or discharge before receipt of laboratory results. The sensitivity of blood culture is estimated to be 50% (*21*), and culture is more likely to miss deep-seated candidiasis in the absence of candidemia. Although
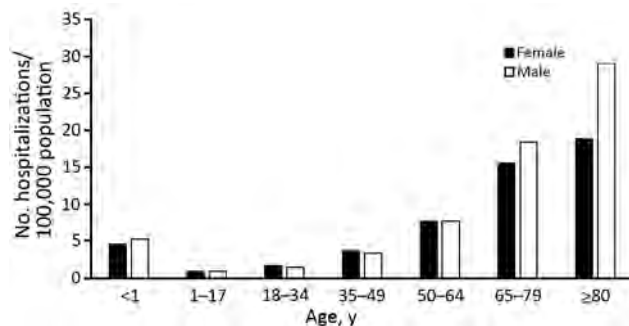
Figure 3. Average annual rate of invasive candidiasis–associated hospitalizations by age and sex, United States, 2002–2012. Neonates (<1 mo of age) were excluded from <1 population. Data were provided by State Inpatient Databases through the Healthcare Cost and Utilization Project maintained by the US Agency for Healthcare Research and Quality. Diagnoses were classified by using Agency for Healthcare Research and Quality clinical classification software (*17*) and multiple codes and ranges from the International Classification of Diseases, 9th Revision, Clinical Modification.
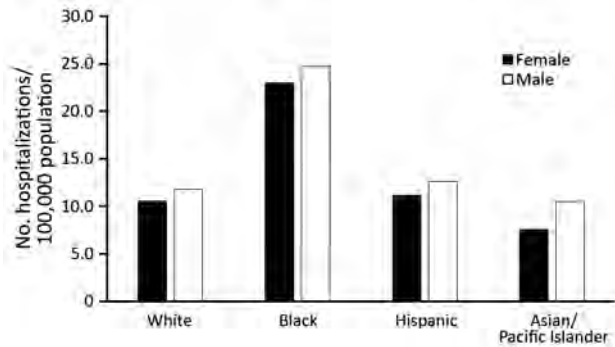
Figure 4. Average annual rate of invasive candidiasis–associated hospitalizations among older age groups (>50 years) by sex and race, United States, 2002–2012. Neonates (<1 mo of age) were excluded from <1 population. Data were provided by State Inpatient Databases through the Healthcare Cost and Utilization Project maintained by the US Agency for Healthcare Research and Quality. Diagnoses were classified by using Agency for Healthcare Research and Quality clinical classification software (17) and multiple codes and ranges from the International Classification of Diseases, 9th Revision, Clinical Modification.

specific to a pediatric population, a cross-sectional analysis found that ICD-9-CM codes for candidemia had a sensitivity of 60% and a specificity >99% specific (22). Invasive infections might persist in organs after infections are cleared from the bloodstream, and ≈8% of candidemia cases show reoccurrence (13). Cultures might take 5–8 days for results to be obtained, such that patients might be discharged or die before receiving results. Thus, patients would not be coded as having candidemia, which would lead to underestimation of illness and death (23).

Adults ≥65 years of age having the highest risk for invasive candidiasis–associated hospitalization and the progressively increasing rate by age of hospitalizations among

adults, with a peak among persons >80 years of age, are also consistent with a previous report of population-based surveillance for candidemia (15). Similarly, the 2-fold higher incidence among black persons has been reported in population-based studies of candidemia in Atlanta and Baltimore. The reasons for this racial disparity are not fully understood. A recent study conducted in 4 US cities found that adjusting for poverty attenuated the association of black race with candidemia; however, a persistent 2-fold racial disparity remained even after this adjustment (24).

The decrease in hospitalizations for invasive candidiasis and deaths from this disease across nearly all age groups is consistent with results from other studies that used similar time frames. Cleveland et al. also reported a major decrease in these parameters in Atlanta and Baltimore during 2008–2013 (15). A major cause of bloodstream infections is central line–associated bloodstream infections. Cleveland et al. found that 85% of candidemia patients had used a central venous catheter ≤2 days before the bloodstream infection culture date (15). Estimates from the Centers for Disease Control and Prevention (Atlanta, GA, USA) identified a marked decrease in central line–associated bloodstream infections during 2001 and 2008–2009. These decreases were attributed to increased state and regional prevention efforts supported by several federal agencies after establishment of a national goal in 2009 to reduce central line–associated bloodstream infections by 50% by 2013 (25).

Few studies have reported on length of stay and associated trends among invasive candidiasis–related hospitalizations. A recent US study reported a mean length of hospital stay of 22 days for persons with candidemia by using the Surveillance and Control of Pathogens of Epidemiologic Importance database (3). We report a median length of stay of 21 days and a major decreasing trend

Table. Primary or secondary diagnosis for invasive candidiasis hospitalizations, United States, 2002–2012*

Diagnosis	No. (%) hospital discharges, n = 138,433
Indicator of invasive candidiasis	
Septicemia or sepsis	99,360 (72)
Complication of surgical procedures or medical care	62,092 (45)
Complication of device, implant, or graft	38,940 (28)
Underlying condition	
Gastrointestinal disorders or conditions	63,470 (46)
Hypertension	54,094 (39)
Diabetes mellitus	35,689 (26)
Kidney disease	34,626 (25)
Cancer	33,359 (24)
Congestive heart failure	30,348 (22)
Nervous system disorders	26,220 (19)
Coronary atherosclerosis and other heart disease	20,085 (15)
Chronic obstructive pulmonary disease and bronchitis	23,850 (17)
Liver diseases	19,888 (14)
Esophageal disorders	14,625 (11)

*Neonates (<1 mo of age) were excluded. Data were provided by State Inpatient Databases through the Healthcare Cost and Utilization Project maintained by the US Agency for Healthcare Research and Quality. Diagnoses were classified by using Agency for Healthcare Research and Quality clinical classification software codes (17) and multiple International Classification of Diseases, 9th Revision, Clinical Modification codes and ranges.

during 2002–2012. A study in 2005 used the AHRQ 2000 Nationwide Inpatient Survey for 28 states to analyze attributable outcomes among adult patients (>18 years of age) with hospital-associated candidemia (*26*). This study reported a mean estimated length of stay of 18.6 days and associated charges of $66,154 in 2000 US dollars. Our reported median hospitalization costs of $46,684 for invasive candidiasis–associated hospitalizations are lower. However, charges probably overestimate actual costs. In addition, median costs limit data extremes from skewing results and provide greater accuracy. Finally, our costs reflect 11 years of hospital discharges, include nonneonatal hospitalizations of patients <18 years of age, and reflect multiple invasive candidiasis codes.

Candida species remain the leading fungal cause of healthcare associated infections and the seventh most common overall pathogen, representing 6% of all healthcare-associated infections; in 2011 an estimated 648,000 patients had ≥1 healthcare-associated infection, representing 4% of all inpatients in the United States (*4*). Although national invasive candidiasis–associated hospitalization rates have been decreasing for men and women since 2005, the incidence of invasive candidiasis–associated hospitalizations remains high and is associated with substantial mortality rates and health costs. Continued research is needed to identify interventions associated with these decreasing trends to further accelerate this observed decrease, including improved prevention and treatment, such as optimum antifungal treatments and timing of medical procedures.

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Epidemiology of Human Anthrax in China, 1955–2014

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Using national surveillance data for 120,111 human anthrax cases recorded during 1955-2014, we analyzed the temporal, seasonal, geographic, and demographic distribution of this disease in China. After 1978, incidence decreased until 2013, when it reached a low of 0.014 cases/100,000 population. The case-fatality rate, cumulatively 3.6% during the study period, has also decreased since 1990. Cases occurred throughout the year, peaking in August. Geographic distribution decreased overall from west to east, but the cumulative number of affected counties increased during 2005-2014. The disease has shifted from industrial to agricultural workers; 86.7% of cases occurred in farmers and herdsmen. Most (97.7%) reported cases were the cutaneous form. Although progress has been made in reducing incidence, this study highlights areas that need improvement. Adequate laboratory diagnosis is lacking; only 7.6% of cases received laboratory confirmation. Geographic expansion of the disease indicates that livestock control programs will be essential in eradicating anthrax.

Anthrax is an acute infectious zoonotic disease caused by the gram-positive, aerobic, nonmotile bacterium *Bacillus anthracis*, which can survive in soil for decades as an extremely resistant form (spores) (*1*). Herbivores become infected when grazing on contaminated land, when bitten by Tabanid flies with contaminated mouthparts, or by ingesting contaminated feed (*2*). Naturally occurring human anthrax infections are caused by contact with infected animals or animal products; ingestion of undercooked infected meat; or exposure to large-scale processing of contaminated hides, wool, and hair in enclosed factory areas (*3*). Injection anthrax has been observed in users of contaminated heroin in western Europe (*4*).

Although there has been a general decrease in the number of anthrax outbreaks in animal populations and in human cases, anthrax still has a nearly worldwide distribution, causes an estimated 20,000–100,000 cases annually, and poses a major public health threat in regions of the Middle East, Africa, central Asia, South America, and Haiti (*5*). In addition, *B. anthracis* is always placed high on the list of potential agents with respect to biologic warfare and bioterrorism because of its robust nature and persistence of spores, the ability of aerosolized spores to readily infect by inhalation, and the high mortality rate for resultant anthrax cases (*6*). *B. anthracis* was used in this context in the anthrax letter events in the United States during 2001 and showed severe consequences (*7*).

Anthrax has probably been present in China for >5,000 years as recorded in ancient Chinese medical books, but few reliable data were available before the People's Republic of China was founded (*8*). Human anthrax was made a reportable disease in China during the 1950s. Over the past 60 years, great progress has been made in control and prevention, including development of human anthrax vaccine in China during the late 1950s and eradication of anthrax in industrial areas during the 1980s (*8*).

To our knowledge, no published literature systematically describes the epidemiology of human anthrax in China. This study was conducted to observe temporal trends, seasonality, and geographic distribution of human anthrax in China during the past 60 years and demographic characteristics during 2005–2014 to identify the current epidemiologic situation and provide information for control and prevention of this disease.

Materials and Methods

National Surveillance Program

The national surveillance program of human anthrax is part of the Chinese Notifiable Disease Reporting System, which was started in the 1950s. During the 1950s–2003, aggregated data for each province (a province in China is similar to a state in the United States) was reported monthly by mail to the Chinese Center for Disease Control and Prevention (China CDC). In 2004, a real-time online nationwide

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reporting system was implemented. Since then, all human anthrax cases were required to be reported online ≤2 hours of diagnosis for inhalational anthrax and ≤24 hours of diagnosis for cutaneous and gastrointestinal anthrax.

Case Definition

All human anthrax cases, including probable and confirmed cases, were diagnosed according to the unified case definitions issued by the Chinese Ministry of Health. The diagnostic criteria for human anthrax cases changed twice during the study period, in 1998 and again in 2008 (online Technical Appendix Table, https://wwwnc.cdc.gov/EID/article/23/1/15-0947-Techapp1.pdf).

Before September 1998, a probable case was defined as a case with clinical manifestations and an appropriate epidemiologic history. A confirmed case was defined as a probable case plus laboratory evidence of *B. anthracis* infection detected by bacterial isolation or demonstration of *B. anthracis* in a clinical specimen by microscopic examination of stained smears.

Since September 1998, a probable case has been defined as a case with clinical manifestations and demonstration of *B. anthracis* in a clinical specimen by microscopic examination of stained smears. A confirmed case is defined as a case with clinical manifestations plus isolation of *B. anthracis* or a ≥4-fold increase in specific antibody titer against *B. anthracis.*

Epidemiologic history included living in areas with reports of confirmed anthrax or having traveled to such places ≤14 days before onset or engaging in occupations that are likely to result in exposure to anthrax. The disease has 3 clinical forms: cutaneous, inhalational and gastrointestinal. We have not detected injection anthrax in China.

Collection and Examination of Specimens

For every suspected anthrax case-patient, appropriate clinical specimens were required to be collected before treatment, including blood for all patients, vesicular fluid for patients with cutaneous anthrax, stools for patients with intestinal anthrax, and sputum or respiratory secretions for patients with inhalational anthrax. Every local CDC (county or prefecture) was required to examine stained smears of clinical specimens by microscopy. The presence of squared-ended, gram-positive, rod-shaped bacteria in chains was considered to be *B. anthracis,* and the corresponding specimens were required to be delivered to the provincial CDC for further bacterial isolation, which was conducted in Biosafety Level 2 laboratories.

In accordance with national surveillance protocol for anthrax (*9*), bacterial isolation was conducted on nutrient agar plates. Specimen were sprayed on plates after pre-processing, which included dilution or sedimentation and centrifugation, and heat-shocking. After incubation for

8–24 hours at 37°C, plates were checked for medium sized bacterial colonies that were hoary and opaque and had a ground-glass–like surface. Phage and penicillin were used to test for sensitivity typical for *B. anthracis*. Because reliable commercial kits for antibody testing are not available, ELISA was rarely used to test for specific antibody against *B. anthracis.* Recently, PCR was also used to provide laboratory evidence of *B. anthracis* infection. However, because there is no unified standard procedure for PCR of *B. anthracis*, PCR was also rarely used in laboratory analysis.

Demographic, Clinical, and Epidemiologic Data

Human anthrax surveillance data included data for 1955–2014 of probable and confirmed cases for all 31 provinces in China. Aggregated data for cases and associated deaths by province and month were available for 1955–2003. During 2004–2014, each human anthrax case was reported online through a standardized form that included basic demographic information (sex, date of birth, address); occupation; diagnosis classification (probable or confirmed); clinical form (cutaneous, inhalational, or gastrointestinal); outcome (survival or death); date of illness onset; and date of death (if applicable). National population data for China during 1955–2014 were obtained from the National Bureau of Statistics of China (*10*).

Data Analysis

We included in the analysis all probable and confirmed human anthrax cases with illness onset during 1955–2014. We calculated the annual incidence rate by dividing the number of human anthrax cases by the corresponding population at the end of a given year and the case-fatality rate by dividing the number of human anthrax–associated deaths by the number of human anthrax cases with illness onset by the end of the same year.

We described spread and emergence of human anthrax during 2005–2014 with different kinds of affected counties as follows. A newly affected county this year was defined as a county that reported human anthrax cases for the first time during that year since 2004, a previously affected county with a new case this year was defined as a county that reported human anthrax cases during and before that year since 2004, and a previously affected county without a new case this year was defined as a county that reported human anthrax cases before that year since 2004 but no case in that year. Any county reporting human anthrax cases since 2004 were designated as a previously affected county.

Descriptive statistics included frequency analyses for categorical variables, medians, and interquartile ranges for continuous variables. We used the χ^2 test for testing differences of proportion for categorical variables. Probabilities were 2-tailed, and p values <0.05 were considered statistically significant. We performed all analyses by using R
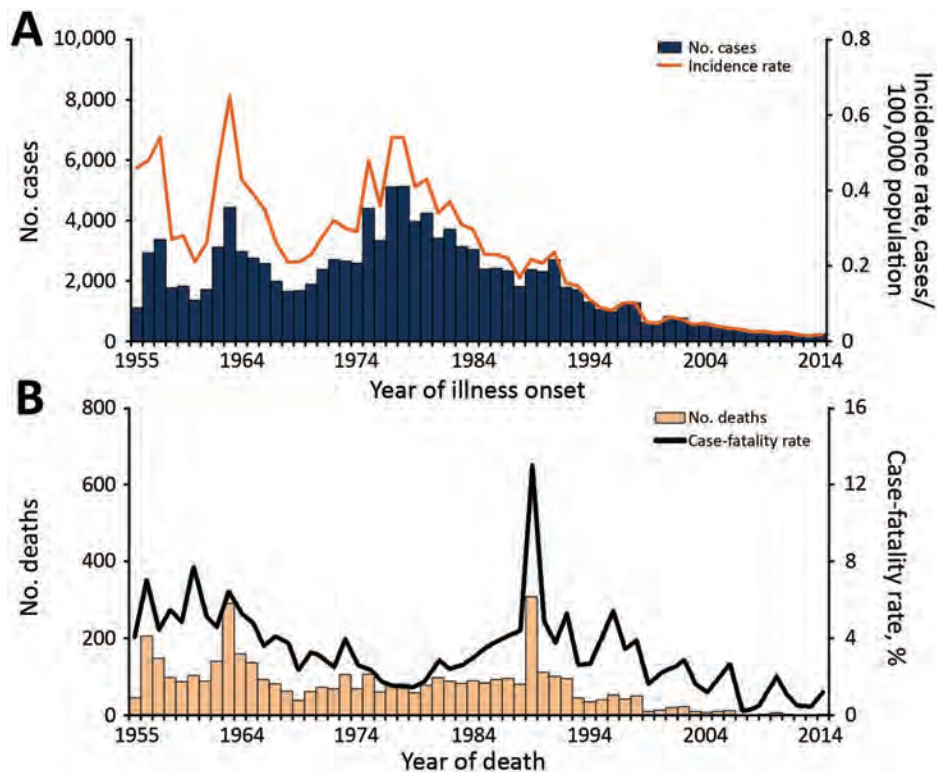
Figure 1. Cases of and deaths from probable and confirmed human anthrax, China, 1955–2014. A) No. human anthrax cases (n = 120,111) and incidence rate (no. cases/100,000 population) by year. B) No. human anthrax deaths (n = 4,341) and case-fatality rate (%) by year.

version 3.0.2 (https://www.r-project.org/) and used ArcGIS version 10.0 (ESRI, Redlands, CA, USA) to plot geographic distribution of cases.

Ethical Approval

This Chinese National Health and Family Planning Commission (Beijing, China) determined that collection of data for human anthrax cases was part of a continuing public health surveillance. Thus, this study was exempt from institutional review board assessment.

Results

Temporal Trend and Seasonality

During 1955–2014, a total of 120,111 probable and confirmed human anthrax cases, including 4,341 fatal cases, were reported to the China CDC; the overall case-fatality rate was 3.6%. Before the 1980s, probable and confirmed human anthrax incidence showed a periodic increase and decrease every 8–10 years. There were 3 major peaks in 1957 (0.54 cases/100,000 population), 1963 (0.65 cases/100,000 population), and 1977–1978 (0.54 cases/100,000 population). Thereafter, incidence decreased until 2013, when it reached a low of 193 cases (0.014 cases/100,000 population) (Figure 1).

The case-fatality rate showed an overall downward trend before the 1980s and then increased to a high of 13.0% in 1989, which was followed by a generally

fluctuating decrease until 2014, when only 3 deaths were reported (Figure 1). Human anthrax cases occurred across the whole year, typically increasing in May, peaking in August (56% of cases occurred during July–September), and decreasing toward November. This pattern was consistent during 1955–2014 (Figure 2).

Geographic Distribution

All 31 provinces had ≥1 probable or confirmed case of human anthrax during 1955–2014. The distribution of cases showed an overall decrease of cases from western to eastern China. From the end of 1970s onward, some large cities, such as Shanghai (1979), Beijing (1984), and Tianjin (1985), and some provinces in eastern China, such as
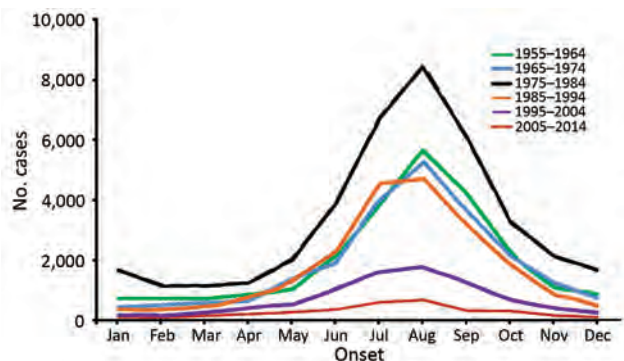
Figure 2. Monthly distribution of probable and confirmed human anthrax cases, China, 1955–2014.

Fujian (1981), Zhejiang (1990), Jiangxi (1996), and Guangdong (1998), gradually stopped reporting human anthrax. In addition, cases were rarely reported in southeastern China (Figure 3).

The cumulative number of counties affected by probable or confirmed human anthrax continued to increase from 188 (6.1% of the 3,074 counties in China) in 2005 to 358 (11.6%) in 2014. During the same period, although 52–88 previously affected counties reported new probable or confirmed human anthrax cases every year, newly affected counties were continuously reported (range 6–71 cases each year) (Figure 4). In contrast, the number of counties reporting confirmed human anthrax cases was much smaller compared with the number of counties affected by probable and confirmed cases. However, the pattern was similar and the cumulative number of affected counties with confirmed human anthrax increased from 41 in 2005 to 104 in 2014 (online Technical Appendix Figure 1).

The counties affected by probable and confirmed human anthrax were located mainly in southwestern and northeastern China. Most newly affected counties were adjacent to previously affected counties. However, there were also newly affected counties not adjacent to previously affected counties in eastern China, such as counties in Shandong, Jiangsu, and Hunan Provinces (Figure 5). The pattern of geographic distribution in affected counties was similar
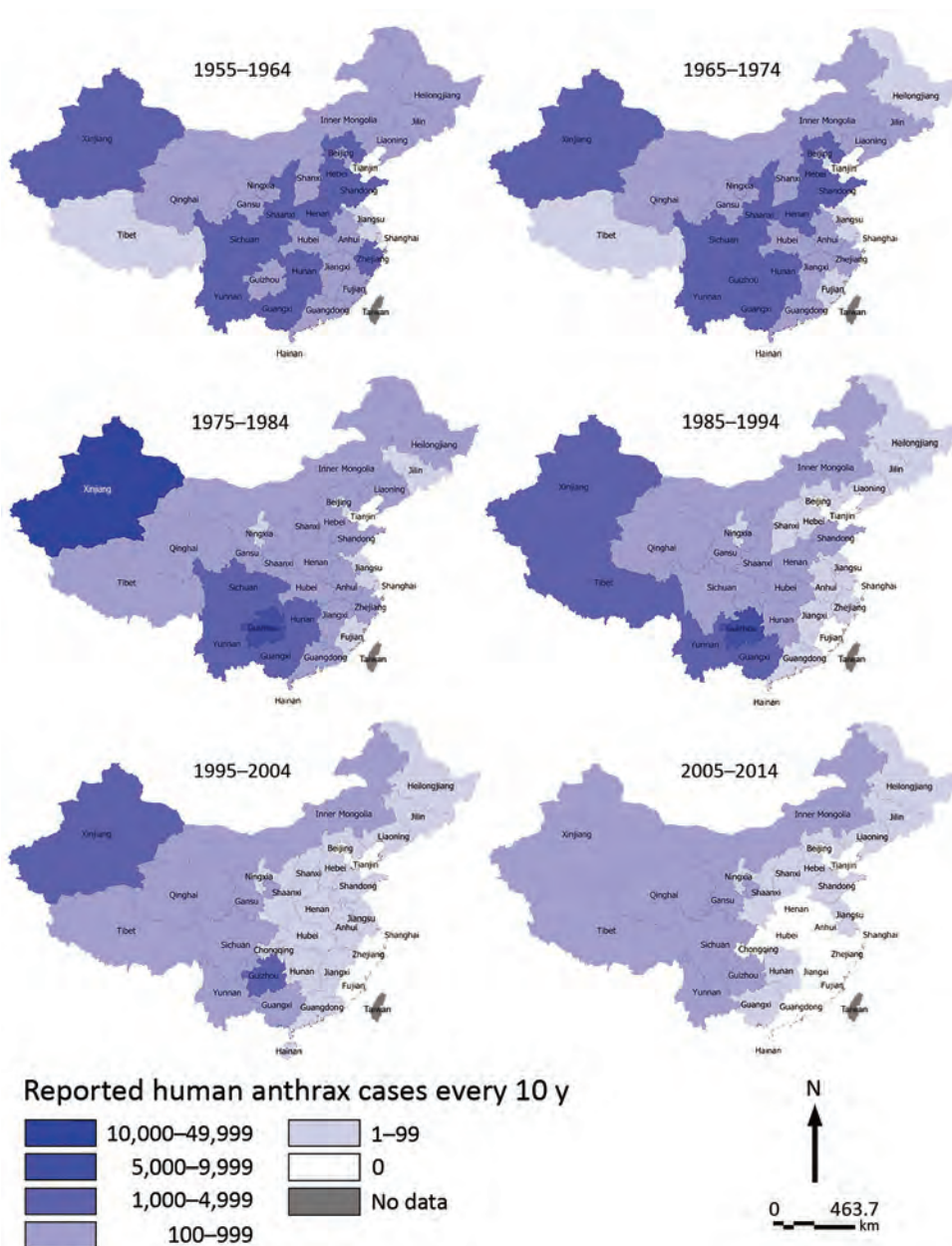
Figure 3. Provincial distribution of probable and confirmed human anthrax cases, China, 1955–2014.
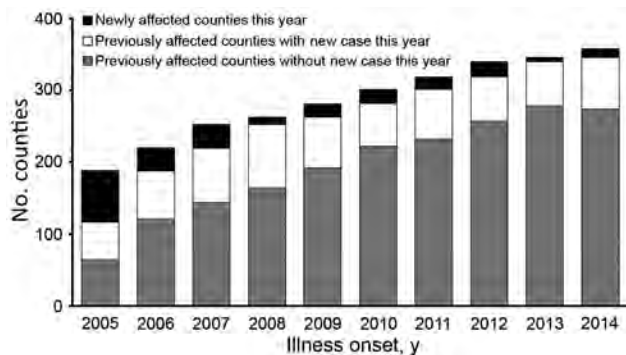
Figure 4. Trends in number of counties affected by probable and confirmed human anthrax, China, 2005–2014.

when only confirmed human anthrax cases were included in the analysis, except that there were fewer counties with confirmed cases (online Technical Appendix Figure 2).

Demographic Characteristics

During 2005–2014, a total of 86.7% of human anthrax cases were found in farmers and herdsmen. Rural cases accounted for 92.4% of all cases (Table). The overall male:female case ratio was 2.8:1, and there was no obvious changing trend during this period. The proportion of urban cases fluctuated around 7%, except for 15% in 2011. In rural areas, younger persons, usually men <34 years of age, were more commonly affected. In urban areas, persons ≥40 years of age were more commonly affected. For affected persons <1–14 and ≥65 y of age, cases were more common in female patients than in male patients (online Technical Appendix Figure 3).

Diagnostics

During 2005–2014, a total of 3,379 human anthrax cases were reported, of which 257 (7.6%) were confirmed cases (Table). The proportion of confirmed cases fluctuated over this period, ranging from 4.8% in 2009 to 11.7% in 2014, and also varied between provinces; the highest was 36.4% in Shanxi and 29.5% in Inner Mongolia, and the lowest was 0% in Jiangsu, Shandong, and Hunan (online Technical Appendix Figures 4, 5). During the same period, 97.7% of national probable and confirmed cases were cutaneous anthrax, which also accounted for most anthrax cases across all provinces (online Technical Appendix Figure 6).

A total of 41 deaths were caused by anthrax; the proportion of fatal cases was 1.9% for confirmed cases and 1.2% for probable cases. The median time from illness onset to diagnosis was 4.0 days (interquartile range [IQR] 2.4–7.0 days), from diagnosis to death, 0 days (IQR 0–1.0 days), and from illness onset to death, 5 days (IQR 3.0–7.0 days). The median time from illness onset to diagnosis was 4.0 days (IQR 2.0–6.7 days) for persons with probable cases and 5.0 days (IQR 3.0–8.3 d) for persons with confirmed cases.

Discussion

We conducted a systematic study of the epidemiology of human anthrax in China during 1955–2014. We believe that this study was useful because of recent epidemiologic changes and rapid socioeconomic changes during the past few decades. This study showed that, since 1990, the incidence rate and case-fatality rate for human anthrax has continued to decrease; only several hundred cases have been reported in the past 10 years. Most cases were in western China and peaked in August, but cases were reported infrequently in eastern China. This study also showed that cutaneous anthrax accounted for 98% of cases, and the largest proportion were in farmers and herdsmen. A low percentage of cases were laboratory confirmed.

The 3 historical peaks for human anthrax all occurred before 1980, after which a general decrease in cases occurred. Before 1980, some cases occurred in fur-processing workers infected by industrial exposure. From the 1960s onward, after implementation of strict quarantine and sanitary measures for animal fur and wool and improved industrial working conditions, the situation gradually improved to the point that after 1980 almost no human anthrax cases were seen in large cities, such as Beijing, Shanghai, and Tianjin. Since then, most anthrax cases have been caused by agricultural exposures. The fact that most anthrax cases were in male farmers or herdsmen further supports this feature of agricultural anthrax in China.

Cattle and sheep were and are the major infection source for human anthrax (2). The number of sheep slaughtered annually in China has increased from 42.419 million in 1980 to 270.995 million in 2012. The number of cattle slaughtered annually has increased from 3.322 million in 1980 to 48.281 million in 2013 (10).

Despite rapid increases in cattle and sheep populations, the case-fatality rate for human anthrax showed a continuous decrease, which suggested some success in control and prevention of this disease in livestock. Practices contributing to this success include timely reporting and detection of anthrax; rapid response to outbreaks and individual cases, which often includes restricting movement of livestock and related products from affected areas, tracing previous sources of possibly infectious livestock, and accordingly alerting related areas and departments; vaccination for possibly affected healthy livestock and related personnel, such as herdsmen, transportation staff, and slaughterhouse workers; prompt disposal of dead animals, bedding, and contaminated materials, for which a guide has been issued that specifies requirements for sites of incineration and burial of livestock carcasses, type of sanitizer and frequency of its use for livestock facilities and equipment, and general hygiene by persons who have contact with diseased or dead animals (8).
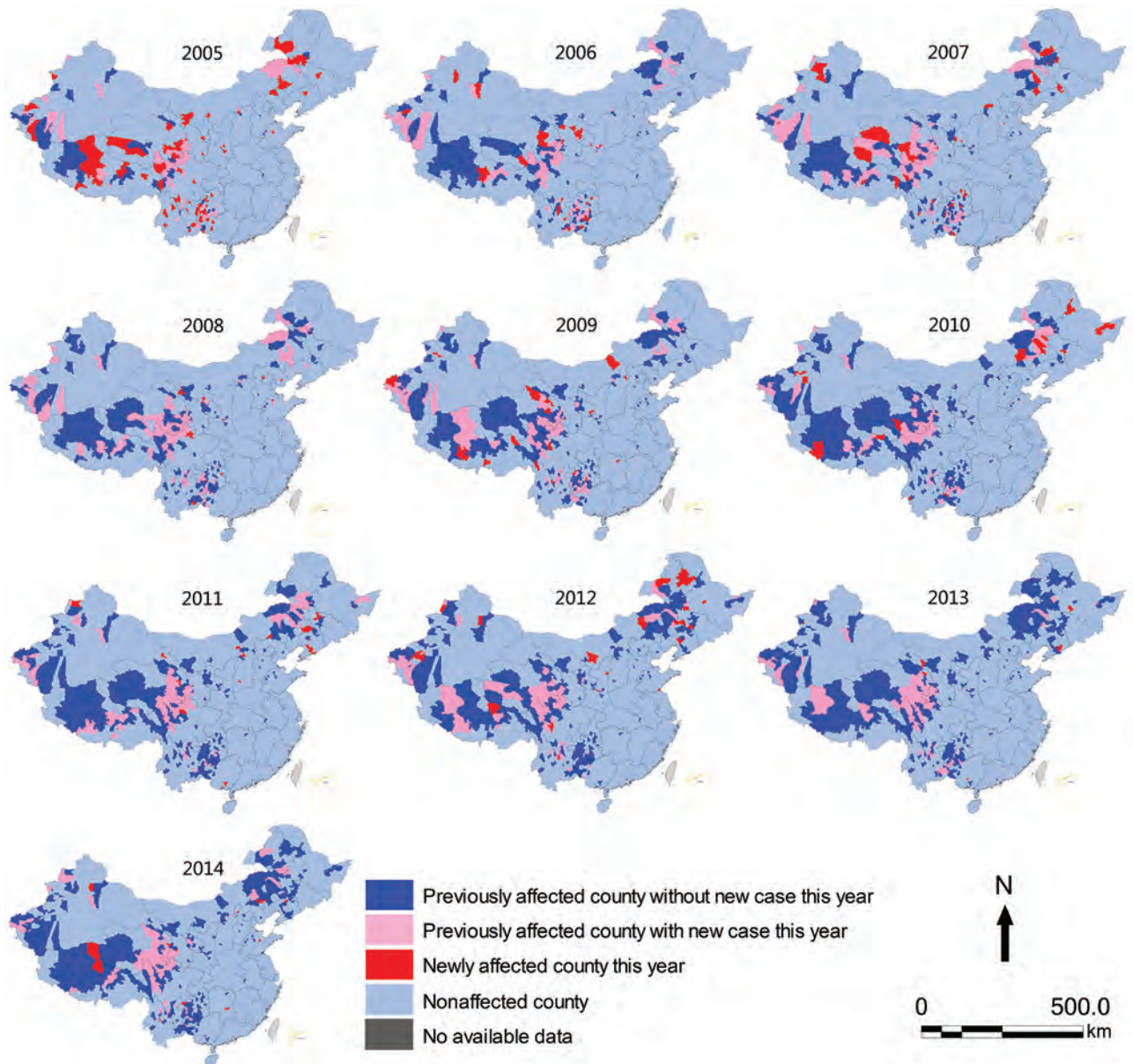
Figure 5. Geographic distribution of counties affected by probable and confirmed human anthrax, China, 2005–2014

However, the present pattern of human cases clearly shows that any further improvement can be achieved only by using a proactive approach to the disease in livestock. This approach includes annual vaccination where outbreaks persist; improved laboratory diagnosis and participation, especially by veterinary diagnostic laboratories; genomic strain identification and mapping; and investigation of anomalous outbreaks (e.g., anthrax is normally a summer disease and outbreaks at other times are more characteristic of contaminated livestock feed). The reason for the decrease in the case-fatality rate for anthrax before 1980 was that this disease was largely industry related and was relatively easy to detect. With improvements

in medical care, the incidence of anthrax showed a general downward trend, but our study showed that, since the 1980s, agricultural anthrax in rural areas has become a serious problem. Because of lack of convenient access to healthcare facilities and awareness of the need to seek early treatment for rural residence, the reported case-fatality rate increased and exceeded the rate during 1950s soon after the People's Republic of China was established. However, one cannot presume that rural anthrax was absent during the earlier period. It is more likely that this disease was not reported, although cases were probably occurring at a level similar to that during the later period of reporting. In 1989, the case-fatality rate reached a high of 13.0%, which was

Table. Demographic and diagnostic characteristics of patients with human anthrax, by diagnosis type, China, 2005–2014*

Characteristic	Probable cases, n = 3,121	Confirmed cases, n = 258	Total, n = 3,379
Sex			
M	2,273 (72.8)	210 (81.4)	2,483 (73.5)
F	848 (27.2)	48 (18.6)	896 (26.5)
Median age, y, (IQR)	38 (27–49)	40 (30–49)	38 (27–49)
Age group, y			
0–14	229 (7.3)	11 (4.3)	240 (7.1)
15–19	158 (5.1)	8 (3.1)	166 (4.9)
20–24	230 (7.4)	20 (7.8)	250 (7.4)
25–29	309 (9.9)	25 (9.7)	334 (9.9)
30–34	352 (11.3)	24 (9.3)	376 (11.1)
35–39	415 (13.3)	34 (13.2)	449 (13.3)
40–44	379 (12.1)	39 (15.1)	418 (12.4)
45–49	282 (9.0)	38 (14.7)	320 (9.5)
50–54	212 (6.8)	18 (7.0)	230 (6.8)
55–59	201 (6.4)	22 (8.5)	223 (6.6)
60–64	167 (5.4)	11 (4.3)	178 (5.3)
≥65	187 (6.0)	8 (3.1)	195 (5.8)
Occupation			
Farmer or herdsman	2,700 (86.5)	231 (89.5)	2,931 (86.7)
Infants or students†	248 (7.9)	12 (4.7)	260 (7.7)
Other‡	173 (5.5)	15 (5.8)	188 (5.6)
Rural residence§	2,889 (92.6)	234 (90.7)	3,123 (92.4)
Fatal outcome	36 (1.2)	5 (1.9)	41 (1.2)
Clinical forms			
Cutaneous	3,055 (97.9)	252 (97.7)	3,307 (97.9)
Inhalational	0	1 (0.4)	1 (0.0)
Gastrointestinal	5 (0.2)	1 (0.4)	6 (0.2)
Unknown¶	61 (2.0)	4 (1.6)	65 (1.9)
Median onset to diagnosis interval, d (IQR)	4.0 (2.0–6.7)	5.0 (3.0–8.3)	4.0 (2.4–7.0)
Year of illness onset			
2005	497 (15.9)	38 (14.7)	535 (15.8)
2006	417 (13.4)	35 (13.6)	452 (13.4)
2007	400 (12.8)	26 (10.1)	426 (12.6)
2008	309 (9.9)	29 (11.2)	338 (10.0)
2009	334 (10.7)	17 (6.6)	351 (10.4)
2010	265 (8.5)	25 (9.7)	290 (8.6)
2011	279 (8.9)	30 (11.6)	309 (9.1)
2012	220 (7.1)	17 (6.6)	237 (7.0)
2013	181 (5.8)	12 (4.7)	193 (5.7)
2014	219 (7.0)	29 (11.2)	248 (7.3)

*Values are no. (%) unless otherwise indicated. Percentages may not total 100 because of rounding. IQR, interquartile range.
†Infants include children attending and not attending kindergarten. Students include primary, secondary, and college students.
‡Includes teacher, laborers, self-employed and unemployed, workers, food industry personnel, retired persons, and cadres of staff.
§If the residence community was connected to seats of county or municipal government through public facilities, residence facilities and other facilities, or mine area, development zone, research institutes, higher education establishments, farming communities or tree farming communities with >3,000 of permanent residents, it was considered an urban residence. Otherwise, the community was considered a rural residence.
¶No information was available.

probably caused by a major outbreak of intestinal anthrax in the Changdu District of Tibet (*11*).

From 1990 onward, a strengthened surveillance program was initiated in western China, and early detection of anthrax facilitated early treatment. This program contributed to a decrease in the case-fatality rate. This rate has decreased to <1% in recent years, which is consistent with the report that the case-fatality rate for cutaneous anthrax is now <1% after treatment (*12*). However, gastrointestinal anthrax has a higher risk for death, which can occur quickly, and this finding would preempt any clinical observations or sampling. These findings might explain the unusually high number of cutaneous cases relative to each gastrointestinal case.

Anthrax has not been eradicated from previously disease-endemic areas in China, and geographic distribution of the disease tends to expand into new areas. Each year, previously affected counties still accounted for a predominant proportion of counties with reports of cases that year. This finding could be caused by strong resistance of *B. anthracis* spores to environmental conditions and persistence of spores in old foci for disease (*13*). However, some provinces, such as Henan, which reported many anthrax cases in the past, has not reported any anthrax cases for ≥10 years, shows that it is possible to control anthrax.

Conversely, newly affected counties were reported every year during the study. Newly affected counties were generally adjacent to previously affected counties, which

indicated a higher risk for importing anthrax from neighboring disease-endemic counties through contaminated livestock feed or movement of animals with latent infections. However, a few counties in Hunan and Jiangsu Provinces in eastern China, which are not near disease-endemic areas, also became newly affected in recent years. Investigations of these incidents showed that they were caused by long-distance transportation of infected livestock from disease-endemic areas. These investigations indicated the need for livestock inspection and ensuring that such livestock have been vaccinated ≥7–10 days before shipment.

As disease control improves, disease reporting also improves, and cases that would have been missed are now detected and reported. However, absence of disease reports is not the same as absence of disease. This situation indicates that provinces and large cities that have not had any anthrax cases in recent years are at risk for reemergence of anthrax. Humans get infected from animals with anthrax, and not vice versa. Therefore, humans will only be safe from this disease when it has been eradicated from livestock. Although eradication will require diligent surveillance and monitoring by the agricultural and veterinary communities, it has been achieved in many countries (5).

Our study had 2 limitations. First, we used data obtained through a passive surveillance system that was based on human health facilities nationwide. Thus, disease burden was probably underestimated because of various reasons, such as not seeking medical care. However, the clinical presentation for cutaneous anthrax makes patients more likely to seek medical care. Second, probable cases without laboratory confirmation of infection are also included in the analysis. Most cases were cutaneous, and their clinical manifestations are easily identified by medical personnel.

Laboratory diagnosis (based on isolation of *B. anthracis*) is not completely reliable. However, *B. anthracis* is susceptible to many antimicrobial drugs, and treatment of this type is commonly used. Such treatment could result in lesions being negative for *B. anthracis*. Although in recent years ELISA and PCR could be used for diagnosing confirmed cases, both techniques were rarely used at the local level because there were no reliable commercial kits for detecting antibodies against *B. anthracis* and no unified standard procedure for PCR of *B. anthracis* is currently available.

Acknowledgments
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Mathematical Modeling of Programmatic Requirements for Yaws Eradication

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Yaws is targeted for eradication by 2020. The mainstay of the eradication strategy is mass treatment followed by case finding. Modeling has been used to inform programmatic requirements for other neglected tropical diseases and could provide insights into yaws eradication. We developed a model of yaws transmission varying the coverage and number of rounds of treatment. The estimated number of cases arising from an index case (basic reproduction number [R_0]) ranged from 1.08 to 3.32. To have 80% probability of achieving eradication, 8 rounds of treatment with 80% coverage were required at low estimates of R_0 (1.45). This requirement increased to 95% at high estimates of R_0 (2.47). Extending the treatment interval to 12 months increased requirements at all estimates of R_0. At high estimates of R_0 with 12 monthly rounds of treatment, no combination of variables achieved eradication. Models should be used to guide the scale-up of yaws eradication.

Yaws is a bacterial infection caused by *Treponema pallidum* subsp. *pertenue* (*1*). The disease predominantly affects children living in poor, remote communities and results in lesions of the skin, bone, and cartilage. Previously, yaws was widespread throughout the tropics (*2*), but in the 20th century a series of control efforts based on mass treatment and case finding led by the World Health Organization (WHO) is estimated to have reduced the burden of cases worldwide by up to 95% (*3*). Despite these efforts, the disease has resurged in several countries in West and Central Africa, the Pacific, and Southeast Asia.

In 2012, a single dose of azithromycin was shown to be effective treatment for yaws (*4*). The availability of a well-tolerated oral agent has prompted WHO to develop a new eradication strategy, known as the Morges strategy, based on community mass azithromycin treatment (*5*). The

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strategy is supported by World Health Assembly resolution 66.12, which calls for eradication of yaws by 2020 (*6*). The strategy combines an initial round of total community treatment (TCT) followed by subsequent active case finding and total targeted treatment (TTT) of newly identified patients and their contacts. Pilot studies have shown that community mass treatment with azithromycin is a highly effective strategy for reducing the community prevalence of yaws (*7*,*8*).

Data are limited to inform the optimum coverage and number of TCT or TTT rounds that are required to achieve elimination (i.e., interruption of transmission) of yaws at a local level to facilitate country-level elimination and ultimately global eradication. In India, a national yaws elimination campaign conducted during 1996–2004 resulted in substantial reduction in the prevalence of yaws, sustained interruption of transmission, and nationwide elimination (*9*). This program consisted of case-finding surveys and treatment with parenteral penicillin conducted every 6 months. Although this approach did not include the initial mass treatment round now recommended as part of the Morges strategy, its success indicates that serial rounds of high-coverage treatment might achieve local elimination.

A recent review of important research questions facing the global yaws eradication program has highlighted the need for more accurate data to inform the optimum number and coverage of rounds of TCT and TTT that will be required to achieve yaws eradication (*10*). Mathematical modeling has been used to inform control efforts for several other neglected tropical diseases (*11–13*) that are also managed by using community mass treatment strategies, and such approaches could be of value for yaws eradication efforts. In particular, this approach might allow a comparison of the differential effects of alternative mass treatment strategies, which would be difficult to assess by empirical randomized controlled trials because of the size and cost of implementing large-scale cluster randomized studies.

Previous mathematical models for yaws (*14*) have assessed the cost-effectiveness of yaws eradication but have not directly addressed the feasibility of achieving this goal or the number of rounds of treatment that would be

required. In this study, we aimed to determine whether the eradication of yaws is feasible based on the Morges strategy and, if it is, the number and coverage of mass treatment rounds needed to achieve the goal.

Methods

We developed a stochastic Markov model of community-level transmission of yaws (Figure 1). This model treats each stage of the disease as a discrete compartment, with persons moving through each compartment as the disease progresses or is treated. Upon infection, susceptible persons acquire primary disease at a rate that is proportional to the transmission rate and the total number of infectious persons. Persons with primary disease can further transition to secondary disease, at which stage they remain infectious, and both those with primary and secondary disease can transition to latent disease, which is not infectious. Last, those with latent disease can relapse back to secondary infectious disease. The model includes a rate of routine treatment for persons with primary or secondary disease, after which they become susceptible to infection again. The model also includes a lower rate of routine treatment for latent disease, after which the patients also become susceptible to infection again. Unlike previous mathematical models of yaws (14), tertiary yaws was not included in the model because such cases are believed not to contribute to transmission (15). Because persons might be reinfected many times, we did not consider them to obtain protective immunity after infection or treatment (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/23/1/16-0487-Techapp1.pdf).

Although some evidence suggests the existence of a nonhuman primate reservoir for yaws in Africa (16), we did not include such infections in our model because there is currently no definitive evidence that the organism responsible for these infections is the same one that causes human yaws or that zoonotic transmission occurs in the real world. We therefore considered the epidemiologic importance of this possible reservoir as minimal when constructing our model.

Population Size

Estimates of the starting population for each compartment were derived from published population-based yaws prevalence studies (7,17). We modeled a discrete closed population without addition or reduction through births or deaths.

Disease Characteristic Variable Estimates

We estimated values for the rates of disease progression between different stages of yaws, including development of and relapse from latent yaws, by using expert opinion, published data, and estimates used in other models (Table 1) (1,14,17). We defined 3 transmission scenarios (low, medium, and high) by using published age-specific
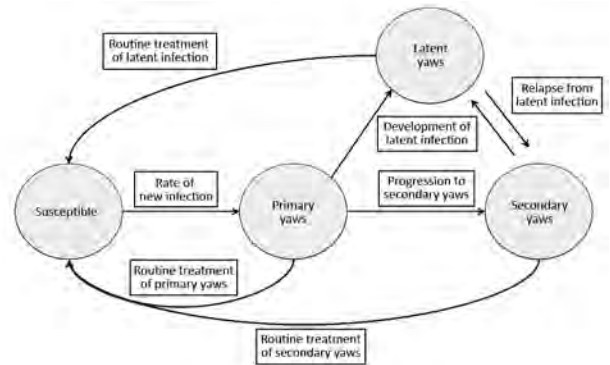
Figure 1. Markov model of yaws transmission. Susceptible persons become infected at a rate dependent on the probability of transmission and the number of persons with infectious primary and secondary yaws. Without treatment, illness progresses from primary disease to either latent yaws or secondary yaws. Persons with latent yaws might relapse to generate secondary cases of infectious yaws in others.

treponemal seroprevalence data (17), expert opinion, and values used in other yaws models (14). Based on these data, we calculated initial estimates of the number of new infections arising from a single index case in a fully susceptible population (the basic reproduction number [R_0]). Based on the seroprevalence data, we generated R_0 estimates of 1.25 (low), 1.83 (medium), and 2.4 (high). These estimates were converted to estimates of the probability of transmission after contact between an infectious person and a susceptible persons (β). The mean R_0 taking account of the full structure of our model, including duration of infection and the size of each starting population, is 1.96, resulting in a mean R_0 of 1.45 (95% CI 1.01–2.14) for the low transmission settings, 1.95 (95% CI 1.38–2.91) for medium, and 2.47 (95% CI 1.7–3.68) for high transmission. We included a variable to represent the likelihood of a person receiving treatment for yaws in the absence of public health interventions based on published data (17).

Mass Drug Administration Variables Estimates

We performed simulation experiments to estimate the impact of a yaws eradication intervention on disease transmission. In line with the Morges strategy (5), we considered 2 program components. In the first component, TCT, all persons were considered to have an equal chance of receiving treatment regardless of their infection status. In the second component, TTT, we considered that the coverage achieved among persons with active infection and those with latent infection might differ. Intervention coverage was modeled independently for TCT, with TTT pertaining to persons with active infection and persons with latent infection over a range of plausible estimates (65%–95% population coverage). Mass treatment compliance was simulated as a random, nonsystematic process (i.e., each person had the same

Table 1. Parameters used in modeling treatment coverage required to achieve yaws eradication

Parameter	Parameter estimate	Source of estimate	Comments
Epidemiologic parameters			
R_0*	1.08–3.32	Derived from published survey data (*17*)	The average number of new cases occurring from a single index case in a fully susceptible population
Monthly probability of progression from primary to secondary disease without treatment	2.78%–5.56%	Derived from expert opinion and previously published models (*1,14*)	All untreated persons with primary disease either develop secondary or latent stage disease, and this occurs over a period of 2–6 mo. Untreated persons with latent cases might relapse for a period of $\geq$5 y and become actively infectious again.
Monthly probability of progression from infectious to latent disease without treatment	13.9%–27.8%		
Monthly probability of relapse from latent disease to infectious stage without treatment	1%–3%		
Population parameters			
Susceptible at baseline	64%	Derived from published survey data (*17*)	Data derived from multiple pre–mass drug administration surveys conducted in communities where yaws is endemic
Primary yaws at baseline	1.5%		
Secondary yaws at baseline	1.5%		
Latent yaws at baseline	33%		
Mass treatment parameters			
Total community treatment coverage†	65%–95%	Expert opinion and published data on coverage achieved in other mass treatment campaigns (*18*)	Coverage estimates were chosen to reflect the range achieved in real-world mass drug administration programs for other neglected tropical diseases
Total targeted treatment coverage of persons with active cases‡	65%–95%		
Total targeted treatment coverage of persons with latent cases‡	65%–95%		
No. rounds of total community treatment†	1–3		
No. rounds of total targeted treatment‡	0–5		

*R_0 (basic reproduction number) is the number of new cases arising from a single index case in a fully susceptible population.
†Total community treatment consists of mass drug administration to all residents in a community where yaws is endemic regardless of clinical or serologic evidence of disease.
‡Total targeted treatment consists of active case finding and treatment of newly identified persons with yaws and their contacts.

chance of receiving treatment, with the likelihood of any 1 person receiving treatment being independent).

We varied the number of treatment rounds of TCT (1–3 rounds) and TTT (0–5 rounds). Where $\geq$1 rounds of TTT were implemented, these rounds followed the final round of TCT. In line with the Morges strategy and real-world pilot implementations (*5,7*), rounds of mass treatment were spaced at 6-month intervals. We also conducted an analysis with rounds of treatment spaced at 12-month intervals to assess whether annual treatment might also be effective.

We derived estimates of the efficacy of single-dose treatment with azithromycin from randomized controlled trials of azithromycin for the treatment of yaws (Table 1) (*4*). After successful treatment, yaws lesions become non-infectious within 24 hours (*1*); therefore, we considered treatment to be immediately efficacious at the time of mass drug administration, with persons reverting to a susceptible state after treatment.

Implementing the Model
The model was implemented in R software (*19*). We performed repeated simulations across a range of assumptions about the rate of transmission (equivalent to low, medium, and high estimates of R_0) and assumptions about mass treatment, varying the coverage and number of mass treatment rounds undertaken (Table 1).

For each combination of disease and intervention parameters, we performed 1,000 simulation experiments.

Within each combination of transmission and treatment assumptions, we varied other disease-specific variables (e.g., rate of progression and relapse and treatment in the absence of intervention) across the range of parameter estimates. The model was run for an initial period of 50 months to achieve a steady state with yaws eradication interventions modeled to commence after this initial period. The model then ran for a further 100 months (online Technical Appendix).

Assessing Outcomes
For each run of the model, we recorded whether eradication was achieved. Eradication was defined as no cases of infectious or latent yaws at the end of the model run. The eradication probability was defined as the percentage of runs within each permutation of model characteristics where eradication was achieved. All analyses were performed by using R version 3.2.2.

Results
The model generated a total of 6,174 simulations of variable mass drug administration strategies. Because each strategy was implemented across a range (n = 3) of assumptions about the force of infection, a total of 18,522 simulations were created. The probability of achieving local interruption of transmission varied substantially across estimates of the force of infection and mass drug administration characteristics.

At the lowest estimates of the force of infection (R_0 = 1.45) and with treatment rounds at 6-month intervals, the minimum treatment thresholds required to have a transmission interruption probability of ≥80% were a coverage of >75% of all populations and ≥8 rounds of treatment (3 rounds of TCT followed by 5 rounds of TTT). Increasing the coverage to 85% reduced the total number of rounds required to 5 (1–3 rounds of TCT followed by 2–4 rounds of TTT) (Figure 2; Table 2). For comparison, when the gap between treatment rounds was extended from 6 to 12 months, a total of 7 rounds of 85% coverage were required (2–3 rounds of TCT and 4–5 rounds of TTT).

At medium estimates of the force of infection (R_0 = 1.95) and with treatment rounds at 6-month intervals , the equivalent thresholds were 90% coverage and a total of 7 rounds of treatment (2–3 rounds of TCT and 4–5 rounds of TTT) (Figure 2; Table 2). When the gap between treatment rounds was increased to 12 months, no combination of treatment variables was predicted to have a transmission interruption probability of ≥80%.

At the highest estimates of the force of infection (R_0 = 3.32) and with treatment rounds at 6-month intervals, a total of 8 rounds (3 rounds of TCT and 5 rounds of TTT) with 95% coverage were required for a ≥80% likelihood of interrupting transmission (Figure 2; Table 2). When the gap between treatment rounds was increased to 12 months, no combination of treatment variables was predicted to have a probability of interrupting transmission of ≥80%.

We considered it plausible that, under field conditions, the coverage of persons with latent infection would not exceed 70% in any given round of TTT, because such cases are not clinically apparent, and adequate coverage might not be achieved by treating the immediate contacts of persons with clinical infection. At lower estimates of the force of infection, a total of 3 rounds of TCT with 85% coverage and 3 rounds of TTT (each with a coverage of persons with active infection of 85% and coverage of persons with latent infection of 65%) was associated with a ≥80% probability of interrupting transmission. If only 1 round of TCT was conducted, then coverage during TCT needed to be 90% and a total of 5 rounds of TTT (each with 90% coverage of persons with active infection and 65% coverage of persons with latent infection) were required. For medium estimates of the force of infection, a total of 8 rounds of treatment (3 rounds of TCT and 5 rounds of TTT) with a coverage of 90% were required. If only 1 round of TCT was undertaken, then 95% coverage was required, followed by 5 rounds of TTT with a 95% coverage of persons with active infection and 70% coverage of persons with latent infection. Under the highest estimate of the force of infection, no combination of treatment variables was associated with a high probability of interrupting transmission.

Discussion

Our study demonstrates that, with implementation of the Morges strategy, interruption of transmission is possible in the setting considered. This finding suggests that eradication of yaws could be achieved, although caution must be applied because variability in the parameter estimates elsewhere could affect the effectiveness of these strategies. The parameter that has the strongest influence on whether elimination can be achieved is the transmission rate; that is, the rate at which infection occurs given contact between a
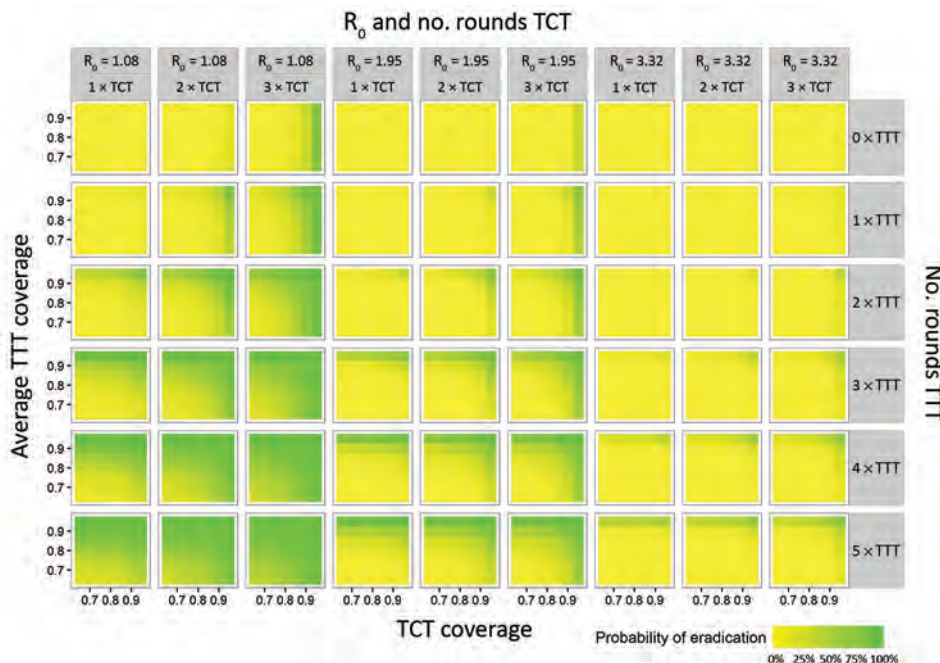
Figure 2. Predicted probability of achieving yaws eradication given variations in the estimate of R_0 (basic reproduction number), total community treatment coverage, number of rounds of total community treatment, total targeted treatment coverage (TTT), and number of rounds of TTT. For this graph, we only show simulations where the coverage of persons with latent cases is the same as the coverage of persons with active cases during TTT. This might overrepresent the actual likelihood of achieving eradication because the coverage of persons with latent cases is probably lower than the coverage of persons with active cases during TTT. R_0, basic reproduction number; TCT, total community treatment; TTT, total targeted treatment.

Table 2. Indicative predicted coverage and number of rounds of treatment required to achieve yaws eradication

Predicted probability by estimated R_0	Treatment every 6 mo		Treatment every 12 mo	
	Coverage required	Total no. rounds*	Coverage required	Total no. rounds*
80% predicted probability of eradication				
Low R_0 (1.45, 95% CI 1.01–2.14)	75%	8	85%	7
Medium R_0 (1.95, 95% CI 1.38–2.91)	90%	7	†	†
High R_0 (2.47, 95% CI 1.7–3.68)	95%	8	†	†
100% predicted probability of eradication				
Low R_0 (1.45, 95% CI 1.01–2.14)	85%	8	95%	6
Medium R_0 (1.95, 95% CI 1.38–2.91)	†	†	†	†
High R_0 (2.47, 95% CI 1.7–3.68)	†	†	†	†

*R_0 (basic reproduction number) is the number of new cases arising from a single index case in a fully susceptible population. For this table, the number of rounds of total community treatment and total targeted treatment is combined (e.g., a total of 7 rounds could be 3 rounds of total community treatment and 4 rounds of total targeted treatment or, alternatively, 2 rounds of total community treatment and 5 rounds of total targeted treatment). Data are indicative only, and in some settings, higher coverage would allow a reduction in the total number of rounds required (see text and Figure 2).
†No combination of treatment variables was associated with the stated eradication probability.

susceptible and an infectious person (online Technical Appendix Figure). We considered 3 different scenarios of the transmission rate of yaws based on serologic data, and our estimate of the feasibility of elimination varied considerably depending on these estimates. Further studies to obtain better estimates of the R_0 in a range of countries where yaws is endemic would be of value to inform improved models and programmatic planning.

A minimum of 8 rounds with coverage of ≥75% seems to be required for a high likelihood of achieving eradication but would prove inadequate at our highest estimates of possible values for R_0. The predictions of our model are broadly in keeping with the real-world findings of the successful yaws elimination program in India (9), where 7 years of consecutive case finding and treatment (analogous to 14 rounds of TTT with 75% coverage) were conducted. In our model, the number of rounds of TTT also had a marked effect on the likelihood of achieving eradication, especially when coverage of persons with latent cases was limited to ≤70%. In these settings, the required number of rounds of treatment to interrupt transmission increased considerably.

Relatively few data are available on the transmission rate of yaws. Even within yaws-endemic countries, the prevalence of yaws varies markedly. Studies in the Pacific have found a seroprevalence of antitreponemal antibodies of >30% in several communities (7,17) and a prevalence of clinical yaws of ranging from 2.5% to 5% in communities before mass treatment. The prevalence of yaws is markedly lower in many yaws-endemic countries in West Africa (20), but limited community-based seroprevalence data are available to inform our understanding of disease transmission there.

We modeled a range of estimates of R_0 from 1.08 to 3.32 based on seroprevalence data and expert opinion. Given the substantial influence of these estimates on the likely outcome of community mass treatment, further studies to better understand disease transmission and how this varies within and between endemic communities would be of value. Ideally, these studies would obtain community-level, age-specific seroprevalence data that could be used

to calculate the force of infection. No perfect serologic marker can be used for this task. Traditional treponemal serology combines a treponemal test, which reflects lifetime exposure but remains positive for life, with a nontreponemal test, the titer of which rises and falls after treatment. It is therefore not possible to use seroprevalence data to distinguish persons who have been infected many times from those who have been infected once, and seroprevalence estimates are likely to underestimate the actual force of infection. For this study, we calculated the force of infection while relying on treponemal serology alone, which should provide a more accurate estimate of the force of infection than if we used dual-positive serology. It remains, however, an imperfect measure.

Our model predicts that high coverage is required in all rounds of treatment to make yaws eradication feasible. Data from the previous WHO and United Nations Children's Fund mass treatment campaign have highlighted the importance of achieving high coverage of persons with latent cases of yaws (21) and that treatment of persons with active cases alone is insufficient to interrupt transmission. These factors were important considerations in the adoption as part of the Morges strategy of an initial round of TCT regardless of the prevalence of active disease in a community. Given the high coverage requirement, particularly of persons with latent cases, and the relatively high fixed-costs of reaching yaws-endemic communities (14) compared with the relatively low costs of generic azithromycin, it might be preferable to conduct multiple rounds of TCT before the switch to TTT. Such a recommendation would be in line with the original Morges strategy (22), which recommended that additional rounds of TCT could be considered if the coverage achieved in the initial round of treatment was <90% or if access to yaws-endemic communities was difficult. A switch to multiple rounds of community mass treatment might also facilitate integration with other neglected tropical diseases mass drug administration programs in countries that are also frequently based on whole community mass treatment (23), although our model predicted a higher probability of achieving eradication with biannual

treatment. Further studies to help determine the optimum strategy for achieving high coverage of persons with latent cases during the TTT phase of eradication efforts should be considered (e.g., studies of the spatial epidemiology of latent yaws cases in relation to persons with active cases in both pre– and post–mass drug administration settings or studies of whether additional mass treatment rounds specifically targeting children might be beneficial).

Our study has several limitations. Most notably, we lack accurate estimates for several disease parameters. The parameters used are derived from expert opinion and data from the Pacific region, and the transmission dynamics of yaws might be different in other regions of the world. However, the disease parameters used in this study are broadly in line with those used by other models of yaws transmission (14). We tested a range of coverage estimates for community mass treatment, but we did not factor in the possibility that some persons might be systematically missed during mass treatment campaigns, a phenomenon that has been observed in control efforts for other neglected tropical diseases (24). The current Morges strategy does not include adjunctive elements, such as water, sanitation, and hygiene interventions, in addition to mass treatment, although some studies suggest that improved access to water and sanitation is associated with a decreased risk for yaws (17). We did not include a secular trend in our model, and such a trend could be anticipated to further increase the likelihood of yaws eradication being achieved. Our model was designed to assess the feasibility of achieving yaws eradication in the near future, driven by the current WHO strategy, and in those conditions any effect of a secular trend could be expected to be minimal compared with the substantial impact of community mass treatment. Previous models have shown that secular trends are unlikely to substantially affect the cost-effectiveness of mass treatment (14); however, those models were based on an assumption of 90%–99% coverage in a single TCT round and 100% coverage of index patients and their contacts in the TTT round. More generally, we used a single-model structure that is simplified by modeling persons as being in 1 of a small number of disease-related compartments at any time and considering contact to occur at random. Uncertainty in model structure relating to disease progression and the probability of contact means that our findings should be interpreted carefully and potentially reassessed as elimination strategies are being applied.

In conclusion, our study assessed the theoretical achievability of worldwide yaws eradication and represents an important milestone in reaching the WHO's eradication target. We have defined programmatic thresholds that might need to be met to achieve yaws eradication and identified key research questions to be addressed to inform refinements of the model and the worldwide roll-out of treatment strategies.

Dr. Marks is a Wellcome Trust Clinical Research Fellow at the London School of Hygiene & Tropical Medicine. His primary research interests include strategies to eradicate yaws and the development of integrated approaches to the control and elimination of neglected tropical diseases.

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etymologia

Bayesian Probability

Ronnie Henry, Martin I. Meltzer

Thomas Bayes (1701–1761) was a Presbyterian Minister, and how he become interested in statistics and probability is uncertain. Bayes presented his famous theorem on probability in "An Essay Towards Solving a Problem in the Doctrine of Chances," which was published posthumously by his friend Richard Price in 1763. Bayes's theorem provides a method of explicitly including prior events or knowledge when considering the probabilities of current events (for example, including a history of smoking when calculating the probability of developing lung cancer). Bayesian approaches use prior knowledge and information (e.g., probabilities) that may help reduce uncertainty in analysis and have therefore been increasingly adopted by analysts in public health.

Thomas Bayes (artist unknown). Public Domain, https://commons.wikimedia.org/w/index.php?curid=14532025

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Estimated Incidence of Antimicrobial Drug–Resistant Nontyphoidal *Salmonella* Infections, United States, 2004–2012

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Patricia M. Griffin, Robert M. Hoekstra

Salmonella infections are a major cause of illness in the United States. The antimicrobial agents used to treat severe infections include ceftriaxone, ciprofloxacin, and ampicillin. Antimicrobial drug resistance has been associated with adverse clinical outcomes. To estimate the incidence of resistant culture-confirmed nontyphoidal *Salmonella* infections, we used Bayesian hierarchical models of 2004–2012 data from the Centers for Disease Control and Prevention National Antimicrobial Resistance Monitoring System and Laboratory-based Enteric Disease Surveillance. We based 3 mutually exclusive resistance categories on susceptibility testing: ceftriaxone and ampicillin resistant, ciprofloxacin nonsusceptible but ceftriaxone susceptible, and ampicillin resistant but ceftriaxone and ciprofloxacin susceptible. We estimated the overall incidence of resistant infections as 1.07/100,000 person-years for ampicillin-only resistance, 0.51/100,000 person-years for ceftriaxone and ampicillin resistance, and 0.35/100,000 person-years for ciprofloxacin nonsusceptibility, or ≈6,200 resistant culture-confirmed infections annually. These national estimates help define the magnitude of the resistance problem so that control measures can be appropriately targeted.

Each year in the United States, nontyphoidal *Salmonella* causes an estimated 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths (*1*). Antimicrobial drug–resistant *Salmonella* is a serious threat to public health (*2*). *Salmonella* infections have been linked to a variety of sources, particularly foods of animal origin (e.g., beef, poultry, eggs, dairy products) and produce (*3–5*). Most antimicrobial drug–resistant nontyphoidal *Salmonella* infections are caused by 4 of the 5 serotypes most commonly isolated during 2004–2012: Typhimurium, Enteritidis, Newport, and Heidelberg (*6–10*). The predominance of these 4 serotypes reflects their ability

to persist in food animals, be transmitted through the food supply, and cause illness in humans (*10,11*).

Most nontyphoidal *Salmonella* infections do not require antimicrobial treatment. However, treatment is recommended for severe infections, including invasive illnesses such as bacteremia and meningitis (*12*). Third-generation cephalosporins (e.g., ceftriaxone) and fluoroquinolones (e.g., ciprofloxacin) are empirically used to treat severe nontyphoidal *Salmonella* infections. Because fluoroquinolones are not routinely prescribed for children, third-generation cephalosporins are particularly important for use in children. Ampicillin remains a useful agent for treating infections documented as susceptible (*12–14*). Adverse clinical outcomes (e.g., increased rates of hospitalization, bloodstream infection, invasive illness, and death) have been associated with resistant infections, and treatment failures have been reported for infections with reduced susceptibility to ciprofloxacin (*5,15–19*).

Estimates of the incidence of resistant *Salmonella* infections are needed to inform policy decisions. The National Antimicrobial Resistance Monitoring System (NARMS) monitors resistance among salmonellae by testing samples of isolates from ill persons and determining the percentage of isolates that display resistance (*8,9*). For extrapolation from resistance percentages to incidence of resistant infections, the incidence of *Salmonella* infections must be known. *Salmonella* incidence data for this calculation are provided by the National Laboratory-based Enteric Disease Surveillance (LEDS) system (*6*). Serotype Heidelberg provides an illustration of why estimates of the incidence of resistant infections are needed. During 2004–2012, the percentage of ceftriaxone-resistant isolates increased from 9% to 22% (*8,9*). At the same time, the incidence of Heidelberg infections declined from 0.60 to 0.31 infections/100,000 population (*6*). Thus, to assess whether the incidence of resistant Heidelberg infections is changing, estimates of the incidence of resistant Heidelberg infections are needed.

Using Bayesian hierarchical models of resistance percentages and *Salmonella* incidence with data from the 2 surveillance systems, we estimated the incidence of culture-confirmed infections caused by nontyphoidal *Salmonella* with resistance to ceftriaxone, nonsusceptibility to ciprofloxacin, and resistance to ampicillin and provide such estimates for major serotypes (*20*). We describe this modeling approach of combining data from the 2 systems to obtain improved estimates and measures of uncertainties.

Methods

LEDS

Clinical laboratories send *Salmonella* isolated from humans to public health laboratories in 50 states and many local health departments for serotyping (*6*). Culture-confirmed *Salmonella* isolates are reported to the Centers for Disease Control and Prevention (CDC) through LEDS (*6*). Excluded from this report are serotypes Typhi and Paratyphi, for which the only reservoir is humans and which account for <1% of *Salmonella* infections in the United States (*6,11,12*). Hereafter, we use the term *Salmonella* to refer to nontyphoidal *Salmonella*.

NARMS

NARMS is a collaboration among CDC, the US Food and Drug Administration (FDA), the US Department of Agriculture, and state and local health departments. NARMS monitors resistance among enteric bacteria isolated from humans, retail meat, and food animals (*8,9*). Public health laboratories of 50 state and 4 local health departments submit a subset (every 20th) of *Salmonella* isolates that they receive from clinical laboratories to the CDC NARMS for susceptibility testing (*8,9*).

From 2004 through 2012, CDC tested *Salmonella* isolates for susceptibility to agents representing 8–9 classes of antimicrobial agents. MICs were determined by broth microdilution (Sensititer; Trek Diagnostics, Westlake, OH, USA) and interpreted by using criteria from the Clinical and Laboratory Standards Institute when available (*8,19*). We defined ceftriaxone resistance as MIC ≥4 μg/mL, ampicillin resistance as MIC ≥32 μg/mL, and nonsusceptibility to ciprofloxacin as MIC ≥0.12 μg/mL; the latter includes resistant and intermediate categories defined by the Clinical and Laboratory Standards Institute (*8,19*).

Resistance Categories for Estimation of Resistance Incidence

We defined 3 mutually exclusive categories of clinically important resistance according to results of testing for ceftriaxone, ciprofloxacin, and ampicillin (Figure 1) (*8,19*): ceftriaxone/ampicillin resistance indicates resistance to ceftriaxone and ampicillin (because all ceftriaxone-resistant isolates are

ampicillin resistant); ciprofloxacin nonsusceptibility indicates nonsusceptibility to ciprofloxacin but susceptibility to ceftriaxone; and ampicillin-only resistance indicates resistance to ampicillin but susceptibility to ceftriaxone and ciprofloxacin. Isolates in each category may be resistant to other agents. Hereafter, we refer to any resistance included in any of these 3 clinically important categories as overall resistance. Unlike the 2013 CDC report, which includes estimates for resistance to ≥5 antimicrobial drug classes, we focused on the 3 agents used to treat invasive infections (*2*).

Bayesian Hierarchical Model

We used 2004–2012 data from NARMS, LEDS, and the US Census Bureau as input in the Bayesian hierarchical model (*6,8,21*). From NARMS, we used resistance proportions calculated as the number of resistant isolates divided by the number of isolates tested per state and year (state-year). We included only fully serotyped isolates. From LEDS, we used the number of culture-confirmed infections reported for state-year. We included all LEDS isolates; for each state, the serotypes of nonserotyped and partially serotyped isolates were imputed on the basis of the observed proportions of 5 serotype categories (Typhimurium, Enteritidis, Newport, Heidelberg, and other) among fully serotyped isolates over the 9 years. We used US Census population data for each state-year to express incidence (infections per 100,000 persons per year [person-years]).
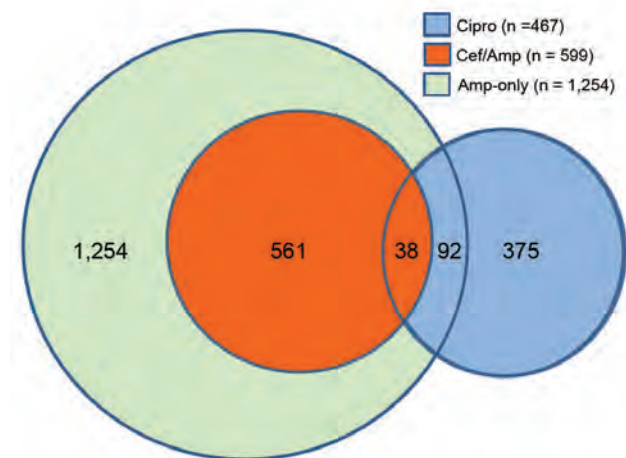
Figure 1. Number of nontyphoidal *Salmonella* isolates with clinically important resistance, by resistance category, United States, 2004–2012. Three mutually exclusive categories were defined. Isolates in each category may have resistance to other agents: 99% of the 599 Cef/Amp isolates, 43% of the 467 Cipro isolates, and 89% of the 1,254 Amp-only isolates were resistant to ≥1 antimicrobial class other than cephems, quinolones, or penicillins. Amp-only, resistant to ampicillin but susceptible to ceftriaxone and ciprofloxacin; Cef/Amp, resistant to ceftriaxone (MIC ≥4 μg/mL) and ampicillin (MIC ≥32 μg/mL); Cipro, nonsusceptible to ciprofloxacin (MIC ≥0.12 μg /mL) but susceptible to ceftriaxone; NTS, nontyphoidal *Salmonella*.

In the Bayesian hierarchical model, we assumed normal distribution for LEDS *Salmonella* incidence data and binomial distribution for NARMS data. The Bayesian hierarchical model of *Salmonella* incidence and resistance data incorporated state, year, and state-year interaction effects. State and year effects used borrowed strength from contiguous states and previous years. Borrowed strength refers to the idea that quantities of interest are related to each other, and information on one can provide information on another (*22*). We excluded Alaska and Hawaii because they are distant from the 48 contiguous states and so the Bayesian hierarchical model could not be well applied. We excluded the District of Columbia because it did not begin submitting isolates to NARMS until 2008 (*9*). In preliminary analyses, we reviewed LEDS *Salmonella* incidence data by state-year to identify outliers that may need modeling adjustments, knowing that some states do not routinely receive all isolates from clinical laboratories (*6*). The models are described in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/23/1/16-0771-Techapp1.pdf).

We generated Bayesian hierarchical model posterior estimates of *Salmonella* infection incidence rates, resistance proportions, and resistant infection incidence rates (resistance incidence) by state-year for each of the 5 serotype categories by using Markov chain Monte Carlo simulations (*22–24*). State-year resistance incidence estimates, expressed per 100,000 person-years, were calculated as follows: ([estimated number of infections for state-year/census population for state-year] × 100,000) × (estimated resistance proportion for state-year). We calculated the means of the 48 state-year mean posterior estimates for each of the 9 study years. We generated overall estimates for 2004–2012 by calculating means and 95% credible intervals (CrIs) from the 9-year mean estimates. We used 2.5th and 97.5th percentiles of 5,000 samples of posterior estimates for the 95% CrIs. For each of the 5 serotype categories, we estimated resistance incidence for the mutually exclusive categories and derived overall resistance incidence estimates by summing them. For all *Salmonella*, estimates were calculated by summing estimates derived for the 5 serotype categories.

As part of model fitting, we plotted observed versus Bayesian hierarchical model–derived (predicted) estimates of *Salmonella* infection incidence, resistance proportion, and resistance incidence by state-year for the 5 serotype categories by resistance category. We assessed the shrinkage of resistance proportions (observed vs. predicted values) related to the number of isolates tested; shrinkage refers to an estimation scheme that borrows strength from related quantities to adjust individual estimates (online Technical Appendix) (*25*). To assess fluctuations over the 9 years of the study, we derived mean estimates and 95% CrIs for 3-year periods (2004–2006, 2007–2009, and 2010–2012) by using an even split of time for simplicity.

Results

Overall *Salmonella* Infection and Resistance Surveillance Data

From 2004 through 2012, the 48 contiguous states reported 369,254 culture-confirmed *Salmonella* infections to LEDS. The periods 2004–2006, 2007–2009, and 2010–2012 accounted for 30%, 33%, and 37% of infections, respectively. Among the isolates from these infections, 87% were serotyped as follows: Enteritidis (19%), Typhimurium (18%), Newport (11%), Heidelberg (4%), and all other serotypes (48%). The remaining 13% were not fully serotyped. These 4 primary serotypes, which were among the 5 most commonly reported to LEDS overall, accounted for 52% of fully serotyped isolates. Of the 48 states, <2% of isolates were not fully serotyped for 10 states, 2%–10% for 27 states, 11%–29% for 5 states, and >62% for 6 states.

From 2004 through 2012, NARMS tested 19,410 *Salmonella* isolates from the 48 states for resistance. The periods 2004–2006, 2007–2009, and 2010–2012 accounted for 30%, 34%, and 36% of isolates, respectively. Most (98%) were fully serotyped as follows: Enteritidis (18%), Typhimurium (17%), Newport (11%), Heidelberg (4%), and other (49%). These 4 primary serotypes, which were among the 5 most common among isolates submitted to NARMS overall, accounted for 51% of fully serotyped isolates. Of the 48 states, <2% of isolates were not fully serotyped for 31 states, 2%–8% for 15 states, and >86% for 2 states.

Overall resistance was detected in 2,320 (12%) isolates. Ampicillin-only resistance was the most common pattern, detected in 1,254 (6.5%) isolates, of which 60% were Typhimurium (Table 1; Figure 1). Ceftriaxone/ampicillin resistance was detected in 599 (3.1%) isolates, of which 33% were Newport, 27% Typhimurium, and 15% Heidelberg. Ciprofloxacin nonsusceptibility was detected in 467 (2.4%) isolates, of which 20% were resistant to ampicillin and 45% were Enteritidis. Only 38 (0.2%) isolates were both resistant to ceftriaxone and nonsusceptible to ciprofloxacin; these were included only in the ceftriaxone/ampicillin resistance category. Most isolates with ceftriaxone/ampicillin resistance, ciprofloxacin nonsusceptibility, or ampicillin-only resistance showed resistance to other agents tested (Figure 1) (*9*). The 4 serotypes accounted for 73% of 2,320 isolates with any clinically important resistance. The percentages of isolates with ciprofloxacin nonsusceptibility and ampicillin-only resistance among not fully serotyped isolates were similar to those among all *Salmonella*.

Surveillance and Resistance Data by State and Year

All 48 states reported *Salmonella* infections to LEDS. Not all states reported infections every year: 47 reported any *Salmonella*, 44 reported Typhimurium, 45 reported Enteritidis, 43 reported Newport, and 39 reported Heidelberg.

Table 1. Nontyphoidal *Salmonella* isolates with clinically important resistance, by serotype and resistance category, United States, 2004–2012*

Resistance category	Typhimurium, no. (%), n = 3,324	Enteritidis, no. (%), n = 3,501	Newport, no. (%), n = 2,175	Heidelberg, no. (%), n = 738	Other fully serotyped, no. (%), n = 9,265	Not fully serotyped, no. (%), n = 407	Total NTS, no. (%), n = 19,410
Cipro†	54 (1.6)	211 (6.0)	7 (0.3)	2 (0.3)	183 (2.0)	10 (2.5)	467 (2.4)
Cef/Amp‡	162 (4.9)	8 (0.2)	198 (9.1)	87 (11.8)	141 (1.5)	3 (0.7)	599 (3.1)
Amp-only§	750 (22.6)	90 (2.6)	25 (1.1)	94 (12.7)	274 (3.0)	21 (5.2)	1,254 (6.5)
Any of the above¶	966 (29.1)	309 (8.8)	230 (10.6)	183 (24.8)	598 (6.5)	34 (8.4)	2,320 (12.0)

*Total NTS isolates include isolates serotyped as Typhimurium, Enteritidis, Newport, and Heidelberg; isolates serotyped as other than these 4; and those not fully serotyped. Amp-only, resistant to ampicillin but susceptible to ceftriaxone and ciprofloxacin; Cef/Amp, resistant to ceftriaxone and ampicillin; Cipro, nonsusceptible to ciprofloxacin but susceptible to ceftriaxone; NTS, nontyphoidal *Salmonella*. Cipro, Cef/Amp, and Amp-only are mutually exclusive categories.
†Nonsusceptible to ciprofloxacin (MIC $\geq$0.12 µg/mL) but susceptible to ceftriaxone, with or without resistance to other agents.
‡Resistant to ceftriaxone (MIC $\geq$4 µg /mL) and ampicillin (MIC $\geq$32 µg/mL), with or without nonsusceptibility to ciprofloxacin or resistance to other agents; of the 599 ceftriaxone-resistant isolates, 38 (0.2% of all NTS isolates) were nonsusceptible to ciprofloxacin.
§Resistant to ampicillin but susceptible to ceftriaxone and ciprofloxacin, with or without resistance to other agents.
¶Nonsusceptible to ciprofloxacin, resistant to ceftriaxone, or resistant to ampicillin.

Many states had wide fluctuations in the annual overall incidence, ranging from 3.1 (Florida) to 28.4 (Mississippi) infections/100,000 person-years.

All 48 states submitted *Salmonella* isolates to NARMS. Not all states submitted isolates every year: 44 submitted any *Salmonella*, 32 submitted Typhimurium, 31 submitted Enteritidis, 23 submitted Newport, and 5 submitted Heidelberg. For Heidelberg and many less common serotypes, small numbers of isolates were tested; in isolates from many states, low or no resistance was detected (e.g., no ceftriaxone resistance among 109 Heidelberg isolates from 19 states). However, very high resistance was assigned to some states for which small numbers were tested (e.g., 1 ceftriaxone-resistant of only 1 tested).

Model Estimates of Annual Resistance Incidence by State

Rates of *Salmonella* incidence in Florida were much lower than those from its 6 closest states. We adjusted for this finding in the Bayesian hierarchical model (online Technical Appendix).

For the 48 states, mean resistance incidence, estimated by serotype and resistance category, varied geographically (Figure 2). For all *Salmonella,* rates (infections per 100,000 person-years) ranged as follows: 0.88–4.69 (median 1.81) for overall resistance; 0.45–2.95 (median 0.94) for ampicillin-only resistance; 0.15–2.20 (median 0.38) for ceftriaxone/ampicillin resistance; and 0.11–0.87 (median 0.33)

Figure 2. Estimated incidence of infection with all NTS and major serotypes with clinically important resistance (no. infections per 100,000 person-years), by state and resistance category, United States, 2004–2012. Estimates were derived by using Bayesian hierarchical models. All NTS includes the 4 major and other serotypes. Isolates in each category may have resistance to other agents. Data on Cipro among Newport (8 isolates), Cipro among Heidelberg (7), and Cef/Amp among Enteritidis (2) were too sparse to use in the Bayesian hierarchical models. Overall resistance was defined as Cipro, Cef/Amp, or Amp-only. Amp-only, resistant to ampicillin (MIC $\geq$32 µg/mL) but susceptible to ceftriaxone and ciprofloxacin; Cef/Amp, resistant to ceftriaxone (MIC $\geq$4 µg/mL) and ampicillin; Cipro, nonsusceptible to ciprofloxacin (MIC $\geq$0.12 µg /mL) but susceptible to ceftriaxone; NTS, nontyphoidal *Salmonella*.

for ciprofloxacin nonsusceptibility. For example, rates of Typhimurium infections with overall resistance were high for many states in the West/Midwest (e.g., Montana, South Dakota, Wyoming, Iowa, Colorado). Rates of Enteritidis infections with ciprofloxacin nonsusceptibility were low for many states in the South (e.g., Mississippi, Arkansas, Louisiana, South Carolina, Alabama).

We observed that the shrinkage of resistance proportions was inversely related to the number of isolates tested, (i.e., more shrinkage with smaller numbers). Examples are shown in the online Technical Appendix.

Model Estimates of Resistance Incidence Overall

Resistance incidence rates were relatively stable, and 95% CrIs overlapped substantially for the 3 periods (Figure 3). For overall *Salmonella* infections (Table 2), we estimated the incidence of resistant culture-confirmed infections per 100,000 person-years for 2004–2012 as follows: 1.93 (95%

CrI 1.60–2.35) for any clinically important resistance, 1.07 (95% CrI 0.86–1.32) for ampicillin-only resistance, 0.51 (95% CrI 0.35–0.70) for ceftriaxone/ampicillin resistance, and 0.35 (95% CrI 0.24–0.51) for ciprofloxacin nonsusceptibility. Newport, Typhimurium, and Heidelberg accounted for 75% of the incidence of ceftriaxone/ampicillin-resistant infections; Typhimurium accounted for 59% of the incidence of ampicillin-only–resistant infections; and Enteritidis accounted for 45% of the incidence of ciprofloxacin-nonsusceptible infections. Overall, the 4 serotypes accounted for 73% of the incidence of *Salmonella* infections with any clinically important resistance.

Discussion

This report provides much-needed national incidence estimates for clinically important antimicrobial drug–resistant *Salmonella* infections in the United States. Overall, we estimate the incidence of such culture-confirmed infections to
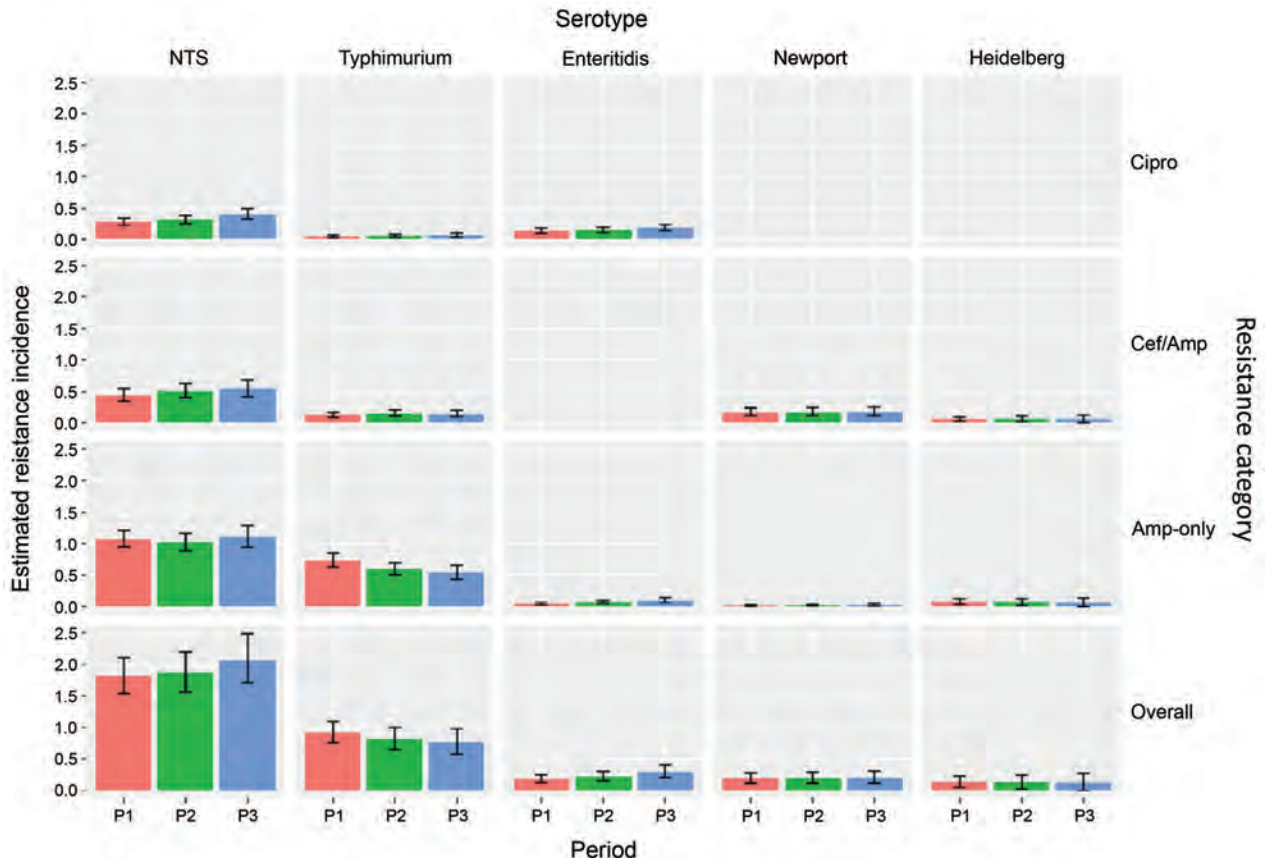
Figure 3. Estimated incidence of NTS infections with clinically important resistance (no. infections/100,000 person-years), by period, serotype, and resistance category, United States, 2004–2012. Estimates were derived by using Bayesian hierarchical models. All NTS includes the 4 major and other serotypes. Three mutually exclusive resistance categories were defined. Isolates in each category may have resistance to other agents. Data on Cipro among Newport (8 isolates), Cipro among Heidelberg (*7*), and Cef/Amp among Enteritidis (*2*) were too sparse to use in the Bayesian hierarchical models. Overall resistance was defined as Cipro, Cef/Amp, or Amp-only. Data were grouped into 3 periods (P): 2004–2006 (P1), 2007–2009 (P2), and 2010–2012 (P3). Error bars indicate 95% credible intervals. Amp-only, resistant to ampicillin (MIC $\geq$32 µg/mL) but susceptible to ceftriaxone and ciprofloxacin; Cef/Amp, resistant to ceftriaxone (MIC $\geq$4 µg/mL) and ampicillin; Cipro, nonsusceptible to ciprofloxacin (MIC $\geq$0.12 µg /mL) but susceptible to ceftriaxone; NTS, nontyphoidal *Salmonella*; P, period.

Table 2. Estimated incidence of nontyphoidal *Salmonella* infections with clinically important resistance, by serotype and resistance category, United States, 2004–2012*

Resistance category	No. infections/100,000 person-years (95% credible intervals)*				
	All NTS	Typhimurium	Enteritidis	Newport	Heidelberg
Cipro†	0.35 (0.24–0.51)	0.05 (0.02–0.10)	0.15 (0.09–0.25)	0.005‡	0.002‡
Cef/Amp§	0.51 (0.35–0.70)	0.14 (0.08–0.23)	0.006‡	0.18 (0.08–0.29)	0.06 (0–0.13)
Amp-only¶	1.07 (0.86–1.32)	0.63 (0.43–0.87)	0.08 (0.03–0.16)	0.02 (0.01–0.05)	0.08 (0–0.18)
Any of the above #	1.93 (1.60–2.35)	0.82 (0.61–1.05)	0.24 (0.14–0.38)	0.20 (0.11–0.32)	0.14 (0.002–0.28)

*Estimates and 95% credible intervals were derived by using Bayesian hierarchical models. Cipro, Cef/Amp, and Amp-only are mutually exclusive categories. Estimates for any clinically important resistance were derived by summing estimates for the mutually exclusive categories. Serotypes other than Typhimurium, Enteritidis, Newport, and Heidelberg were combined in an "other" category. For all NTS, estimates were derived by summing those derived for the 4 major serotypes and other category. Amp-only, resistant to ampicillin but susceptible to ceftriaxone and ciprofloxacin; Cef/Amp, resistant to ceftriaxone and ampicillin; Cipro, nonsusceptible to ciprofloxacin but susceptible to ceftriaxone; NTS, nontyphoidal *Salmonella*.
†Nonsusceptible to ciprofloxacin (MIC $\geq$0.12 μg/mL) but susceptible to ceftriaxone, with or without resistance to other agents.
‡Only 8, 7, and 2 isolates of Enteritidis, Newport, and Heidelberg, respectively, showed this resistance pattern; thus, state-year data were too sparse to use in the Bayesian hierarchical models. Crude estimates are shown, calculated as mean incidence for the serotype multiplied by mean resistance proportion over the 9 y.
§Resistant to ceftriaxone (MIC $\geq$4 μg/mL) and ampicillin (MIC $\geq$32 μg/mL), with or without nonsusceptibility to ciprofloxacin or resistance to other agents.
¶Resistant to ampicillin but susceptible to ceftriaxone and ciprofloxacin, with or without resistance to other agents.
#Nonsusceptible to ciprofloxacin, resistant to ceftriaxone, or resistant to ampicillin.
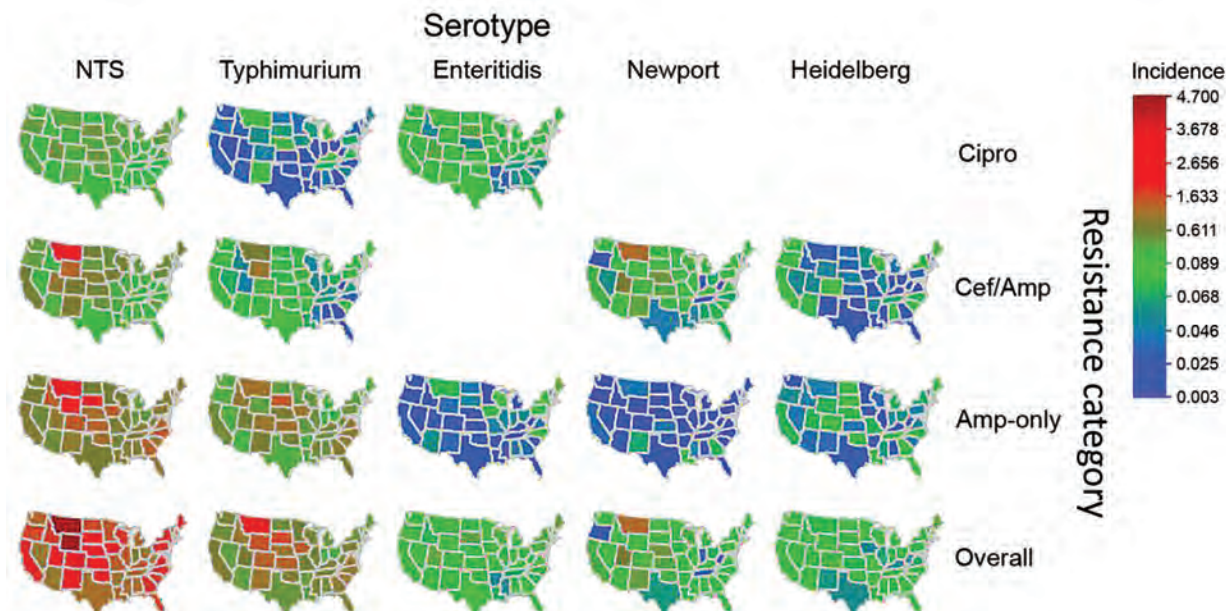
be ≈2/100,000 person-years. Clinically important resistance is strongly linked to specific serotypes. Enteritidis accounts for about half the incidence of ciprofloxacin-nonsusceptible infections; Newport, Typhimurium, and Heidelberg for three fourths of the incidence of infections with resistance to both ceftriaxone and ampicillin; and Typhimurium for more than half the incidence of infections with ampicillin-only resistance. Many of these isolates with clinically important resistance are also resistant to other agents (*8,9*). Although these 4 serotypes account for about half of culture-confirmed *Salmonella* infections, they account for nearly three fourths of the incidence of clinically important resistant infections (*6,9*). This finding suggests that strategies to reduce the incidence of infections caused by these 4 serotypes could have a larger effect on reducing the incidence of resistant *Salmonella* infections overall.

Using the Bayesian hierarchical model, we improved the estimation of resistance incidence by addressing issues related to missing and sparse state data, particularly for certain combinations of serotypes and resistance. Both surveillance databases showed great variation in reporting by state and year; these variations are probably associated with testing only small numbers of isolates in certain states, underreporting, and incomplete serotyping (*6,8*). Therefore, crude estimates based on observed data could lead to biased estimation. We mitigated these issues by statistically borrowing strength from neighboring states and previous years (*22*). We present observed and predicted state resistance incidence estimates by year (online Technical Appendix) to illustrate how our Bayesian hierarchical model smooths state-to-state variability of observed data. We used an estimation scheme called shrinkage, which moved disparate estimates toward a common central value, leading to a more robust set of estimates (*25*). We noted that the shrinkage of resistance proportions was inversely related to the number of isolates tested (online Technical Appendix Figure 1).

Our analysis has limitations. Because LEDS is a passive surveillance system, underreporting probably occurs in most states (*6*); it was marked in Florida, and we adjusted for this only in the Bayesian hierarchical model (online Technical Appendix). We assumed that populations under surveillance are defined by the US Census population data, although populations are mobile and illnesses are sometimes reported by the state in which they are diagnosed rather than the state in which the patient resides (*6,21*). The proportion of isolates that were not fully serotyped varied by state and was much higher in LEDS than NARMS. This finding suggests that isolates submitted to NARMS were more likely to be serotyped; regardless, we found similar distributions of major serotypes in LEDS and NARMS. Our approach of imputing missing serotypes of nonserotyped and partially serotyped LEDS isolates by state is reasonable because of the similar distribution of major serotypes in NARMS and LEDS. We did not include serogroup information when imputing partially serotyped isolates; such an approach would not alter our estimates. However, refined methods for imputing partially serotyped isolates could be useful for other analyses.

Because we created mutually exclusive categories, our incidence estimates for ciprofloxacin nonsusceptibility and for ampicillin-only resistance do not include all *Salmonella* with ciprofloxacin nonsusceptibility and ampicillin resistance, respectively. Isolates resistant to ceftriaxone and ampicillin, of which there were many, and those resistant to ceftriaxone and nonsusceptible to ciprofloxacin, were included only in the ceftriaxone/ampicillin resistance category. Furthermore, we do not provide estimates for resistance to trimethoprim-sulfamethoxazole, which can be used for noninvasive infections (*12*); during 2004–2012, <2% of *Salmonella* isolates were resistant to trimethoprim-sulfamethoxazole, 79% of which were also resistant to ceftriaxone or ampicillin, or nonsusceptible to ciprofloxacin (*8*; CDC, unpub. data).

Surveillance data capture culture-confirmed infections only, which represent a fraction of all infections (*6,8,9*). Our estimates total ≈6,200 culture-confirmed *Salmonella* infections with clinically important resistance annually (*21*). CDC has estimated that for every laboratory-confirmed case of *Salmonella*, there are many other undetected cases; the most recent estimate is 29 infections for every 1 culture-confirmed case (*1*). Because persons with resistant infections are at increased risk for more serious illness that may result in medical attention, such infections may be more likely than susceptible infections to be detected through culture-based surveillance (*15–18,26*). The ratio of undetected to detected resistant infections has not been estimated.

We found marked state-to-state variation in the incidence of resistant infections. Additional modeling, taking into account the varying distributions of infections by geography, serotype, demographic subgroup, and season, would be needed to help elucidate the reasons (*27,28*). Infections among older persons have been associated with increased rates of invasive illness and hospitalization, which may be more likely to be detected; thus, these estimates may represent a higher proportion of older patients than actually exists (*13,16,21,26*). Estimates are based on resistance among all *Salmonella* isolates, which are mostly isolated from fecal samples (*9*). Therefore, these estimates of resistant infections represent mostly noninvasive infections, only a fraction of which may require antimicrobial treatment (*9,12*). About 27% of patients with culture-confirmed salmonellosis are hospitalized (*1*). If patients with resistant infections are more likely to be hospitalized, these estimates may disproportionately reflect hospitalized patients (*15–18*).

For our estimates, we used data based on current laboratory methods, reporting, and isolate submission practices in states. With increasing use of culture-independent diagnostic tests by clinical laboratories, we anticipate changes in reporting and submission of isolates to public health laboratories (*29*). These changes would warrant model adjustments for future estimation and assessment of changes over time.

Annual NARMS reporting of resistance percentages remains a useful approach for tracking resistance, particularly emerging resistance in serotypes in low numbers of tested isolates (*8*). The method we have developed (using 2 data sources) provides a way to understand changes in the incidence of resistance especially for serotypes like Heidelberg, which is decreasing in incidence but increasing in the proportion resistant to ceftriaxone (*6,8,9*). By estimating resistance incidence rather than percentage of resistant isolates, we remove a major confounder to interpretation of estimated resistance levels. Our 95% CrIs incorporate uncertainties associated with missing and sparse data. However, our results go a long way toward this understanding. The overlapping 95% CrIs for ceftriaxone-resistant Heidelberg that we found for the 3 periods suggest that incidence rates were relatively stable during 2004–2012. A future, more detailed analysis could assess resistance incidence trends in Heidelberg and other serotypes.

Antimicrobial drug use in food-producing animals is a major driver of—although not the only contributor to—resistant *Salmonella* infections. An example is the contribution of third-generation cephalosporin use in poultry to ceftriaxone resistance among Heidelberg infections of humans (*30–32*). FDA has taken actions to contain the spread of antimicrobial-resistant bacteria and prolong the usefulness of antimicrobial agents, including a strategy for limiting antimicrobial use in food animals to therapeutic uses and agents administered under veterinary supervision (*9,33*). Even more stringent actions are being applied in the European Union (*9,34*). Reservoirs of infection vary by serotype, and resistant infections have been linked to a variety of sources and exposures (*7,17,35–37*). For example, an outbreak of multidrug-resistant (MDR) Typhimurium infections with resistance to ampicillin was linked to consumption of contaminated ground beef (*17,35*). MDR Newport infections with resistance to ceftriaxone were linked to exposure to infected dairy cattle and consumption of contaminated ground beef (*14,36*). Infections with Enteritidis that are nonsusceptible to ciprofloxacin have been associated with international travel (*37*). Recently, MDR strains of other serotypes, including I 4,[5],12:i:- and Dublin, have become an increasing concern; these serotypes have been linked to swine and cattle sources, respectively (*8,38*). NARMS needs to continue to monitor emerging resistance patterns by serotype. The 4 major serotypes that have been driving the incidence of resistant infections should continue to be high priorities in combating resistance.

National incidence estimates of resistant *Salmonella* infections are needed to track progress to support the US President's Executive Order to combat antibiotic-resistant bacteria (*39,40*). Such estimates help define the magnitude of the resistance problem, target prevention efforts, and assess whether control measures are working. Further development of these methods can be used to assess progress from control measures.

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Oral Cholera Vaccine Coverage during an Outbreak and Humanitarian Crisis, Iraq, 2015

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During November–December 2015, as part of the 2015 cholera outbreak response in Iraq, the Iraqi Ministry of Health targeted ≈255,000 displaced persons ≥1 year of age with 2 doses of oral cholera vaccine (OCV). All persons who received vaccines were living in selected refugee camps, internally displaced persons camps, and collective centers. We conducted a multistage cluster survey to obtain OCV coverage estimates in 10 governorates that were targeted during the campaign. In total, 1,226 household and 5,007 individual interviews were conducted. Overall, 2-dose OCV coverage in the targeted camps was 87% (95% CI 85%–89%). Two-dose OCV coverage in the 3 northern governorates (91%; 95% CI 87%–94%) was higher than that in the 7 southern and central governorates (80%; 95% CI 77%–82%). The experience in Iraq demonstrates that OCV campaigns can be successfully implemented as part of a comprehensive response to cholera outbreaks among high-risk populations in conflict settings.

As of 2015, ≈3.2 million internally displaced persons (IDPs) were dispersed throughout Iraq due to increased activity by an armed antigovernment group and subsequent counter-insurgency operations by the Iraq government and coalition forces, and Iraq was hosting >200,000 Syrian refugees due to protracted fighting in Syria between the government and several opposition groups (1). The risk of communicable disease epidemics in Iraq is heightened due to the large numbers of displaced populations residing in camps, informal settlements, or temporary placement sites (collective centers). These sites are usually overcrowded and have inadequate shelter arrangements and limited access to sanitation facilities, safe drinking water, safe food, and basic healthcare services. Such risk factors, coupled with austerity measures and the effect of those measures on health services, have contributed to transmission of cholera in Iraq.

On August 30, 2015, cholera was confirmed in Iraq's southern governorate, Diwaniya, and on September 15, an outbreak was declared by the Iraq Ministry of Health (MoH); activation of the Cholera Control and Command Center followed the outbreak declaration. The outbreak continued to rapidly spread throughout the country, and by October 2015, a total of 1,656 laboratory-confirmed cases of *Vibrio cholerae* 01 Inaba had been reported from 15 of 18 governorates; 1,000 (60%) of these cases were reported in Babylon and Baghdad, which are in southern and central governorates.

Oral cholera vaccines (OCVs) are recommended by the World Health Organization (WHO) as a complementary strategy for comprehensive cholera prevention and control in addition to the primary intervention of safe water, sanitation, and hygiene (WaSH) measures. Three OCVs are currently prequalified by WHO: Dukoral, Shanchol, and Euvichol (2,3). In early 2013, a global OCV stockpile was established with initial support from several donors and endorsed for funding support through Gavi, the Vaccine Alliance (4). The stockpile, which is intended to provide rapid deployment of OCVs in emergency and outbreak situations, is managed by the International Coordinating Group that comprises 4 decision-making partners: the International Federation of Red Cross and Red Crescent Societies; Médecins Sans Frontières; United Nations Children's Fund; and WHO, which also serves as the Secretariat (5,6).

When the 2015 cholera outbreak began in Iraq, the Iraq MoH and implementing partners immediately began planning a vaccination campaign using the bivalent OCV Shanchol (7–9) to complement WaSH and other cholera control measures. The 2-dose OCV campaign targeted ≈255,000 persons ≥1 year of age living in selected refugee camps, IDP camps, and collective centers because of increased vulnerability to cholera due to living conditions. This deployment of ≈510,000 OCV doses in Iraq was the largest to date from the global OCV stockpile for outbreak and humanitarian response. As part of the recommended

monitoring and evaluation activities for these deployments, the MoH requested partners to conduct a vaccination coverage survey to evaluate vaccine uptake, OCV campaign awareness, reasons for vaccine acceptance or nonacceptance, and any adverse events reported after the campaign. We report results of the coverage survey and key lessons learned from the Iraq experience.

Methods

Study Setting

Because of the large numbers of IDPs and the limited supply of OCV, the vaccination campaign in Iraq was limited to IDP camps at full capacity or overcrowded and to all refugee camps and collective centers. The OCV campaign was conducted during October 31–November 5, 2015 (round 1), and December 7–9, 2015 (round 2). Campaign dates were chosen beyond the 2-week minimum interval between OCV doses to accommodate a polio vaccination campaign that was conducted between the 2 OCV campaign rounds. Vaccination teams were trained by WHO and MoH staff and composed of at least 1 vaccinator, recorder, and crowd controller. Experiences from the polio vaccination teams and infrastructure supported the implementation of this campaign during a public health emergency. Vaccination strategy included a combination of fixed sites (i.e., large health centers) and mobile teams for door-to-door vaccine delivery. The vaccine cold chain was maintained, and vaccines were transported using a sufficient number of vaccine carriers and ice packs for a door-to-door strategy. The coverage survey was conducted during December 14–16, 2015, immediately after the second round of the campaign, by WHO, the US Centers for Disease Control and Prevention, and the Iraqi Red Crescent Society.

Study Design

We designed a stratified multistage cluster survey to obtain representative OCV coverage estimates among selected camps in Iraq's governorates that were targeted during the 2015 campaign. The sampling universe was stratified first by governorate and then by refugee camp, IDP camp, or collective center within a governorate. Within each household, all persons in each of 3 designated age groups (1–4, 5–14, and ≥15 years of age) were interviewed.

We performed sample size calculations based on an estimated 2-dose coverage of 75%, an intraclass correlation of 0.2, an average household size of 6, and a nonresponse rate of 5%. Based on these assumptions, we estimated a design effect of 2 due to household clustering. To achieve 8% precision in the group of 1- to 4-year-old children for the northern and southern/central regions, we estimated that ≈120 households per governorate would need to be sampled and allocated the sample equally to each governorate. We expected to yield a coverage estimate with a precision of 4.7% for each governorate.

Within each governorate, we proportionally allocated our sample based on the estimated population size of each refugee camp, IDP camp, or collective center. For logistical reasons, we excluded camps that had a population of <500 persons. A total of 35 camps and collective centers were eligible for sampling, but in the governorates of Anbar and Baghdad Karkh, we selected only the 2 largest camps due to security concerns, logistical challenges, and access issues in the southern and central regions. All eligible camps in the northern region were selected. Overall, we selected 27 refugee camps, IDP camps, and collective centers in 10 governorates for this survey; 3 governorates were in the northern region (Dahuk, Erbil, Sulaymaniya), and 7 were in the southern and central regions (Najaf, Baghdad Karkh, Kerbala, Salah Addin, Anbar, Wasit, Babil).

Within a selected camp, the allocated numbers of households were systematically sampled using a predetermined skip interval, which we calculated as the estimated number of households in the camp divided by the proportionally allocated sample size. Survey teams used a start, selected randomly between the first household at the corner of the camp and the nth household, based on the predetermined sampling interval. Once the interview at the first household was completed, the interviewers moved on to the next household, based on the sampling interval. Selected households that were excluded because of ineligibility (if consent was not given or if no one was present at the household after 3 attempted visits) were counted toward the sample size per camp; that is, selected households were not replaced for nonresponse or refusal reasons. In camps or collective centers where population size was larger than estimated, the survey team continued to sample households using the predetermined skip interval.

In each selected household, all eligible persons were interviewed; for younger children, information was collected from parents or the primary caregiver. If any of the household members were absent during the first visit, teams attempted to revisit the household at least twice at a later time. Respondents were categorized by 3 age groups: 1–4 years, 5–14 years, and ≥15 years to match administrative recording via tally sheets for the campaign and to align with data from previous campaigns in other settings (9–12). All data were collected using the Survey123 application (Esri, Redlands, CA, USA) installed in electronic tablets for real-time data entry and global positioning system (GPS) tracking of survey teams. Electronic data entry and GPS tracking of survey teams were used to remotely monitor the spatial pattern of selected households and data quality during this humanitarian crisis in a complex security environment.

Analytic Methods

We used survey procedures in SAS version 9.3 (SAS Institute, Cary, NC, USA) for data analysis. Data were weighted to ensure that each individual in the sampling frame had an equal probability of selection and to adjust for potential nonresponse bias. We had to account for 3 different stages of weighting: 1) probability of selection by taking the inverse of the sampling rate, 2) household nonresponse rate by calculating the inverse of the governorate-wide household response rate, and 3) individual nonresponse by calculating the inverse of the response rate within a household. The Iraq MoH approved this survey as a program evaluation activity.

Results

Response Rate and Household Characteristics

We selected 1,240 households in the targeted camps and collective centers to survey; 99% (1,226 households; 5,007 persons) participated. The governorate of Dahuk had the lowest household response rate (93%), followed by Erbil and Baghdad Karkh (99% each); the remaining 7 governorates all had a 100% response rate. Among 5,007 individual-level survey respondents in the 10 governorates, 51% were female, 10% were 1–4 years of age, 22% were 5–14 years of age, and 69% were ≥15 years of age (Table 1). The median number of residents per household was 4 (interquartile range 3–5). The governorate of Anbar did not report household-level questions and therefore was excluded from the household-level analysis. Overall, 12% of households reported using an unimproved primary water source, 36% reported using an unimproved secondary water source, and 4% reported having an unimproved sanitation facility (Table 1). Among all households, 22% reported sharing

sanitation facilities with ≥4 other households, and 4% reported not having soap for handwashing.

OCV Coverage

Among the 5,007 respondents from the 10 governorates, 87% reported 2-dose OCV coverage, and 7% reported 1-dose coverage (Table 2). Two-dose coverage was similar among male (86%) and female (88%) respondents and among age groups: 85% among children 1–4 years of age, 89% among children 5–14 years of age, and 87% among persons ≥15 years of age (Table 2). When vaccination coverage was stratified by sex and age group, the lowest 2-dose coverage was among boys 1–4 years of age (83%) and the highest was among girls 5–14 years of age (89%). OCV campaign vaccination cards were available for 79% of persons who reported being fully vaccinated; these cards indicated that 47% had received 2 doses, and 32% had received 1 dose. Among the respondents who reported receiving 2 doses, 27% had only 1 dose recorded on their vaccination cards. Among the respondents who reported receiving OCV, 90% reported receiving the vaccine at their residential structure, 6% at a health facility, 3% at school, and 1% at a market.

Two-dose OCV coverage in the northern governorates (91%) was higher than that in the southern and central governorates (80%), and 1-dose coverage in the northern governorates (6%) was lower than that in the southern and central governorates (10%) (Table 3). Among the northern governorates, 2-dose OCV vaccination coverage ranged from 90% in Dahuk to 93% in Erbil and Sulaymaniya; however, greater variability was seen between the southern and central governorates, where 2-dose coverage ranged from 21% in Babil to 98% in Anbar (Figure; Table 3).

Table 1. Individual and household characteristics for oral cholera vaccination survey respondents in refugee camps, internally displaced persons camps, and collective centers targeted for vaccination, Iraq, 2015

Characteristic	No.	Weighted estimate, % (95% CI)
Individual level, n = 5,007		
Sex		
Male	2,487	49 (47–51)
Female	2,500	51 (49–53)
Age, years		
1–4	650	10 (9–11)
5–14	1,235	21 (19–24)
≥15	3,117	69 (66–71)
Household level, n = 1,226		
Water sources*		
Unimproved primary water source	458	12 (10–14)
Unimproved secondary water source	666	36 (31–42)
Sanitation facilities		
Unimproved†	85	4 (2–6)
Shared with ≥4 other households	366	22 (18–26)
Lacked soap for handwashing	86	4 (2–7)

*Unimproved water sources include unprotected well; unprotected spring water; river, stream, lake, irrigation, or canal water; bottled water; water truck; and water vendor.
†Unimproved sanitation sources include pit latrines without cement slab; bucket toilet; hanging toilet or hanging latrine; and canal, open, bush, and field defecation.

Table 2. Estimated oral cholera vaccine coverage, by vaccinee age, among persons in refugee camps, internally displaced persons camps, and collective centers targeted for vaccination, Iraq, 2015*

Vaccination group, no. doses	All vaccinees		Male vaccinees		Female vaccinees	
	No. vaccinated	Weighted estimate, % (95% CI)	No. vaccinated	Weighted estimate, % (95% CI)	No. vaccinated	Weighted estimate, % (95% CI)
Overall						
2 doses	3,523	87 (85–89)	1,735	86 (83–89)	1,777	88 (85–90)
1 dose	745	7 (6–9)	363	8 (6–11)	380	7 (5–9)
1–4 y of age						
2 doses	407	85 (81–88)	213	83 (77–88)	191	85 (81–89)
1 dose	89	5 (3–7)	50	6 (4–9)	38	4 (2–7)
5–14 y of age						
2 doses	931	89 (85–92)	487	88 (83–92)	442	89 (84–93)
1 dose	171	7 (5–11)	88	9 (5–14)	83	6 (3–11)
>15 y of age						
2 doses	2,183	87 (84–90)	1,034	86 (83–89)	1,143	88 (84–91)
1 dose	483	8 (6–10)	224	8 (6–12)	258	8 (6–10)
*Zero doses and incomplete data are not shown.						

Reasons for Not Being Vaccinated

The 2 most common reasons for not receiving vaccine during the first or second OCV vaccination round were being absent during the campaign (first round 35%, second round 39%) and teams not visiting the respondents' residential structures (first round 30%, second round 36%) (Table 4). Other reasons for not being vaccinated during the first round were unavailability of vaccine (11%), lack of faith in the vaccine (4%), and being sick during the campaign (3%). The reasons for not being vaccinated during the second round were similar: unavailability of vaccine (2%), sick during the campaign (9%), and absence of the decision-maker at home at the time of the vaccinators' visit (5%). In the 3 governorates with the lowest coverage (Baghdad Karkh, Kerbala, and Babil), 46% of respondents stated vaccination teams did not visit their residential structure, and 22% stated they were absent during the campaign.

Adverse Events following Vaccination

Adverse events within 14 days of receiving either the first or second dose of OCV were reported by 21% of respondents. The most commonly reported adverse events were minor, primarily abdominal pain (9%), fever (5%), vomiting (3%), and diarrhea (2%). Only 1 person reported rash following vaccination. No severe adverse events were reported.

OCV Campaign–Associated Messaging

Most vaccine recipients reported having received information about the OCV campaign through television (19%),

Table 3. Estimated oral cholera vaccine coverage, by region and governorate, among refugee camps, internally displaced persons camps, and collective centers targeted for vaccination, Iraq, 2015*

Region, governorates	Frequency	No. doses	Weighted estimate, % (95% CI)
Northern, N = 154,396	1,340	2	91 (87–94)
		1	6 (4–9)
Dahuk, n = 98,327	351	2	90 (84–94)
		1	7 (4–12)
Erbil, n = 34,675	476	2	93 (90–96)
		1	4 (2–7)
Sulaymaniya, n = 21,394	513	2	93 (89–96)
		1	6 (4–10)
Southern and central, N = 78,046	3,667	2	80 (77–82)
		1	10 (8–12)
Anbar, n = 41,070	539	2	98 (93–99)
		1	2 (1–6)
Wasit, n = 2,300	523	2	91 (85–95)
		1	6 (3–12)
Salah Addin, n = 6,000	528	2	81 (74–86)
		1	16 (11–22)
Najaf, n = 14,000	572	2	74 (67–81)
		1	10 (6–16)
Baghdad Karkh, n = 6,055	471	2	37 (29–44)
		1	28 (20–36)
Kerbala, n = 6,017	529	2	30 (22–38)
		1	35 (28–42)
Babil, n = 2,604	505	2	21 (15–27)
		1	31 (24–39)
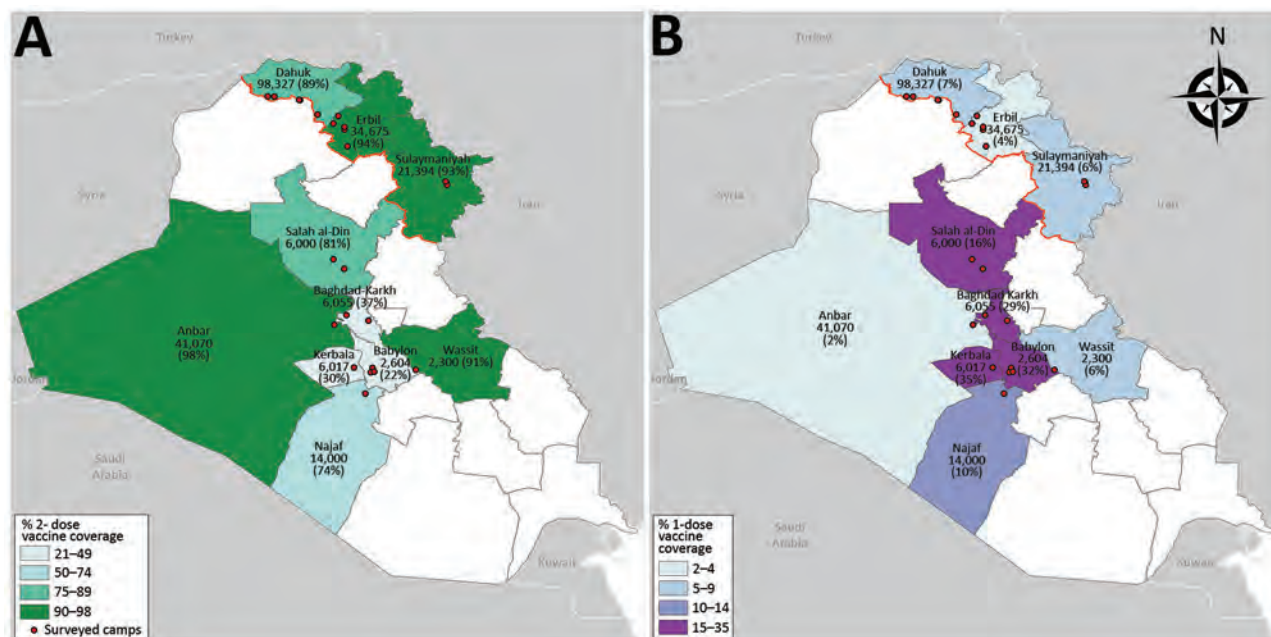
Figure. Location of camps and collective centers where persons were surveyed and vaccinated during a cholera outbreak and humanitarian crisis, Iraq, 2015. Numbers indicate targeted population; estimated 2-dose (A) and 1-dose (B) oral cholera vaccine coverages are shown in parentheses. White indicates governorates where surveys and vaccination were not conducted; black outlining indicates governorates; red line indicates border between the northern region and the southern and central regions of Iraq.

neighbors or friends (13%), radio (12%), health staff (7%), or posters or banners posted before or during the campaign (7%) (Table 5). In addition, 55% of respondents reported receiving other cholera prevention messages, such as handwashing (33%), thoroughly cooking food (14%), boiling water (15%), and washing vegetables and fruits (13%).

Discussion

We describe the context of an OCV campaign in Iraq that was conducted during an acute humanitarian emergency and cholera outbreak and results from an OCV coverage survey in the vaccine-targeted areas. The primary objective of vaccination in an acute humanitarian emergency is to rapidly reduce disease risk to protect a population during periods of extreme vulnerability (13). The risk for cholera epidemics among displaced populations during a humanitarian crisis can be elevated, especially due to massive population movements and overcrowding. Limited access to clean water, adequate sanitation, and shelter are also risk factors associated with cholera epidemics. The World Health Assembly (WHA) and WHO have recommended OCV use in the context of a humanitarian emergency to reduce morbidity and mortality from cholera, where indicated. In 2011, because of worldwide increases in cholera incidence, the WHA adopted resolution WHA 64.15, which called for implementation of an integrated and comprehensive approach to cholera control, including rapid provision of safe water, adequate case management at health facilities, strengthened case detection through

early-warning surveillance and laboratory confirmation, and cholera vaccination (14).

The use of the global OCV stockpile was a positive experience during the humanitarian crisis and outbreak response in Iraq. The rapidity of the OCV response activity is highlighted by the short time that passed between cholera detection and implementation of the OCV campaign. During ≈1 month, cholera was detected, a request for OCV was submitted to the International Coordinating Group, the decision to provide OCV was made, vaccine was deployed and arrived in country, and the first round of the OCV campaign was planned and implemented. Excellent collaboration and coordination was seen among partners, not only for campaign implementation but also for evaluation activities.

Administrative coverage data have several limitations for immunization programs in general, usually because of issues with population denominator estimates (15,16). Coverage surveys can help verify administrative data and provide helpful insights into the reasons for vaccine acceptance or nonacceptance and the effectiveness of social mobilization activities. Two-dose coverage among targeted camps in Iraq was high (87%) compared with OCV campaigns conducted in other conflict settings (10,17,18) and with campaigns conducted in more stable, conflict-free settings (19–22).

Coverage among the OCV-targeted camps in Iraq's northern governorates was high; national authorities thought this high coverage reflected the commitment and dedication of country staff and partners and the use of

Table 4. Five most common reasons for not receiving oral cholera vaccine among persons in refugee camps, internally displaced persons camps, and collective centers targeted for vaccination, Iraq, 2015

Reasons for non-vaccination	Frequency	Weighted estimate, % (95% CI)
First dose		
Was absent during campaign	175	35 (27–43)
Teams did not visit my house	284	30 (23–38)
Vaccines not available	99	11 (7–18)
No faith in vaccine	22	4 (2–9)
Was sick	24	3 (2–5)
Second dose		
Was absent during campaign	148	39 (28–51)
Teams did not visit my house	419	36 (26–46)
Vaccines not available	38	2 (1–3)
Was sick	22	9 (4–18)
Decision-maker not at home	2	5 (2–18)

adaptive vaccination strategies during campaign implementation. Certain governorates were able to attain high coverage (e.g., 98% in Anbar) due to the strongly captive nature of the closed camps and collection centers, which restricted movement of the populations in or out. Compared with the northern governorates, the southern and central governorates had lower 2-dose coverage, especially in Baghdad Karkh, Kerbala, and Babil. Civil strife, heavy rains, and challenges in program management might have played a role. Although 2 OCV doses are recommended, a recent single-dose trial in Bangladesh showed promising results (23), and a modeling study showed that single-dose coverage may be especially useful for interrupting disease transmission in outbreak situations that present challenges to population access (24). Furthermore, vaccine thermostability data that support considerations for the use of controlled temperature chains for OCV may help eliminate stringent cold chain requirements, thereby simplifying vaccine delivery (25).

OCV campaign vaccination cards did not accurately portray 2-dose vaccination status for all respondents, even though the coverage survey was implemented immediately after the campaign. This finding suggests a need to remind vaccine recipients to bring back vaccination cards for the second round and to improve vaccination card recording training for vaccination teams. Previous OCV campaigns in Haiti that reported higher card-documented 2-dose coverage (51%–70%) emphasized the value of keeping vaccination cards for receiving the second vaccine dose (21). Where feasible, the use of serologic studies may also be helpful in validating reported coverage.

A unique feature of the coverage survey in this complex security environment during a humanitarian crisis was the use of electronic tablets for data collection and GPS tracking of survey teams, which enabled remote review of data quality, spatial tracking of teams, and immediate feedback for corrective actions. In addition to using fixed posts at health facilities, the Iraqi MoH adopted a door-to-

Table 5. OCV campaign information sources and cholera-associated messages reported seen or heard by survey respondents in refugee camps, internally displaced persons camps, and collective centers targeted for vaccination, Iraq, 2015*

Information source and type	Frequency	Weighted estimate, % (95% CI)
Source for information about campaign		
Television	1,046	19 (15–24)
Neighbors or friends	599	13 (10–16)
Radio	315	12 (9–17)
MoH staff or vaccinators	579	7 (5–10)
Poster or banner	521	7 (5–9)
Schools	88	3 (2–6)
Community mobilizer	62	3 (2–6)
SMS text messages	57	3 (2–5)
Mosque announcements	62	1 (0.3–3)
The Internet	40	<1 (<0.1–0.2)
Received nonvaccine information during campaign	2,410	55 (49–61)
Nonvaccine information heard or seen during campaign		
Wash hands with soap and water	2,023	33 (28–39)
Cook food thoroughly	930	14 (11–18)
Wash vegetables and fruits	1,413	13 (10–16)
Boil water	794	15 (12–18)
Clean cooking utensils and vessels	635	6 (4–8)
Dispose of human waste properly	519	5 (4–7)
Drink and use water treated with chlorine products	334	4 (3–7)
Go to health center if I have cholera	312	4 (3–6)
Take ORS if I have cholera	163	3 (1–6)
Do not know or do not remember	35	<1 (0.1–0.4)

*MoH, Ministry of Health; OCV, oral cholera vaccine; ORS, oral rehydration solution; SMS, short message service.

door strategy for both rounds of the OCV campaigns. This strategy was based on the MoH's familiarity with the use of this method in previous polio and measles campaigns. The door-to-door strategy explains the finding that most respondents (90%) were vaccinated at residential structures. Furthermore, strategies to include extended evening hours and additional sites at camp entrances and nearby markets helped capture male persons ≥15 years of age, a group that has experienced the lowest coverage rates in other campaign settings (20,21). However, given that one of the most frequent reasons for not being vaccinated was that vaccination teams did not visit residential structures, clearer messaging may be needed for the dual strategy of providing vaccine door-to-door and at fixed posts.

Less than 10% of respondents reported receiving information about the campaign from MoH staff or community mobilizers, which may have been because of the complex management structure of numerous partners and nongovernmental organizations within the targeted camps. It was encouraging to note that at least half of all survey participants reported receiving educational information on cholera other than OCV (e.g., WaSH measures) during the campaign. Advance communications to health clinics and nongovernmental organizations to strengthen social mobilization and messaging activities, especially WaSH and vaccine integration, will be helpful for comprehensive cholera prevention and control in the future.

The camps and collective centers selected for the OCV campaigns were targeted because they were overcrowded or at full capacity, and it was believed that such facilities were most at risk for cholera because of high population density. However, our survey results showed that >85% of respondents had access to improved primary water sources, improved sanitation facilities, and soap. Future campaigns may also consider inclusion of communities outside of camps, which may have worse WaSH conditions than their in-camp counterparts. Many communities were not targeted in this campaign because the supply of vaccine at the global level was limited. Efforts to increase the global OCV supply available for outbreaks and humanitarian emergencies will help expand access to more settings in need.

Our survey had limitations. First, survey enumerators from southern and central governorates were unable to attend a centralized training and therefore relied on training by local supervisors, which may have resulted in varied training. Second, the survey results by region and governorate should only represent the selected camps and collective centers surveyed. However, access to the camps and centers was severely limited during the campaign and monitoring activities because of safety issues; access was especially difficult in the 3 governorates with the lowest coverage, where vaccination teams had difficulty visiting residential structures. Hence, it is possible that the camps

that were excluded from the survey due to access issues were also the ones that were missed during the campaign. We were not able to visit smaller camps in southern and central governorates because they were in areas of heavy conflict, and therefore the assessment is not representative of these areas.

The OCVs Shanchol and Euvichol are relatively new additions to cholera and acute humanitarian response activities, although there have been some prior examples of their use in emergency settings (18,20,26). Given the current vaccine supply limitations, monitoring and evaluation activities form an integral component of OCV stockpile use. These activities provide information to help determine the most appropriate use of vaccine; the factors associated with vaccine acceptance; and, where possible, the effect of vaccine use on disease transmission. Although it was determined that extensive evaluations would not be possible in Iraq, partners did agree that a coverage survey was feasible and would provide insights into campaign implementation, strategies, and acceptability and key lessons learned in this unique setting. Because an oral polio vaccine campaign was scheduled around the same time as the OCV campaign, the 2 OCV rounds were scheduled about a month apart. No data are currently available regarding co-administration of oral polio vaccine with OCV; such data are critically needed to optimize vaccine delivery in resource-limited settings without compromising the effectiveness of either vaccine. Nevertheless the use of polio eradication assets and activities to support public health emergencies contributed to the success in implementing the OCV campaign in Iraq. This experience of optimizing polio eradication assets, infrastructure, and experience in this survey was unique and proves the principle of the legacy of polio eradication efforts in action (27).

Vaccination is one of the most basic and critical health interventions for protecting vulnerable populations during emergencies. The Iraq experience has shown that OCV campaigns can be part of a comprehensive response to cholera outbreaks among populations at high-risk in conflict settings. OCV use in humanitarian emergencies should complement the foundational public health interventions (i.e., appropriate case management, enhanced environmental control, improved WaSH measures, and social mobilization) of all cholera prevention and control programs (14).

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Dr. Lam was a medical epidemiologist in the Emergency Response and Recovery Branch, Division of Global Health Protection, Center for Global Health, Centers for Disease Control and Prevention, while this research was being conducted. His work focuses primarily on vaccine-preventable diseases among displaced populations during humanitarian emergencies.

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Modeling Tool for Decision Support during Early Days of an Anthrax Event

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Health officials lack field-implementable tools for forecasting the effects that a large-scale release of *Bacillus anthracis* spores would have on public health and hospitals. We created a modeling tool (combining inhalational anthrax caseload projections based on initial case reports, effects of variable postexposure prophylaxis campaigns, and healthcare facility surge capacity requirements) to project hospitalizations and casualties from a newly detected inhalation anthrax event, and we examined the consequences of intervention choices. With only 3 days of case counts, the model can predict final attack sizes for simulated Sverdlovsk-like events (1979 USSR) with sufficient accuracy for decision making and confirms the value of early postexposure prophylaxis initiation. According to a baseline scenario, hospital treatment volume peaks 15 days after exposure, deaths peak earlier (day 5), and recovery peaks later (day 23). This tool gives public health, hospital, and emergency planners scenario-specific information for developing quantitative response plans for this threat.

Population exposure to aerosolized *Bacillus anthracis* spores is one of the most potentially catastrophic public health emergencies (*1*). The 2001 US anthrax attack, in which inhalation anthrax (IA) affected 11 persons and killed 5, led to multiple mass antimicrobial prophylaxis campaigns and considerable healthcare activity (*2*). Data in the first few days of such an event may be limited, leading to uncertainty regarding the scale of the event and difficulty making response decisions.

Public health officials lack widely available tools for rapidly estimating the number of cases, projecting medical surge, and evaluating response options during an anthrax event. Several efforts have evaluated response options in predefined scenarios, which are useful for planning but not during a response (*3–6*). Two other models have attempted to predict the number and timing of IA cases after exposure to aerosolized *B. anthracis* spores; 1 evaluated response options (*7,8*). However, neither model estimates the surge of patients in the healthcare system,

and both models have constraints that limit their practical utility. Walden and Kaplan built a model that presumes equal probability of various event sizes and requires at least 5 days of case data before robust estimates of final attack sizes can be calculated (*8*). This timing may be insufficient given the US Cities Readiness Initiative (CRI) guideline that postexposure prophylaxis (PEP) dispensing be completed within 48 hours of event detection (*9*). The back-calculation techniques of Egan et al. permit estimation of the final outbreak size after a certain number of observed cases under different PEP assumptions (*7,10*). Although these models can be reconciled with the CRI timeline, they were not designed for direct use by public health practitioners (use requires the R coding language and understanding of maximum-likelihood functions), and the earlier work assumes 90% PEP uptake by the infected population, which is an overestimation ($\geq$25%) of the probable public response (*11*).

An alternative method for predicting the scale of IA events is plume modeling, which calculates the number of exposed persons by estimating the geographic spread of dispersed *B. anthracis* spores. Plume models require knowledge (or estimates) of the number of spores released, release timing and location, population densities, meteorologic data (e.g. wind speed and direction), and inhaled spore volume. It is unclear whether plume modeling is sufficiently timely and robust to guide local response decisions.

We therefore developed a modeling tool, called Anthrax Assist, to provide public health officials with rapid projections of IA cases and response decision support during an aerosolized anthrax event. This tool can assist with responding to an anthrax event (or designing and conducting locally tailored training exercises) by providing critical information in the first few days of response.

Methods

Tool Overview

We used Excel 2010 (Microsoft Corporation, Redmond, WA, USA) to construct Anthrax Assist (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/23/1/15-1787-Techapp1.pdf). Anthrax Assist is composed of

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Table 1. Anthrax Assist models and associated inputs, outputs, and public health decisions supported*

Model	Inputs	Outputs	Decision informed
Epidemic Curve	1) Case counts by illness-onset date 2) Incubation period distribution	1) Cumulative caseload 2) Unmitigated epidemic curve	How the event unfolds: 1) Size of event 2) How quickly people become ill
PEP Impact	1) Epidemic curve (output from Epidemic Curve model) 2) Dispensing plan 3) Effectiveness 4) Population needing prophylaxis	1) Cases prevented by PEP 2) PEP-mitigated epidemic curve	1) Initiate a PEP campaign and when to begin 2) How much PEP to dispense 3) Dispensing resource requirements
Healthcare Impact	1) Unmitigated epidemic curve (output from Epidemic Curve model) or PEP-mitigated epidemic curve (output from PEP Impact model) 2) Disease progression 3) Treatment-seeking behavior 4) Treatment effectiveness and availability	1) Hospital demand curves: a) ED surge b) treatment load 2) Deaths curve 3) Recovered curve	1) Treatment guidance: a) messaging to public b) standards of care 2) Set treatment priorities 3) Mobilize medical care resources
*ED, emergency department; PEP, postexposure prophylaxis.			

3 linked models (Table 1). The Epidemic-Curve model combines daily case counts with incubation distributions to project the future number and timing of symptomatic IA cases in a nonvaccinated population. The PEP Impact model estimates the potential decrease in the projected trajectory of future cases (output from the Epidemic-Curve model) resulting from a PEP dispensing campaign. The Healthcare Impact model uses the projected unmitigated or PEP-mitigated incidence curves to project the size and timing of peak healthcare utilization and associated patient outcomes. Users can readily change a number of input values to reflect a desired attack scenario or response strategy (Table 2). To illustrate the models, we developed an attack scenario and used it to evaluate estimates resulting from various outbreak detection scenarios (using 1, 2, or 3 days of initial case count data) and PEP response strategies (Table 3).

Calculations

Epidemic-Curve Model

We base our IA incubation distribution on the Wilkening model, which plots the probability of becoming symptomatic over a 60-day period for a given infectious dose of *B. anthracis* spores (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/23/1/15-1787-Techapp2.pdf) (*13*). We combine this incubation probability distribution with the number of detected IA cases at a given time to calculate the total projected number of ill persons (final case count [FCC]) by using the following equation:

FCC = no. cases detected by day *t* / proportion of infected persons expected to become symptomatic by day *t*

where *t* is the number of days from the date of the first symptomatic case to the time of analysis. The numerator is obtained through public health disease surveillance, and the denominator is obtained from the incubation probability distribution. We then generate an epidemic curve by distributing the FCC over each day of the outbreak according to the incubation probability distribution.

We assume a single, localized release that causes near-simultaneous population exposure. Because public health authorities will probably not know the average inhaled spore dose among affected persons, we designed the model to calculate a range of plausible outbreak sizes from a range of spores inhaled per person. To illustrate the model, we used a median value of 360 spores/person (range 1–8,000), resulting in a median incubation period of 6.9 days (range 10.3–5.0) (Table 2; online Technical Appendix 2).

PEP Impact Model

The PEP Impact model uses median projected daily case counts (output from the Epidemic Curve model) to estimate the potential effects of a PEP campaign. This effect is calculated as the product of the number of persons who become symptomatic on any given day *t*; the effectiveness of PEP on day *t* (which is a product of antimicrobial efficacy and adherence); and the probability that an infected, asymptomatic person receives antimicrobial prophylaxis on or before day *t*. We calculate the probability that a person receives PEP on day *t* by multiplying the PEP uptake (proportion of persons seeking antimicrobial drugs) by the daily antimicrobial dispensing throughput and then dividing by the population targeted for PEP (Table 2). The FCC with a PEP campaign is the sum of detected cases and daily PEP-mitigated case count projections. We express PEP effect as both a difference measure (cases averted) and as a proportion (cases averted divided by the unmitigated FCC). We assume that symptomatic persons seeking PEP are referred for medical treatment and do not receive PEP (*21*). We further assume that all of the population suspected to be exposed would be targeted for PEP because there is no definitive PEP triage process for IA beyond exposure risk (Table 2).

In accordance with US CRI guidelines, we assume that PEP dispensing is completed in 2 (range 1–2) days after the

Table 2. Inputs and parameter values for all Anthrax Assist models*

Parameter	Baseline value	Range†	User adjustable‡	Reference
Epidemic-Curve model				
Case counts for days 1, 2, 3§	20, 10, 70	1–4 days of data	Yes	(12)
Median inhaled spore count, no.¶	360	1–8,000	Yes	(13,14)
Median incubation, d ± SD	6.9 ± 1.8	10.3–5.0 ± 2.2–1.6	Yes	(13)
Population size of the impacted jurisdiction, no.	500,000		Yes	Assumed
PEP Impact model				
Size of population to receive prophylaxis	500,000		Yes#	Assumed
PEP throughput at full capacity, daily	250,000		Yes	Assumed**
Delay to PEP campaign start, d††	2	1–2	Yes	(9)
Ramp-up period until PEP campaign throughput reaches full capacity, d	0		Yes	Assumed**
PEP campaign duration at full throughput capacity, d	2	1–4	Yes	Assumed**
PEP uptake, %‡‡	65	40–90	Yes	(11)
Antibiotic efficacy, %	90		Yes	(15–17)
Adherence to PEP regimen at event day 60, %	40	25–80	Yes	(18)
Time until antimicrobials are protective, d	1		No	(15–17)
Healthcare Impact model				
Public health messaging starts, d of event§§	2		Yes	Assumed
Proportion seeking care relative to public health message timing, by disease state				(2)
During prodromal stage, %	40 before; 80 after		Yes	
During fulminant stage, %	95 before; 95 after		Yes	
Daily transition fraction from prodromal to fulminant illness, by outcome				(19)
Eventually recover, %	20		No	
Eventually die, %	50		No	
Maximal length of prodromal illness, by outcome				(19)
Eventually recover, d	5		No	
Eventually die, d	2		No	
Length of fulminant illness among untreated, d	0		No	Assumed
Length of fulminant illness among treated who die, d¶¶	1		No	(19)
Median ± SD of normal distribution of length of treatment among those who recover, d¶¶	18 ± 3		No	(19)
Recover with treatment, by stage of illness when treatment initiated, %##				Assumed
Prodromal, %	80		Yes	
Fulminant, %	20		Yes	
Prodromal who recover after fulminant illness, %***	50		Yes	(2)

*Amerithrax, anthrax attacks in the United States during 2001; CRI, Cities Readiness Initiative; PEP, postexposure prophylaxis.
†Values provided were used in our evaluation of the influence of the number of days of case data on Epi-Curve projections (case counts parameter), to create high and low final case count estimates (median inhaled spore count and median incubation parameters), and to evaluate various PEP scenarios (all PEP-Impact model parameters) (Table 3). Range values used in the univariate sensitivity analysis of PEP parameters differ (Table 4).
‡Anthrax Assist user can readily change the input value.
§Case counts from the first 3 days of the 1979 Sverdlovsk (USSR) anthrax event epidemic curve inflated by a factor of 10. When 4 days of case counts are used (Table 4), the fourth day of counts is 40.
¶360 spores is a dosage estimated to have occurred during the 1979 Sverdlovsk (USSR) anthrax event (13). One spore represents the minimum possible infectious dose, and 8,000 is a plausible high dose (14).
#Cannot exceed the value of the Epidemic-Curve model "Population size of the impacted jurisdiction" parameter. When less, proportionately fewer infected persons are eligible for PEP protection.
**Value chosen so that PEP dispensing is in accordance with US CRI guidelines and is completed within 2 days after the decision to initiate PEP (9).
††Determined by counting days from date of earliest illness onset (i.e., event day 1).
‡‡Percentage of population targeted to receive PEP who actually obtain and start PEP.
§§Public health messaging only influences treatment-seeking behavior in the absence of a PEP campaign or prior to campaign initiation.
¶¶Same length assumed for patients initiating treatment in the fulminant versus prodromal stage of illness.
##Assumes an improved treatment effectiveness compared with the 2001 US anthrax attacks as a result of clinical experience gained in treating inhalation anthrax cases in the United States since and the recent availability of intravenous antitoxin; in addition to the full complement of medical resources used during the 2001 attacks: an acute-care bed and the associated medical care staff (including respiratory therapists), pleural fluid drainage, mechanical respiratory ventilation, and intravenous antimicrobial drugs. In the United States in 2001, 6 (67%) of 9 persons who sought treatment during the prodromal stage of illness recovered (however, 2 who died did not receive antimicrobial drugs with activity against *Bacillus anthracis* until they exhibited fulminant illness), and both persons who sought care during fulminant illness died (2,20).
***On the basis of 6 survivors during the 2001 Amerithrax attacks who sought treatment during the prodromal illness stage: cases 2, 3, 4, 7, 8, 9 (2,20). Progression to fulminant illness was defined as severe symptomatic disease characterized by respiratory distress requiring pleural effusion drainage, or mechanical ventilation, marked cyanosis, shock, or meningoencephalitis.

decision to initiate PEP (9). Following SteelFisher et al., we also assume that of the population targeted to receive PEP, 65% (range 40%–90%) actually start taking PEP (11). Everyone starting PEP is assumed to fully adhere to the regimen on the first day. After that, adherence decreases linearly to 40% (range 25%–80%) at the conclusion of the event (online Technical Appendix 2) (18). Last, we assumed 90% (range 10%–90%) antimicrobial drug efficacy and that this level of protection is achieved 1 day after initiation of the regimen (15,16) (Table 2).

Scenario (description)	Logistics components	Drug-use components
Scenario 1 (no PEP)	Not applicable	Not applicable
Scenario 2 (ideal)	1-day delay,† 1-day campaign	90% uptake,‡ 80% adherence§
Scenario 3 (practical: logistics follow CRI guidance, and utilization data based on the Amerithrax attacks)	2-day delay,† 2-day campaign	65% uptake,‡ 40% adherence§
Scenario 4 (constrained)	2-day delay,† 4-day campaign	40% uptake,‡ 25% adherence§

*Amerithrax, anthrax attacks in the United States during 2001; CRI, Cities Readiness Initiative; PEP, postexposure prophylaxis.
†Delay days are determined by counting the days from the date of earliest illness onset (i.e., event day 1). Public health messaging also begins on the same day as the campaign. The delay dictates the number of days of case data potentially available as input. Two days of case data are available as input in Scenario 2, and 3 days are available as input in Scenarios 3 and 4.
‡Proportion of the population targeted by public health officials to receive PEP who actually obtain and start PEP (11).
§Proportion fully adhering to the PEP regimen on event day 60 (18).

Healthcare Impact Model

To calculate the demand for medical care, we used a compartmental model (based on one reported by Zaric et al.) and used the review of IA cases by Holty et al. to select the rates of patients' transitions through illness stages (6,19) (Figure 1; online Technical Appendix 2). This model is used to calculate daily patients initiating treatment, peak daily treatment caseload (i.e., census of hospitalized patients receiving treatment for IA), and the day of peak treatment caseload.

In this model, medical intervention is required for recovery from symptomatic IA, and only patients with fulminant disease can die. We define treatment effectiveness as the percentage of patients who recover after receiving some type of medical intervention and pattern it after the 2001 US IA events. As such, treatment is 4 times more effective when started in the prodromal (80%), rather than fulminant (20%), stage of illness (Table 2). However, the probability that a patient in the fulminant stage seeks healthcare (95%) is roughly twice that for someone in the prodromal stage (40%) (22). In addition, we varied the likelihood that any patient seeks healthcare by the timing of public health messaging regarding screening and treatment recommendations. We assume that the proportion of persons in the prodromal stage who seek care would double as a result of widespread media attention (80% vs. 40%) (2) (Table 2). Last, we assume treatment effectiveness values based on full availability of medical countermeasures and resources at the time of treatment and no delay in access to care once sought (Table 2).

During the 2001 US IA event, treatment duration was highly associated with treatment outcomes (22). Thus, for those who recover, we assume a normal distribution with a
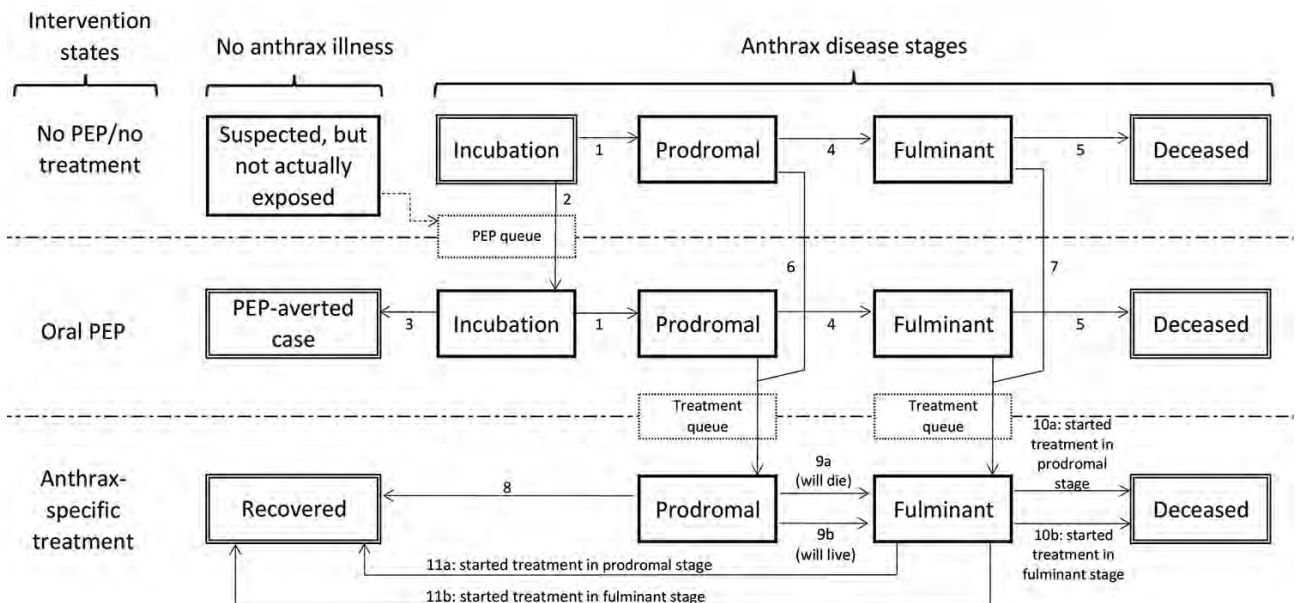
Figure 1. Anthrax Assist model disease stages, intervention states, and transitions. Persons begin in the top Incubation state and may transition via the numbered arrows from one state to another until they eventually reach an outcome state (doubled-walled boxes). All persons with untreated infection will progress to deceased. Recovery is possible only through effective oral PEP (averted case) or anthrax-specific treatment (recovered). Transitions are governed by the 3 Anthrax Assist models as follows: Epidemic-Curve model, transition 1; PEP Impact model, transitions 2 and 3; Healthcare Impact model, transitions 4–11. Suspected, but Not Actually Exposed cases are shown here because of their role in diluting the incubating population seeking PEP (dashed transition arrow). PEP and Treatment queues (dashed outline boxes) are depicted to reflect the necessary interactions persons must have with the public health and healthcare systems to transition between treatment states. PEP, postexposure prophylaxis.

mean of 18 (SD 3) treatment days from the date of transition to the fulminant stage of illness or from the sixth day of prodromal illness for patients whose illness does not progress to the fulminant stage. For patients who eventually recover from fulminant illness (in treated and yet-nontreated populations), we assume a 20% transition each day so that all have transitioned to the fulminant stage after 5 days in the prodromal stage. Among those who eventually die, half transition to the fulminant stage on the first day of symptoms and the other half on the next day. When treatment is not sought, we assume that death occurs on the same day as the transition to fulminant illness.

Scenarios

To illustrate use of the models, we created an attack case series scenario patterned after the 1979 Sverdlovsk, USSR, event, in which at least 70 people died of IA after accidental aerosol release of *B. anthracis* spores from a bioweapons facility (Table 2) (*12*). We created this Sverdlovsk-like case series by multiplying each day's case count from the Sverdlovsk event by 10, resulting in a 40-day, 700-patient case series (online Technical Appendix 2).

To illustrate the accuracy of the Anthrax Assist FCC projections under realistic conditions of limited reported case data in the first days of an event, we first ran the Epidemic-Curve portion of Anthrax Assist by using only the first 3 days of case data as input (20, 10, and 70 cases, respectively), then by using 2 days of case data, and then only the first day's cases. To examine the effect of the number of days of case data on the accuracy of our FCC projection, we also incrementally added a day of case data, beyond the first 3 days, until the projection was within 10% of the true FCC.

Next, to evaluate prophylaxis response options, we developed 4 PEP scenarios by varying components of the PEP campaign implementation (logistics) and the public response to the campaign (utilization) (Table 3). Scenario 1 (no PEP) is an event without a PEP campaign. Scenario 2 (ideal) is an event wherein early detection of the event (e.g., through biosensors) and positive public perception results in a 1-day campaign starting 1 day after detection, 90% uptake, and 80% adherence at the event's conclusion. Scenario 3 (practical) is an event in which PEP dispensing logistics follow current public health guidance and PEP utilization is based on data from the 2001 US IA event, resulting in a 2-day campaign starting 2 days after detection, 65% uptake, and 40% adherence at the event conclusion. Scenario 4 (constrained) is an event in which logistics hurdles (e.g. staffing shortages, traffic congestion [*3,23*]) and poor public perception impede rapid PEP coverage, resulting in a 4-day campaign starting 2 days after detection, 40% uptake, and 25% adherence at event conclusion. Hereafter, the baseline scenario comprises

PEP scenario 3 and the Healthcare Impact model values in Table 2.

Sensitivity Analyses

We conducted 2 sensitivity analyses. We first evaluated the influence of individual PEP-related parameters on outputs from the models as follows: prophylaxis campaign duration of 1–6 days at full throughput capacity, delay of 3–6 days until PEP campaign starts, a range of 15%–90% for PEP uptake, a range of 10%–90% for antimicrobial efficacy, and a range of 15%–90% for adherence to the regimen at the conclusion of the event. These ranges encompass reported values (*3,4,11,18,24*).

In our second sensitivity analysis, we altered the Epidemic-Curve model inputs used in the baseline attack scenario to illustrate potential data limitations and surveillance inaccuracies that might occur during an actual event. Doing so involved comparing estimates using the full complement of the initial 3 days of case data with a scenario in which 60% of cases are reported. This level of underreporting represents the plausible difficulties often encountered when initially collecting outbreak data.

Results

For the scenario that uses the first 3 days of case data, no PEP campaign, and early public messaging, the tool projects a median 60-day FCC of 1,164 (66% higher than actual FCC, plausible range 675–1,612; Figure 2, panel A), 35% event mortality (408 deaths), and a peak hospital caseload of 692 patients on day 15 (Table 5). Running the same scenario with only 2 days of case data (i.e., 20 followed by 10 cases) yields a median FCC estimate of 1,441 (106% higher than actual FCC, range 963–1,464) (Figure 2, panel B), 35% event mortality (506 deaths), and a peak hospital caseload of 856 on day 14. Using only the first day of case data (20 cases) yields a median FCC estimate of 27,555 (3,800% greater than actual FCC, range 10,993–36,603), 35% event mortality (1,688 deaths), and a peak hospital caseload of 2,871 on day 14. In contrast, when 4 days of case data are used, the FCC projection (median 750, range 435–1,175) falls within 10% of actual FCC.

Irrespective of the number of days of case data available, the estimated effects of PEP ranged from ≈25% cases averted in scenario 4 (constrained) to 79% in scenario 2 (ideal) (*54*). These PEP effects are equally reflected in the percentage of averted deaths (Table 5). Even with rapid event detection, an aggressive PEP campaign, and unlimited treatment resources one-third of deaths expected under the unmitigated scenario will still occur (calculated as the ratio of deaths in PEP scenario 2 [ideal] to deaths in PEP scenario 1 [no PEP], using 3 days of case data) (Table 5).
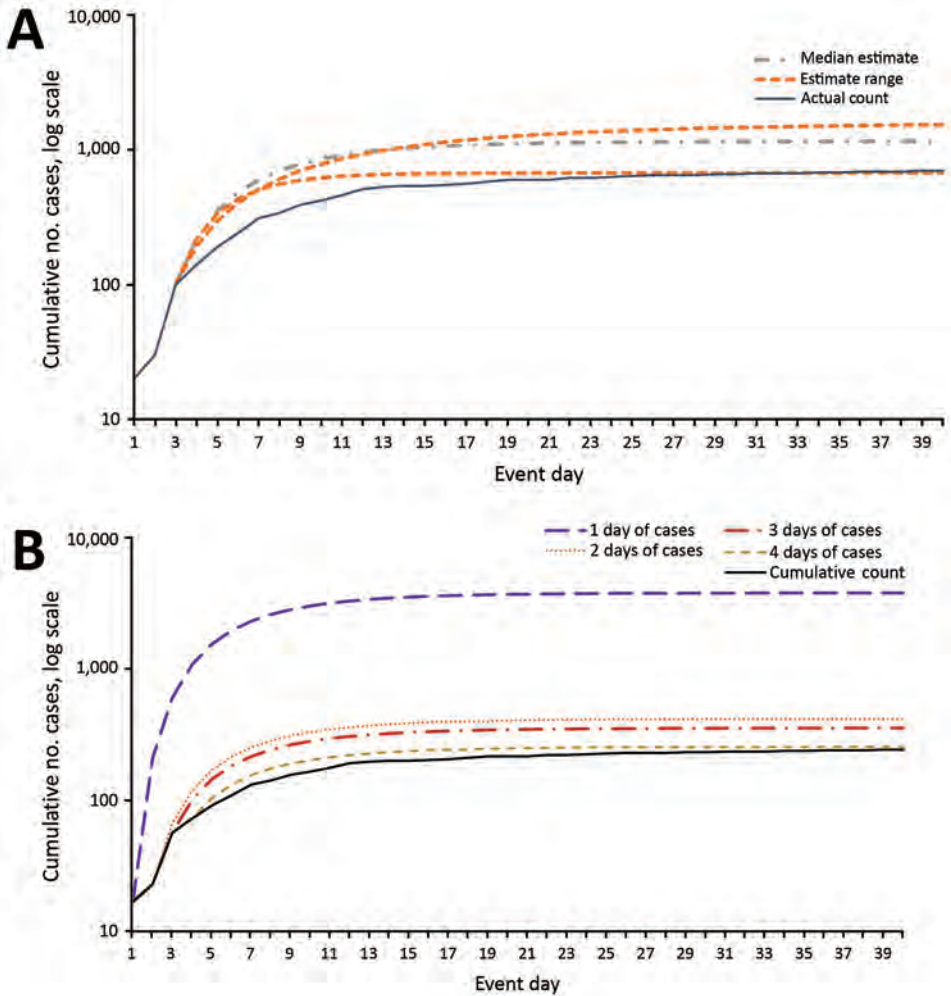
Figure 2. Comparison of the estimated cumulative epidemic curve by using 3 days of surveillance data with the actual event curve (A), and comparison of the median estimated cumulative epidemic curve with the actual event curve, by days of surveillance data available (B). Actual case data are case counts from the 1979 Sverdlovsk (USSR) anthrax outbreak (*12*), inflated by a factor of 10. Estimates were produced by using the first days of case data from that event (20 cases on day 1, 10 on day 2, 70 on day 3, and 40 on day 4) and other Epidemic-Curve model values listed in Table 2.

In the baseline scenario, treatment initiation and deaths peak early in the event (days 4 and 5, respectively), and treatment load and recoveries peak later (days 15 and 23, respectively) (Figure 3). The treatment load curve exhibits a plateau-like shape because of the extended length of time required to treat and recover from IA.

Sensitivity Analysis

Influence of Individual PEP Campaign Factors
The decision to take PEP (uptake) is the most influential PEP-related parameter (Figure 4). Projected cases averted differ as much as 59% when results using the lowest and highest
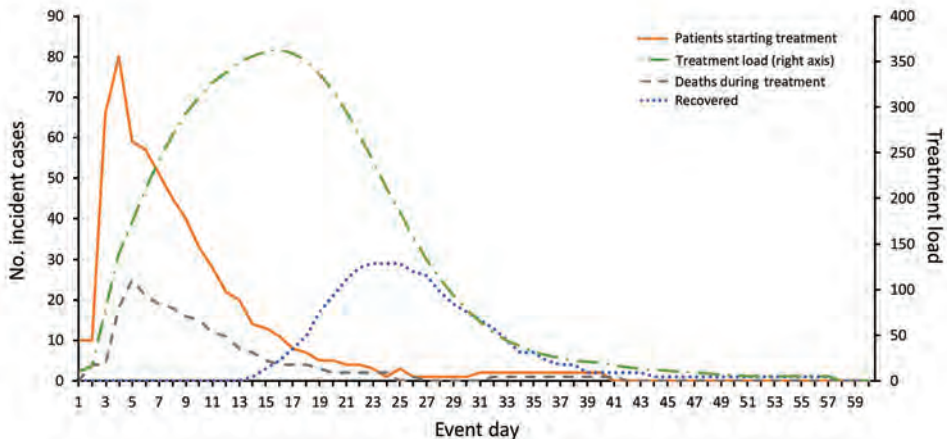
Figure 3. Projected daily patients seeking treatment, daily treatment load, and treatment outcomes by event day (baseline scenario). Estimates were calculated by using values shown in Table 2. Base case scenario is the same as PEP Evaluation Scenario 3 (practical) (Table 3). PEP, postexposure prophylaxis.
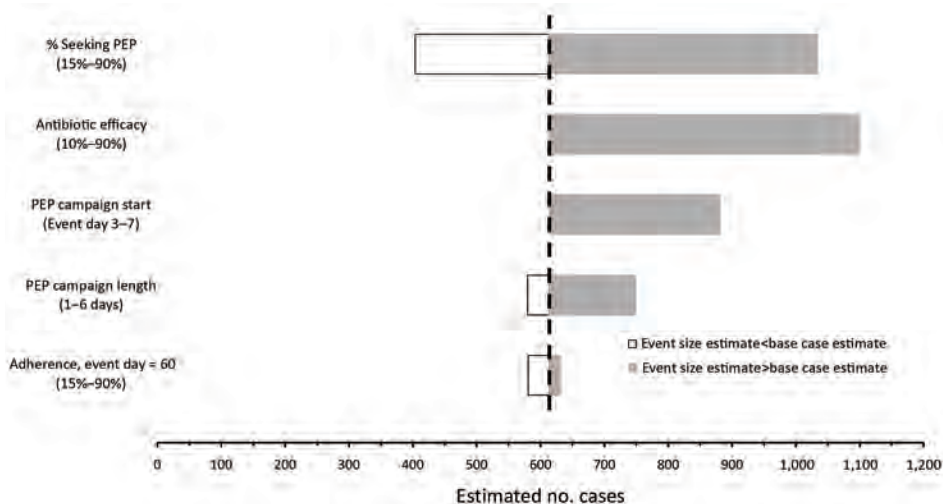
Figure 4. Final case count estimates comparisons to the baseline scenario estimate (614 cases) for selected PEP campaign parameter ranges. The base case estimate was produced using data from the first 3 days of the 1979 Sverdlovsk (USSR) anthrax outbreak (*12*), inflated by a factor of 10. All other values used in calculations are shown in Table 2. PEP, postexposure prophylaxis.

plausible PEP uptake values (12% and 71%, respectively) are compared (Table 4). In contrast, adherence (at day 60) to the PEP regimen exhibits the least direct influence on averted cases and deaths. Averted cases differ by only 5% when the lowest and highest plausible adherence values are used (50% and 55%, respectively) in the baseline scenario. This small difference results from the fact that most infected persons become symptomatic well before declining adherence can affect PEP effectiveness (online Technical Appendix 2).

Effects of Data Limitations

Because of the underlying model structure, case count inaccuracies are reflected in the final event size projections proportionately to the level of overreporting or underreporting. For example, when the first 3 days of detected cases are 40% underreported in the Sverdlovsk-like scenario (60 cases instead of 100: 12 cases with illness onset event day 1, 6 cases on day 2, and 42 cases on day 3), the median event size caseload was projected to be 698 (range 405–967), 40% less than the 1,164 cases projected in the original scenario.

Discussion

Our modeling tool provides estimates of future IA caseloads over time and quantifies the effects of various prophylaxis and treatment response options. By integrating projections of the event scale with interventions reflecting healthcare utilization and patient outcomes, our tool permits evaluation of responses during the first days of a real or simulated event.

The accuracy of our FCC projections improves with the number of days of case data available and may provide estimates sufficient for response decisions when 3 days of data are available (Figure 2, panel B). FCC projections made before day 3 probably overestimate the eventual FCC, which may be informative for policymakers (online Technical Appendix 2).

The results of our PEP and Healthcare Impact models are consistent with reports showing the benefits of initiating PEP as early as possible after exposure recognition (3,4,6,7,25). Zaric et al. (6) calculated 45.3% event mortality if a 65% effective PEP campaign was completed within 3 days after a 2-day detection delay; our comparable event mortality is 37.1% (by adjusting the associated parameter values in our baseline scenario). In highly effective PEP scenarios, Brookmeyer et al. (25) and Baccam et al. (3) separately calculated 16%–17% event mortality (we calculated 11% by adjusting our baseline PEP scenario to match theirs), if 100% effective drugs were used after a 2-day delay, 2-day dispensing campaign, 25% final adherence, and 90% inferred uptake (from 90% initial adherence used by Baccam et al., because uptake was not a parameter in either the Baccam or Brookmeyer model).

Unlike prior efforts to evaluate PEP strategies (3,4,6,7,25), our model includes a PEP uptake parameter in our evaluation of PEP strategies (Table 3; Figure 4). In our model, daily PEP uptake percentage by infected persons deteriorates as the number of unexposed persons requiring PEP increases and when the daily campaign throughput capacity cannot accommodate the increase (because uninfected persons dilute the infected population seeking PEP) (online Technical Appendix 2).

The hospital occupancy estimates generated with our Healthcare Impact model are unique among published IA models (Figure 3). This output can support pre-event and intra-event collaboration between public health officials and healthcare system leaders. It also suggests that balancing efforts to allocate countermeasures between public health and healthcare delivery will be a dynamic process that would benefit from daily reassessments of caseloads and responder capabilities.

Our baseline scenario results in lower mortality than was reported for the 2001 US anthrax attacks (37% vs.

Table 4. Effects of individual PEP campaign factors*

Variable	Median projected caseload with PEP campaign, no.	Projected averted cases from campaign start, no. (%)†	Projected averted deaths, no. (%)‡	Peak hospitalizations, no.
Days required to provide PEP to entire target population				
1	580	583 (55)	190 (47)	339
2§	614	550 (52)	183 (45)	363
3	651	513 (48)	166 (41)	385
4	680	483 (46)	160 (39)	405
5	723	441 (41)	142 (35)	431
6	749	415 (39)	135 (33)	448
Delay to PEP campaign start, d¶				
2§	614	550 (52)	183 (45)	363
3	681	482 (45)	156 (38)	402
4	753	411 (39)	131 (32)	450
5	821	343 (32)	107 (26)	494
6	881	283 (27)	88 (22)	535
PEP uptake, %#				
15	1,034	130 (12)	47 (12)	618
40	824	340 (32)	111 (27)	489
65§	614	550 (52)	183 (45)	363
90	404	760 (71)	259 (63)	235
Antimicrobial efficacy, %				
10	1,099	64 (6)	19 (5)	653
50	857	307 (29)	97 (24)	508
90§	614	550 (52)	183 (45)	363
Adherence to regimen at event day 60, %				
15	631	533 (50)	174 (43)	370
40§	614	550 (52)	183 (45)	363
65	597	566 (53)	184 (45)	353
90	581	583 (55)	192 (47)	342

*Estimates were calculated by using values shown in Table 2. Base case scenario is the same as PEP evaluation scenario 3 (practical) using 3 days of case data (Table 3). Without a PEP campaign, the median projected caseload would be 1,164 (Table 3, Scenario 1 [no PEP]) using 3 days of case data. PEP, postexposure prophylaxis.
†% = PEP averted cases/(median attack size estimate without a PEP campaign – cases detected to date)
‡% = PEP averted deaths/(median attack size deaths estimate without a PEP campaign). This calculation assumes no deaths within the first 3 event days.
§Baseline scenario value (Table 2).
¶Determined by counting days from date of earliest illness onset (i.e., event day 1).
#Percentage of population targeted to receive prophylaxis who actually obtain and start prophylaxis.

45%) (*22*), a result of our assumption of improved treatment effectiveness for persons initiating treatment during the fulminant stage of illness (20% vs. 0) (Table 2). In a large event, in which FCC exceeds treatment resources, treatment effectiveness would deteriorate. Anthrax Assist allows responders to alter effectiveness values (assume crisis standards of care) with regard to local treatment capacity.

Anthrax Assist has limitations. We do not account for gastrointestinal and cutaneous forms of *B. anthracis* infection (online Technical Appendix 2). We assume a uniform exposure dosage and a consistent relationship between dose and incubation period across patient types, which may mask logistically relevant temporal variability of illness onset (earlier cases associated with higher inhaled spore counts and vice versa); furthermore, some evidence suggests that certain populations (e.g., children, pregnant women) may be more susceptible to infection or may progress through disease stages differently. Similarly, we do not fully address the consequences of a surge of worried-well patients or the routine demands for healthcare by new and existing patients. Last, although the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices recommends anthrax vaccine as part of the PEP regimen (*26*), we do not include vaccine in our PEP Impact model under the assumption that adherence to the full 60-day PEP regimen effectively protects against infection and to assess the effects of decreasing adherence.

Some limitations result from data uncertainties. For example, our Epidemic-Curve model does not pinpoint the timing and location of a release and cannot distinguish between prolonged, short, or multiple releases (online Technical Appendix 2). This model is also sensitive to case surveillance uncertainty. To address this uncertainty, Anthrax Assist accepts simultaneous input of up to 3 case series variations. Thus, users can inflate or deflate counts on the basis of perceived underreporting or overreporting, can assign cases to different illness-onset dates, and can examine the influence on outputs. Last, in the absence of a compelling alternative, we rely on the Wilkening analyses of the Sverdlovsk outbreak for our incubation distribution (*13,27*), which is not without criticism (online Technical Appendix 2). By definition, our Epidemic-Curve model FCC estimates demonstrated high accuracy when applied to the Sverdlovsk-like attack scenario (Figure 2). Use of a Sverdlovsk-like

Table 5. PEP effects by number of days of surveillance data available and different scenarios of PEP distribution, uptake, and adherence*

Days of baseline case data, scenario	Median projected caseload, no.	Cases averted by PEP, no. (%)	Peak treatment load, no.	Median projected deaths, no.	Deaths averted by PEP, no. (%)
2†					
Scenario 1 (no PEP)	1,441	Not applicable	856	506	Not applicable
Scenario 2 (ideal)	324	1,117 (79)	188	124	382 (75)
Scenario 3 (practical)	760	681 (48)	447	279	227 (45)
Scenario 4 (constrained)	1,084	358 (25)	648	385	121 (24)
3‡					
Scenario 1 (no PEP)	1,164	Not applicable	692	408	Not applicable
Scenario 2 (ideal)	323	841 (79)	191	123	283 (70)
Scenario 3 (practical)	614	550 (52)	363	225	183 (45)
Scenario 4 (constrained)	875	289 (27)	521	316	92 (23)
4§					
Scenario 1 (no PEP)	750	Not applicable	440	270	Not applicable
Scenario 2 (ideal)	269	481 (79)	161	103	165 (62)
Scenario 3 (practical)	481	332 (54)	244	163	107 (40)
Scenario 4 (constrained)	572	178 (29)	334	215	55 (20)

*Case data are from the 1979 Sverdlovsk (USSR) anthrax outbreak (12), inflated by a factor of 10. Estimates were calculated by using values shown in Table 2, except for the selected PEP Impact model parameters varied to create the PEP scenarios analyzed here: these are identified in Table 3. PEP, postexposure prophylaxis.
†Day 1, 20 cases; day 2, 10 cases.
‡Day 1, 20 cases; day 2, 10 cases; day 3, 70 cases.
§Day 1, 20 cases; day 2, 10 cases; day 3, 70 cases; day 4, 40 cases.

scenario should not be seen as a liability, however, because no evidence suggests that any future IA event would have a substantially different epidemiological profile and our tool permits users to specify other incubation distributions. Because its projections are relatively precise (differences between the highest and lowest FCC estimates are never larger than the estimate itself), Anthrax Assist enables responders to avoid having to consider response options based on event sizes, which differ on a log scale (as with other methods [8]).

In conclusion, Anthrax Assist gives public health officials the ability to examine the future scale and consequences of alternative responses to a newly detected anthrax event. This modeling tool mirrors public health practice by using disease surveillance data and permits responders to update projections as new data arrive from the field. The results of our illustrative scenarios underscore the value of integrating epidemic curve projections with decision-based modeling of PEP use and healthcare resource planning. Furthermore, Anthrax Assist highlights the realistic benefit of public health countermeasures and the value of optimizing public perception of PEP.

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Analysis of Anthrax Immune Globulin Intravenous with Antimicrobial Treatment in Injection Drug Users, Scotland, 2009–2010

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We studied anthrax immune globulin intravenous (AIG-IV) use from a 2009–2010 outbreak of *Bacillus anthracis* soft tissue infection in injection drug users in Scotland, UK, and we compared findings from 15 AIG-IV recipients with findings from 28 nonrecipients. Death rates did not differ significantly between recipients and nonrecipients (33% vs. 21%). However, whereas only 8 (27%) of 30 patients at low risk for death (admission sequential organ failure assessment score of 0–5) received AIG-IV, 7 (54%) of the 13 patients at high risk for death (sequential organ failure assessment score of 6–11) received treatment. AIG-IV recipients had surgery more often and, among survivors, had longer hospital stays than did nonrecipients. AIG-IV recipients were sicker than nonrecipients. This difference and the small number of higher risk patients confound assessment of AIG-IV effectiveness in this outbreak.

B*acillus anthracis* is identified as a select agent subject to select agent regulations (*1–3*) and as a potential bioweapon that presents a high risk to the US public (*4,5*). Production of lethal toxins and edema toxins by *B. anthracis* is central to the bacterium's pathogenesis (*6–8*). The Centers for Disease Control and Prevention (CDC) guidelines now recommend that patients with clinical evidence of systemic anthrax disease receive an antitoxin agent in combination with antimicrobial agents (*9*).

Anthrax immune globulin intravenous (AIG-IV; current trade name Anthrasil, manufactured by Emergent BioSolutions Inc., Rockville, MD, USA) is one of the few antitoxin agents approved by the Food and Drug Administration (FDA) and included in the Strategic National Stockpile (*10*). It is a polyclonal human antibody prepared from the serum of persons previously vaccinated with anthrax vaccine adsorbed (BioThrax; Emergent BioSolutions, Gaithersburg, MD, USA). Because of the infrequency of invasive *B. anthracis* infection, AIG-IV approval was based on its efficacy in anthrax animal models in combination with safety data from healthy humans (*11,12*). Therefore, although AIG-IV has been the only antitoxin therapy administered clinically since the 2001 US anthrax outbreak, its actual efficacy in humans is unknown. Reviewing clinical experiences where AIG-IV has been administered for anthrax is important to inform future use of this agent and of antitoxin agents in general.

Although AIG-IV use has been reported in 3 isolated anthrax cases (*13–16*), the largest clinical experience with it came during an outbreak of *B. anthracis* soft tissue infection in injection drug users in the United Kingdom during 2009–2010. These cases were secondary to heroin injections contaminated with the same *B. anthracis* strain (*17–24*). Of the 52 confirmed cases in this outbreak, 47 occurred in Scotland, and 15 of these persons received AIG-IV through the coordinated efforts of CDC, Health Protection Scotland (HPS), and the Scottish National Anthrax

Outbreak Control Team. Although the epidemiology of this outbreak and the clinical characteristics of a subgroup of 27 patients has been reported, a review of experience with AIG-IV itself and its effects on recovery has not (*18,25*). Here we examine that experience in 15 recipients and 28 nonrecipients of the agent.

Methods

Approval
This study used de-identified data collected during routine hospital care of patients. The Office of Human Subjects Research from the Clinical Center at the National Institutes of Health (Bethesda, MD, USA) determined the study to be exempt from institutional review board review.

AIG-IV Availability, Distribution, and Administration
Representatives of CDC and the Scottish National Anthrax Outbreak Control Team directly involved with the 2009–2010 UK anthrax outbreak provided information about how AIG-IV was distributed and administered. Data from a previously published survey of physicians caring for patients during the outbreak were also reviewed (*18*).

Clinical Characteristics and Outcomes Comparing AIG-IV Recipients and Nonrecipients
Data regarding the clinical characteristics and outcomes of patients came from 2 sources. One was clinical data CDC obtained under its AIG-IV emergency investigational new drug application (E-IND). The other was from a previous survey of physicians caring for patients during the outbreak that had sought information about the disease characteristics, care, and outcome of patients (*18*). This previous survey did not compare AIG-IV recipients and nonrecipients.

Lethal Factor Level Determinations
Lethal factor (LF) toxemia was quantified at CDC's Clinical Chemistry Branch, Division of Laboratory Sciences (Atlanta, GA, USA), by using a validated mass spectrometry method that reports specific LF endoproteinase activity in nanograms per milliliter of serum. The LF mass spectrometry assay had precision of 8%–14%, accuracy of 92%–98%, and 100% diagnostic sensitivity and specificity (*26*).

Data Analysis
We analyzed parameters for which >50% of patients had data reported for that parameter. The sequential organ failure assessment (SOFA) scores analyzed were those recorded by physicians caring for patients (*27*). Categorical data were analyzed with χ^2 if not sparse or with Fisher exact test if otherwise. Normally distributed continuous data were analyzed by calculating the mean ± SE and

compared between groups by using 2-sample *t* tests. Otherwise, data were summarized with medians (interquartile range [IQR]). Times from exposure to symptom onset; from symptom onset to hospital admission; and from hospital admission to anthrax diagnosis, surgery, AIG-IV administration, and intensive care unit (ICU) or hospital discharge were compared with Wilcoxon rank sum tests, and physical and laboratory findings not normally distributed were log-transformed and then compared by using 2-sample *t* tests. To assess the trend of LF over time, we used a linear regression model with a common slope and different intercepts for survivors and nonsurvivors. LF levels were $\log_{10}$-transformed, and a random subject effect was used to account for repeated measures. We considered 2-sided p values ≤0.05 to be significant without adjusting for multiple comparisons. All analysis were done by using SAS version 9.3 (SAS Institute, Cary, NC, USA).

Results

Outbreak

During the *B. anthracis* outbreak in the United Kingdom during December 9, 2009–July 12, 2010, a total of 47 confirmed cases were reported in Scotland from 14 hospitals. A case was defined as confirmed on the basis of a positive bacterial culture or PCR result from blood or tissue or on paired serology samples showing increasing antibody protective antigen or LF titers (*25*).

AIG-IV Availability, Distribution, and Administration

During the outbreak, 15 patients received AIG-IV under the CDCs E-IND, and all treatments were from the same AIG-IV batch (Cangene Corp., Winnipeg, MB, Canada). CDC's Division of Strategic National Stockpile provided the first set of AIG-IV doses for use on December 18, 2009, and a limited number of AIG-IV doses were available throughout the remainder of the outbreak. HPS distributed AIG-IV during the outbreak. Clinicians identified patients for AIG-IV treatment in accordance with criteria outlined in the E-IND and published by HPS (*28*). To be eligible to receive AIG-IV, patients had to have laboratory confirmation of *B. anthracis* infection based on positive cultures or PCR results; visualization of gram-positive bacilli from blood, tissue, or a normally sterile site; or other confirmed evidence of anthrax (e.g., positive paired serologic results as noted earlier) consistent with *B. anthracis* infection (Table 1). Having met these criteria, however, whether a patient was administered AIG-IV was at the discretion of the treating physicians and their impression that a patient would or would not benefit from this treatment.

Under the E-IND, patients receiving AIG-IV were assessed, and baseline status was recorded before AIG-IV

administration. All patients received a single similar dose (420 U) of AIG-IV. Patients were then monitored during and after the infusion until discharge. Monitoring during infusion frequently occurred in the ICU.

Initial Clinical Characteristics of AIG-IV Recipients and Nonrecipients

For 43 patients, data were available for review and analysis, including for all 15 AIG-IV recipients. Times from contaminated heroin exposure to symptom onset and from symptom onset to hospitalization did not differ significantly between AIG-IV recipients and nonrecipients (Figure 1; Table 2). Age, sex, smoking status, excessive alcohol use, hepatitis C status, and use of different routes or limbs for drug injection did not differ significantly (Table 2). The proportion of patients who had only localized skin or limb complaints or only generalized complaints or a combination of localized and generalized complaints did not differ significantly between AIG-IV recipients and nonrecipients (Table 2). Among AIG-IV recipients, neither the time from exposure to AIG-IV treatment between survivors and nonsurvivors (median [IQR] 8 [6.5–11.0] days vs. 5.5 [3.0–7.0] days; p = 0.12] nor the time from symptom onset to treatment (4 [1–8] days vs. 4 [3–5] days; p = 0.59] differed significantly.

On initial physical examination, AIG-IV recipients had lower body temperature than nonrecipients (mean ± SE 36.3°C ± 0.4 vs. 37.3°C ± 0.3; p = 0.05), but heart and respiratory rates and blood pressure, and Glasgow coma scores (GCS) did not differ significantly (Table 3). Although a smaller proportion of recipients had limb edema

Table 1. Clinical criteria for administering anthrax immune globulin intravenous during an outbreak of anthrax in injection drug users, Scotland, UK, 2009–2010

Criteria

1. Systemic illness in a heroin user with ≥1 of the following:
    a. Severe cellulitis, especially accompanied by substantial soft tissue edema
    b. Sudden onset of sepsis with no other obvious source
    c. Meningitis, which might also be characterized by subarachnoid hemorrhage
    d. Respiratory symptoms (suspect inhalational anthrax)
    e. Gastrointestinal symptoms (suspect gastrointestinal anthrax);

OR

2. Features clinically compatible with cutaneous, inhalation, or gastrointestinal illness with systemic effects (including malaise, myalgias, or fever).

In addition to 1 or 2:
    Laboratory confirmation by isolation or visualization of a gram-positive bacillus consistent with *Bacillus anthracis* from blood, tissue, or a normally sterile site or other laboratory-confirmed evidence of anthrax infection after discussion with a local microbiologist or the Special Pathogens Reference Laboratory, Health Protection Agency, Porton Down, UK;

AND
    An epidemiologic link to a documented anthrax exposure (such as being a heroin injecting drug user).

(7 [47%] of 15 vs. 21 [84%] of 25; p = 0.03] and limb pain (7 [47%] of 15 vs. 17 [89%] of 19; p = 0.01), neither the presence nor type of skin finding differed significantly between the groups (Table 3). Although a greater proportion of nonrecipients had confusion (6 [35%] of 17 vs. 0 of 13; p = 0.02), other nonskin and nonlimb findings did not differ significantly between groups.

We compared results of initial laboratory results of AIG-IV recipients and nonrecipients. Recipients had higher total levels than nonrecipients for leukocytes

(median [IQR] 18.9 [9.5–23.2] vs. 10.9 [8.6–14.1] cells × 10^3 cells/μL; p = 0.02); neutrophils (15.4 [7.4–19.5] vs. 7.6 [5.2–10.0] × 10^3 cells/μL; p = 0.008); blood urea nitrogen (8.6 [7.1–13.9] vs. 4.3 [3.7–6.0] mmol/L; p = 0.01); creatinine (102.0 [84.0–189.0] vs. 75.5 [64.0–89.5] mmol/L; p = 0.04); and bilirubin (13.5 [9.0–17.0] vs. 8.0 [5.0–11.0] μmol/L; p = 0.02) but lower levels for bicarbonate (mean ± SE 20.7 ± 0.9 vs. 24.4 ± 1.2 mmol/L; p = 0.02); alkaline phosphatase (85 [56–97] vs. 100 [74–189] U/L; p = 0.04); total protein (46 [41–62]
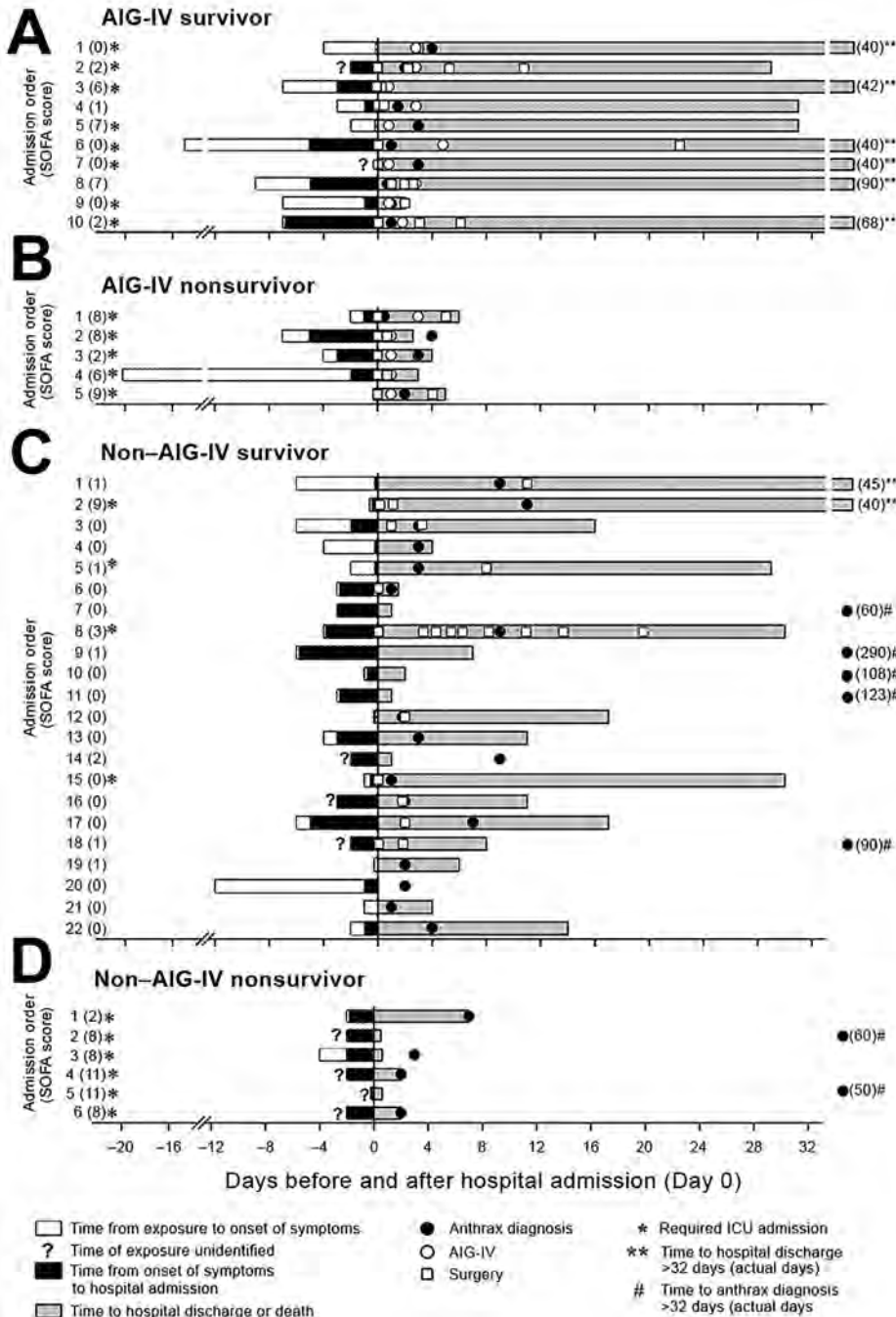
Figure 1. Key events during the illness courses of 15 patients who received AIG-IV (10 survivors, 5 nonsurvivors) and 28 patients who did not receive AIG-IV (22 survivors, 6 nonsurvivors) from the time of their suspected exposure to contaminated heroin to the time of discharge from hospital or to death, Scotland, UK, 2009–2010. A) AIG-IV recipient who survived. B) AIG-IV recipient who died. C) AIG-IV nonrecipient who survived. D) AIG-IV nonrecipient who died. AIG-IV, anthrax immune globulin intravenous; ICU, intensive care unit; SOFA, sequential organ failure.

Table 2. Medical history of AIG-IV recipients and nonrecipients, Scotland, UK, 2009–2010*

Variable	AIG-IV nonrecipient	AIG-IV recipient	p value
Presentation and clinical history			
Days from exposure to symptom onset, median (IQR)	1 (0.0–4.0), n = 18	2 (1.0–4.5), n = 12	0.19
Days from symptom onset to hospitalization, median (IQR)	2 (1.0–3.0), n = 25	2.0 (1.0–5.0), n = 14	0.55
Age, y, mean ± SE	34.2 ± 1.5, n = 28	37.5 ± 1.6, n = 15	0.18
Male sex	60.7 (17/28)	73.3 (11/15)	0.41
Smoker	94.4 (17/18)	81.8 (9/11)	0.54
Alcohol user	33.3 (5/15)	58.3 (7/12)	0.19
Hepatitis C infection	60 (9/15)	77.8 (7/9)	0.66
Drug injection route and site			
Intravenous	50 (14/18)	40 (6/15)	0.53
Intramuscular	21.4 (6/28)	46.7 (7/15)	0.16
Arm	39.3 (11/28)	26.7 (4/15)	0.41
Groin	25 (7/28)	26.7 (4/15)	1.00
Buttock	7.1 (2/28)	26.7 (4/15)	0.16
Leg	10.7(3/28)	6.7 (1/15)	1.00
Presenting complaints			
Local†	67.9 (19/28)	53.3 (8/15)	0.54
General‡	17.9 (5/28)	20.0 (3/15)	
Both%	14.3 (4/28)	26.7 (4/15)	

*Values are % patients (no. patients with the finding/no. patients for whom data were available) except as indicated. n values indicate number of patients for whom data were available. AIG-IV, anthrax immune globulin intravenous; IQR, interquartile range.
†Skin lesion, limb swelling, or limb pain.
‡Fever, diaphoresis, confusion, seizures, lethargy, or malaise.

vs. 67 [65–75] g/L; p = 0.001); and albumin 27.6 ± 2.6 vs. 38.3 ± 1.1 g/L; p<0.0001) levels (Table 4). Other laboratory parameters did not differ significantly between the 2 groups (Table 4).

On microbiological examination, a higher proportion of AIG-IV recipients than nonrecipients had positive blood cultures (10 [71%] of 14 vs. 8 [32%] of 25; p =

0.02) and positive blood PCR results (8 [80%] of 10 vs. 5 [29%] of 17; p = 0.02) for *B. anthracis* (Table 5). Other microbiological data did not differ significantly between the 2 groups (Table 5). The time to anthrax diagnosis was shorter for AIG-IV recipients than for nonrecipients (median [IQR] 2.0 [1.0–3.0] vs. 3.5 [2.0–30.5]; p = 0.006] (Figure 1; Table 5).

Table 3. Initial physical findings of recipients and nonrecipients of AIG-IV, Scotland, UK, 2009–2010*

Physical finding	AIG-IV nonrecipient	AIG-IV recipient	p value
Vital signs [reference value]†			
Temperature, °C, mean ± SE [36.1–37.2°C]	**37.3 ± 0.3, n = 28**	**36.3 ± 0.4, n = 15**	**0.05**
Systolic BP, mmHg, mean ± SE [90–140 mm Hg]	113.6 ± 3.8, n = 28	117.2 ± 5.0, n = 15	0.57
Diastolic BP, mmHg, mean ± SE [60–90 mm Hg]	65.9 ± 3.0, n = 28	68.6 ± 4.3, n = 15	0.59
Mean BP, mmHg, median (IQR) [70–100 mm Hg]	83.5 (74.3–90.7), n = 28	88.0 (67.7–93.3), n = 15	0.57
Heart rate, beats/min, mean ± SE [60–100 beats/min]	104.0 ± 4.7, n = 28	102.4 ± 5.3, n = 15	0.83
Respiratory rate, breaths/min, mean ± SE [12–20 breaths/min]	18.8 ± 1.4, n = 28	19.9 ± 1.7, n = 13	0.64
Glasgow coma score, median (IQR) [15]	15 (15–15), n = 28	15 (15–15), n = 15	0.56
Skin and limbs			
Skin lesion	85.2 (23/27)	73.3 (11/15)	0.43
Ulcer	35.3 (6/17)	10 (1/10)	0.20
Exude	27.8 (5/18)	18.2 (2/11)	0.68
Limb mottling	26.7 (4/15)	60 (6/10)	0.12
Eschar	17.7 (3/17)	18.2 (2/11)	1.00
Local pain	100 (19/19)	85.7 (12/14)	0.17
Localized edema	95.8 (23/24)	93.3 (14/15)	1.00
Local erythema	87.0 (20/23)	92.3 (12/13)	1.00
Limb pain	**89.5 (17/19)**	**46.7 (7/15)**	**0.01**
Limb edema	**84 (21/25)**	**46.7 (7/15)**	**0.03**
Findings other than skin and limb			
Fever	60.9 (14/23)	66.7 (8/12)	1.00
Diaphoresis	63.6 (7/11)	40 (4/10)	0.39
Lethargy	64.3 (9/14)	41.7 (5/12)	0.25
Nausea	29.4 (5/17)	30 (3/10)	1.00
Abdomen pain	6.7 (1/15)	20 (2/10)	0.54
Confusion	**35.3 (6/17)**	**0 (0/13)**	**0.02**

*Values are % patients (no. patients with the finding/no. patients for whom data were available) except as indicated. n values indicate no. patients for whom data were available. Bold indicates statistical difference between AIG-IV recipients and nonrecipients. AIG-IV, anthrax immune globulin intravenous; BP. blood pressure; IQR, interquartile range.

Table 4. Initial laboratory findings of AIG-IV recipients and nonrecipients, Scotland, UK, 2009–2010*

Laboratory test [reference value]	Non-AIG-IV	AIG-IV	p value
Complete blood counts and differentials			
Leukocytes, × 10⁹ cells/L, median (IQR) [4–11 × 10⁹/L]	**10.9 (8.6–14.1), n = 27**	**18.9 (9.5–23.2), n = 15**	**0.02**
Neutrophils, × 10⁹ cells/L, median (IQR) [2–7 × 10⁹/L]	**7.6 (5.2–10.0), n = 26**	**15.4 (7.4–19.5), n = 15**	**0.008**
Lymphocytes, × 10⁹ cells/L, median (IQR) [1–3 × 10⁹/L]	1.8 (1.3–2.5), n = 25	2.0 (1.3–2.7), n = 15	0.78
Hemoglobin, g/dL, mean ± SE [12–18 g/dL]	14.0 ± 0.8, n = 27	14.8 ± 1.5, n = 15	0.61
Hematocrit, %, mean ± SE [35%–50%]	41 ± 2, n = 23	42 ± 4, n = 13	0.86
Platelets, × 10⁹/L, mean ± SE [150–450 × 10⁹/L]	214 ± 19, n = 24	181 ± 24, n = 15	0.29
Coagulation parameters and C-reactive protein			
Prothrombin time(s), median (IQR) [11–13.5 s]	11.0 (10.0–14.0), n = 15	12.8 (12.0–15.0), n = 14	0.94
Partial thromboplastin time(s), median (IQR) [25–35 s]	26.7 (24.0–36.0), n = 10	33.8 (30.0–39.0), n = 14	0.63
International normalized ratio, median (IQR) [0.8–1.1]	1.1 (1.0–1.3), n = 11	1.1 (1.0–1.3), n = 11	0.36
C-reactive protein, nmol/L, median (IQR) [<95 nmol/L]	21 (8–49), n = 25	32 (17–52), n = 14	0.24
Serum electrolytes and glucose			
Sodium, mmol/L, median (IQR) [135–145 mmol/L]	137 (132–139), n = 27	135 (131–136), n = 15	0.11
Chloride mmol/L, median (IQR) [96–108 mmol/L]	100 (96–101) , n = 13	102 (101–103), n = 14	0.76
Potassium, mmol/L, mean ± SE [3.5–5.3 mmol/L]	4.26 ± 0.13, n = 28	4.35 ± 0.22, n = 13	0.69
Calcium, mmol/L, median (IQR) [2.25–2.5 mmol/L]	2.3 (2.0–2.3), n = 28	2.1 (2.0–2.3), n = 12	0.97
HCO3⁻ , mmol/L, mean ± SE [22–28 mmol/L]	**24.4 ± 1.2, n = 11**	**20.7 ± 0.9, n = 11**	**0.02**
Glucose, mmol/L, median (IQR) [3.6–6.0 mmol/L]	6.5 (5.6–8.1), n = 16	7.8 (5.3–8.9), n = 10	0.69
Renal and liver functions			
Blood urea nitrogen, mmol/L, median (IQR) [2.5–7.8 mmol/L]	**4.3 (3.7–6.0), n = 28**	**8.6 (7.1–13.9), n = 15**	**0.01**
Creatinine, mmol/L, median (IQR) [40–130 μmol/L]	**75.5 (64.0–89.5), n = 28**	**102.0 (84.0–189.0), n = 15**	**0.04**
Bilirubin, μmol/L, median (IQR) [5–17 μmol/L]	**8.0 (5.0–11.0), n = 25**	**13.5 (9.0–17.0), n = 14**	**0.02**
Alanine aminotransferase, U/L, median (IQR) [<50 U/L]	18.5 (14–36.5), n = 16	28.0 (11.0–40.0), n = 14	0.69
Alkaline phosphatase, U/L, median (IQR) [30–130 U/L]	**100 (74–189), n = 15**	**85 (56–97), n = 11**	**0.04**
Total protein, g/L, median (IQR) [60–80 g/L]	**67 (65–75), n = 12**	**46 (41–62), n = 13**	**0.001**
Albumin, g/L, mean ± SE [35–55 g/L]	**38.3 ± 1.1, n = 25**	**27.6 ± 2.6, n = 14**	**<0.0001**

*n values indicate no. patients for whom data were available. Bold indicates significant differences between AIG-IV recipients and nonrecipients. AIG-IV, anthrax immune globulin intravenous; IQR, interquartile range.

Treatments of AIG-IV Recipients and Nonrecipients

The median time to AIG-IV treatment in recipients was 1 day (IQR 1–3 days) (Figure 1; Table 6). No adverse events were documented during AIG-IV administration. A greater proportion of AIG-IV recipients than nonrecipients had surgery either on the day of (11 [73%] of 15 vs. 5 [18%] of 28; p = 0.0003) or at any time during [14 [93%] of 15 vs. 11 [39%] of 28; p = 0.0006] hospital admission (Figure 1; Table 6). AIG-IV recipients received more types of antimicrobial drugs than did nonrecipients (mean ± SE 5.3 ± 0.2 vs. 3.0 ± 0.2; p < 0.0001). Administration of mechanical ventilation or vasopressors did not differ between the groups (p≥0.36; data not shown).

Outcomes

Five (33%) of 15 AIG-IV recipients and 6 (21%) of 28 nonrecipients died, and these death rates did not differ significantly (p = 0.47) (Figure 1). However, in patients overall, risk for death at admission as reflected by the SOFA score was greater for AIG-IV recipients than for nonrecipients, although this finding did not reach statistical significance (median [IQR] 2 [0–7] vs. 0.5 [0–2.5]; p = 0.14). However, SOFA scores were not distributed equally between recipients and nonrecipients (Figure 2). Of the 30 patients with a SOFA score of 0–5 (indicating a low risk for death), only 8 (27%) received AIG-IV (p = 0.01, against the null hypothesis that 50% of these patients received AIG-IV).

On the other hand, of the 13 patients with a SOFA score of 6–11 (indicating a higher risk for death), 7 (54%) received AIG-IV (p = 0.78, against the null hypothesis that 50% of these patients received AIG-IV). Death rates did not differ between AIG-IV recipients and nonrecipients either for patients with SOFA scores of 0–5 (1 [13%] nonsurvivor of 8 recipients vs. 1 [5%] of 22 nonrecipients; p = 0.47) or for patients with SOFA scores of 6–11 (4 [57%] of 7 recipients vs. 5 [83%] of 6 nonrecipients, p = 0.56]. For patients with SOFA scores of 6–11, the median score was higher in nonrecipients than recipients (8.5 [8–11] vs. 7 [6–8]; p = 0.03).

For survivors, duration of ICU and hospital stays were longer for recipients than for nonrecipients (median [IQR] for ICU stay, 4.5 [0.9–19.0] vs. 0 [0–0]; p = 0.0008; for hospital stay, 38.0 [31.0–42.0] vs. 9.5 [2.0–17.0]; p = 0.001) (Figure 1; Table 6). For nonsurvivors, the time to death was longer for recipients than for nonrecipients in a pattern approaching significance (4.0 [3.0–5.0] vs. 1.3 [0.6–2.0]; p = 0.07) (Figure 1; Table 6). Of the 6 nonsurvivors not receiving AIG-IV, 3 died within 24 h and 2 within 48 h after admission, times possibly too short for AIG-IV acquisition and treatment. Furthermore, nonsurvivors receiving AIG-IV had significantly higher GCS (better neurologic function) than nonrecipients (15 [15–15] vs. 9 [6–14]; p = 0.04). Consistent with this finding, all 4 head computed tomograms reported from patients in the outbreak were in nonrecipient nonsurvivors, and all showed evidence of

Table 5. Microbiology data and the time to confirmatory anthrax diagnosis for recipients and nonrecipients of AIG-IV, Scotland, UK, 2009–2010*

Laboratory test	AIG-IV nonrecipient	AIG-IV recipient	p value
Blood culture	**32 (8/25)**	**71.4 (10/14)**	**0.02**
Wound culture	46.2 (6/13)	33.3 (3/9)	0.67
Tissue culture	54.6 (6/11)	70 (7/10)	0.66
Blood PCR	**29.4 (5/17)**	**80 (8/10)**	**0.02**
Blood protective antigen antibody	81.3 (13/16)	66.7 (4/6)	0.59
Blood lethal factor antibody	62.5 (10/16)	57.1 (4/7)	1.00
Days to diagnosis, median (IQR)	**3.5 (2.0–30.5), n = 28**	**2.0 (1.0–3.0), n = 13**	**0.006**

*Values are % patients (no. patients with the finding/no. patients for whom data were available) except as indicated. n values indicate no. patients for whom data were available. Bold indicates significant differences between AIG-IV recipients and nonrecipients. AIG-IV, anthrax immune globulin intravenous; IQR, interquartile range.

subarachnoid hemorrhage (3 patients) or high attenuation material caused by subarachnoid hemorrhage or purulence (1 patient). Two of these nonrecipients died within 15 h after admission and 2 by 48 h. In addition, although all nonsurvivors receiving AIG-IV had at least 1 surgery, no nonsurvivor not receiving AIG-IV had surgery (Figure 1).

Four Patients from the Outbreak for Whom Data Were Unavailable

None of the 4 patients for whom data were unavailable received AIG-IV. Of these, 2 survived and 2 did not (*25*). Therefore, across all 47 patients in Scotland, the proportion of patients dying did not differ significantly between AIG-IV recipients and nonrecipients (5 [33%] of 15 vs. 8 [25%] of 32; p = 0.73).

LF Levels in AIG-IV Recipients

LF levels were available for 5 nonsurvivors and 7 survivors receiving AIG-IV and from no nonrecipients. These levels were examined over the period they were available for both nonsurvivors and survivors (10 h before and up to 50 h after AIG-IV administration). Before AIG-IV treatment, LF levels trended to be higher but did not differ significantly between nonsurvivors and survivors (p = 0.42) (Figure 3). Two survivors had LF levels <0.1 ng/mL, noticeably lower

than levels of other patients. After AIG-IV treatment, levels trended slightly lower and with a common slope approaching significance (p = 0.08).

Discussion

The experience with AIG-IV during the 2009–2010 anthrax outbreak in injection drug users in Scotland is, to our knowledge, the largest single experience with this agent. Despite AIG-IV efficacy in animal models (*11,12*), death rates did not differ significantly between 15 patients who did and the 28 who did not receive therapy. However, several criteria indicated that AIG-IV recipients were sicker than nonrecipients, and this difference confounds an assessment of the efficacy of AIG-IV.

Although SOFA scores seemed to suggest that AIG-IV recipients were at higher risk than nonrecipients for death, this trend did not reach significance (p = 0.14). However several laboratory findings differed significantly between the 2 groups and were consistent with more severe disease in AIG-IV recipients: lower temperature, serum bicarbonate, total protein, and albumin and higher leukocytes, neutrophils, blood urea nitrogen, creatinine, and bilirubin. Patients with a low risk for death (SOFA score <5) were less likely to receive AIG-IV (8 of 30; p = 0.01). In contrast, the proportions of recipients and

Table 6. Treatment after hospital admission and durations of ICU and hospital stay in survivors and nonsurvivors who did and did not receive AIG-IV, Scotland, UK, 2009–2010*

Treatment characteristic	AIG-IV nonrecipient	AIG-IV recipient	p value
Treatments after hospital admission			
Days to AIG-IV receipt, median (IQR)	NA	1 (1–3), n = 15	NA
ICU care	**35.7 (10/28)**	**86.7 (13/15)**	**0.001**
Receipt of antimicrobial drugs	100 (28/28)	100 (15/15)	1.00
No. antimicrobial drugs/patient during hospital stay, mean ± SE‡	**3.0 ± 0.2, n = 28**	**5.3 ± 0.2, n = 15**	**<0.0001**
Surgery on day of admission	**17.9 (5/28)**	**73.3 (11/15)**	**0.0003**
Surgery during hospital stay	**39.3 (11/28)**	**93.3 (14/15)**	**0.0006**
Days to surgery, median (IQR)	1 (0–2), n = 11	0 (0–0.33), n = 14	0.24
Vasopressors	13.3 (2/15)	33.3 (4/12)	0.36
Mechanical ventilation	33.3 (5/15)	50 (7/14)	0.36
Duration of ICU and hospital stay, median (IQR)			
Survivors' ICU stay, d	**0, n = 22**	**4.5 (0.9–19.0), n = 10**	**0.0008**
Nonsurvivors' time to death, d	1.3 (0.6–2.0), n = 6	4.0 (3.0–5.0), n = 5	0.07
Survivors' hospital stay, d	**9.5 (2.0–17), n = 22**	**38 (31–42), n = 10**	**0.001**

*Values are % patients (no. patients with the finding/no. patients for whom data were available) except as indicated. n values indicate no. patients for whom data were available. Bold indicates significant differences between AIG-IV recipients and nonrecipients. AIG-IV, anthrax immune globulin intravenous; ICU, intensive care unit; IQR, interquartile range; NA, not applicable.
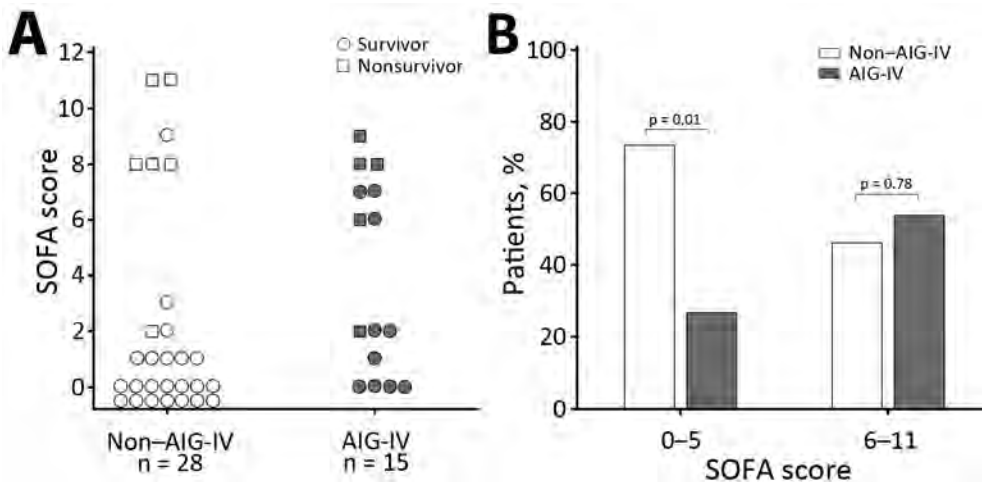
Figure 2. SOFA scores of injection drug users who did and did not receive AIG-IV, Scotland, UK, 2009–2010. A) Individual SOFA scores for patients who did or did not receive AIG-IV and did or did not survive. B) Proportion of patients who did or did not receive AIG-IV for those who had admission SOFA scores of 0–5 and a low risk for death and for those with SOFA scores of 6–11 and a higher risk for death. AIG-IV, anthrax immune globulin intravenous; SOFA, sequential organ failure.

nonrecipients with a higher risk for death (SOFA scores ≥6) did not differ. The number of patients with higher SOFA scores was too small to assess AIG-IV effects in this subgroup. Consistent with the possibility that AIG-IV recipients were sicker than nonrecipients, among survivors, ICU and hospital stay were significantly longer for recipients, suggesting longer recovery from more severe disease. More AIG-IV recipients than nonrecipients had surgery to manage their disease, and a greater proportion of AIG-IV recipients had blood cultures positive for *B. anthracis*, suggesting a higher bacterial load.

For at least 3 reasons, AIG-IV treatment might have been directed to more severely ill patients. First, the sensitivity of various diagnostic laboratory criteria for documenting anthrax can vary on the basis of the severity of infection. Positive blood cultures, suggestive of significant bacterial load, often were available early during the patient's course (i.e., within ≤1 day), resulting in timely consideration of AIG-IV. In contrast, confirmation based solely on paired serum samples, reflecting less severe infection, required far longer for results to be available (often weeks), thus mitigating against AIG-IV use. Second, criteria for AIG-IV stipulated that patients have evidence of systemic and therefore more severe illness. Third, outbreak treatment teams reported in personal communications to authors of this article (L.N. and M.B.) that AIG-IV was considered a limited resource and that treatment was directed to patients with evidence of more severe infection but with a likelihood of survival.

For at least 2 possible reasons, some patients with high SOFA scores did not receive AIG-IV. First, disease might have progressed too quickly in some nonsurvivors for AIG-IV to be made available; 3 nonrecipients died within 24 h after seeking care and 2 within 48 h. Second, among nonsurvivors, nonrecipients had significantly poorer neurologic status at admission, as

assessed by GCS scores, than did AIG-IV recipients, and none underwent surgery, suggesting that care might have been limited in these patients. In fact, 4 of these patients had evidence of subarachnoid hemorrhage soon after seeking care. In personal communications, caregivers reported withholding AIG-IV in patients with severe neurologic deficits.

Thus, on the basis of experience during this outbreak in injection drug users, whether AIG-IV provides benefit for anthrax-related soft tissue infection is unclear. This form of infection has only recently been identified and has received little preclinical study. Debridement was effective in a mouse model of subcutaneous anthrax, but that study did not investigate the efficacy of antitoxin therapies (*29*). The preclinical models on which FDA based its approval of AIG-IV all used aerosolized bacterial challenge to simulate inhalational anthrax (*11,12*). In these studies, AIG-IV added to the protective effects of antimicrobial drugs when both treatments were administered after the onset of lethality, in trends that approached significance (*11*). The prior clinical experience with AIG-IV also has been restricted to inhalational disease (3 cases) or gastrointestinal disease (1 case) contracted by inhalation or ingestion of spores (*13–16*). Although 3 of these 4 patients survived, an overall survival rate higher than previously reported with these forms of disease, to what extent this might have been related to AIG-IV treatment or other factors is unknown. Studies examining the effects of AIG-IV or other antitoxin agents when combined with antimicrobial drugs and debridement in animal models are necessary to better define the optimal therapeutic approach for this newly identified form of anthrax. Prompt and aggressive antimicrobial therapy and surgical debridement if necrotic tissue requires it, remain the mainstays of management for soft tissue infection when anthrax is suspected. However, on the basis of animal efficacy studies with other forms of anthrax, as well as human
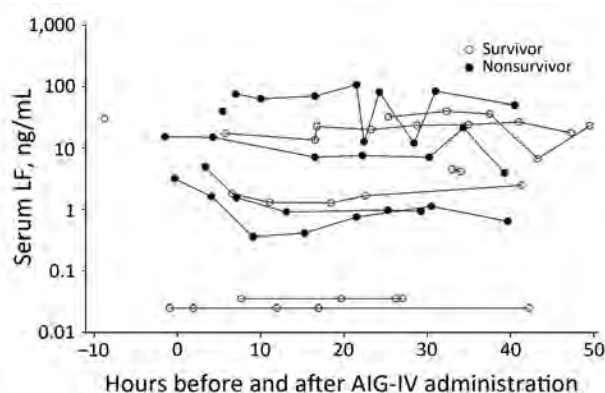
Figure 3. Individual LF levels (nanograms per milliliter) in 12 anthrax patients receiving AIG-IV from 10 h before until 50 h after treatment administration, Scotland, UK, 2009–2010. AIG-IV, anthrax immune hlobulin intravenous; LF, lethal factor.

safety studies, AIG-IV or other approved antitoxin agents still might be considered as adjunctive therapy when clinical suspicion is high for systemic anthrax.

LF levels trended higher in nonsurvivors than survivors before AIG-IV administration, but this difference was not significant. Although there was a small but close to significant reduction in LF levels after AIG-IV administration, without data from nonrecipients, determining whether this decline reflects the effect of antibody treatment or the course of the infection itself is not possible. LF levels could have been influenced by previous antimicrobial use. Although current LF detection is based on serum and plasma levels, the relationship between these and tissue levels is currently under investigation. Tissue levels ultimately might be more instructive than serum and plasma levels for disease treatment.

No adverse events related to AIG-IV administration were reported to CDC during this *B. anthracis* outbreak. The number of recipients was relatively small, however, and given these patients' severity of disease, identifying adverse effects of treatment without an equivalent control group would be difficult.

This study has limitations. First, data were obtained retrospectively and not completely for all patients. However, parameters were analyzed and presented only if available from >50% of patients. Furthermore, the SOFA score on which stratification of AIG-IV treatment and survival data was based, and which is a well-regarded gauge of disease severity and lethality risk (27), was available for all 43 patients analyzed. Second, data were not available regarding decisions about whether individual patients should or should not receive AIG-IV treatment. Third, the overall number of patients available for analysis was small. However, our analysis addressed the single largest experience with an antitoxin agent for treating anthrax since the

introduction of antimicrobial drugs during the 1940s and the routine use of modern ICU support in the early1960s (30).

In conclusion, guidelines now recommend treatment with agents inhibiting the effects of lethal and edema toxins for patients with a high likelihood of having systemic anthrax infection. AIG-IV is one of the few antitoxin agents that has received FDA approval and been included in the Strategic National Stockpile. Documenting the clinical experience with anthrax antitoxin agents is critical for further defining this therapeutic approach. Whether AIG-IV treatment is effective for systemic anthrax soft tissue infection related to drug injection cannot be answered with currently available data.

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Sequelae and Other Conditions in Ebola Virus Disease Survivors, Sierra Leone, 2015

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We rapidly assessed the health of Ebola virus disease (EVD) survivors in Kenema, Sierra Leone, by reviewing medical charts of all patients attending the Survivor Clinic of Kenema Government Hospital. Data were abstracted on signs and symptoms at every attendance. As of November 2015, a total of 621 attendances by 115 survivors with laboratory-confirmed EVD were made to the Survivor Clinic. Most (60.9%) survivors were women. Survivors' median age was 28 years (range 0.25–70 years). Survivors attended the clinic a median of 5 times (range 1–21 times) each, and the median time from EVD discharge to attendance was 261 days (range 4–504 days). The most commonly reported signs and symptoms among the 621 attendances were headache (63.1%), fever (61.7%), and myalgia (43.3%). Because health needs of EVD survivors are complex, rapid chart reviews at survivor clinics should be repeated regularly to assess the extent of illness and prioritize service delivery.

After Ebola virus disease (EVD) emerged in Sierra Leone in 2014, a total of 8,704 confirmed cases and 3,955 deaths had been reported as of the declaration of EVD transmission-free status on November 7, 2015 (*1*). Approximately 4,000 survivors are in the country, and evidence of the frequency and duration of sequelae over an extended period is limited in this cohort.

Recently published studies highlight the occurrence of post-EVD complications, such as uveitis (*2,3*) and encephalopathy (*4*), but these are isolated investigations. Evidence on the prevalence of these and other sequelae is increasing. There is a growing body of evidence on the burden of these and other sequelae, including 1 report based on a survey of 81 survivors in Kenema (Sierra Leone), which found that nonspecific symptoms, such as arthralgia, headache, and myalgia, persist for months after recovery from acute EVD (*5–7*).

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Many survivors also face EVD-related stigma and rejection from their communities (*5,8*) and suffer with posttraumatic stress disorder, depression, or anxiety (*5,7–9*). Furthermore, new ocular problems, such as uveitis, are commonly reported among survivors (*2,5,6,10*). A summary of the literature on these sequelae was presented in a recently published review article, but evidence from the 2014–15 West Africa outbreak had a maximum duration of follow-up of 1 year (*11*).

To rapidly assess the health of survivors in Kenema District, we reviewed the medical charts of all patients of the Survivor Clinic at Kenema Government Hospital (KGH) to determine the frequency of possible EVD-related sequelae among persons in this cohort, many of whom had been in convalescence for >1 year. Secondarily, we determined the frequency of diseases diagnosed within this group.

Materials and Methods

Setting

KGH is the major Ministry of Health and Sanitation, Government of Sierra Leone, referral hospital in Kenema District, the third most populous district in Sierra Leone (population 609,873 [Sierra Leone Population and Household Census 2015, https://www.statistics.sl/wp-content/uploads/2016/06/2015-Census-Provisional-Result.pdf]). In October 2014, an EVD Survivor Clinic was opened at KGH to provide treatment and care at no cost to survivors. This nurse-led clinic has support from an on-call medical doctor and provides treatment for minor complaints and mental health counseling. The Ministry of Social Welfare, Gender and Children's Affairs of the Government of Sierra Leone maintains a register of EVD survivors in Kenema to facilitate the provision of benefits from the government and nongovernmental organization partners. As of November 2015, records from the Ministry of Social Welfare, Gender and Children's Affairs in Kenema indicated that 162 EVD survivors were registered to receive benefits there.

Data Entry

After a rapid review of the literature, we developed an electronic proforma to collect demographic data and signs

and symptoms previously reported as EVD sequelae using Microsoft Excel 2010 (Microsoft Corp., Redmond, WA, USA) (*5*). A close-ended list of signs and symptoms was used for consistent data collection. This list included fever because signs and symptoms reported with fever might indicate other acute infectious diseases, rather than EVD sequelae. Data were then abstracted from individual hard copy medical charts for all patients who attended the Survivor Clinic during November 10–13, 2015, by a medical doctor (A.O.V.) and entered by the lead author (H.M.).

For each patient, we collected name, sex, age, address, and date of discharge from KGH after the diagnosis and treatment of EVD and for each of their attendances during convalescence, their complaints (signs and symptoms), and differential diagnoses. Each sign and symptom was collected as a binary variable (yes/no), and the differential diagnoses were entered as open-ended text (verbatim from the medical chart); this text was later queried to generate a list of diseases and conditions by searching for selected text. After entry, a unique identifier was generated for each patient, and personally identifiable information was stripped from the dataset and stored separately on a password-protected terminal at the World Health Organization in Kenema. These patients' identities were then cross-referenced against the national viral hemorrhagic fever database developed by the US Centers for Disease Control and Prevention (Atlanta, GA, USA) (*12*) and maintained by the Kenema District Health Medical Team EVD Response Centre to confirm these patients had laboratory-confirmed EVD.

Data Analysis

Data analysis was performed on the anonymized dataset and restricted to patients with confirmed EVD. We calculated the time (days) from EVD discharge to attendance date and determined attendance-level frequencies of signs and symptoms (first among all attendances, then among attendances during which fever was not reported). To determine which signs and symptoms were associated with febrile presentation, we assessed associations between each and fever (yes/no) using univariate generalized estimating equations (GEE) logistic regression to account for the clustering of attendances by patient. We also determined associations between fever and age (quartiles), sex, and time since EVD discharge (quartiles) using univariate GEE logistic regression. Similarly, to determine whether signs and symptoms varied between children (<18 years of age) and adults (≥18 years of age), we determined associations between age and each sign and symptom using univariate GEE logistic regression, then in GEE models adjusted for the time since discharge. All data cleaning, management and analysis were performed by using Stata version 13.1 (StataCorp LP, College Station, TX, USA). We considered p values <0.05 to be statistically significant.

Ethics Statement

This review of medical charts was conducted as part of the response to the outbreak of EVD, a public health emergency of international concern (*13*). Patient consent was not sought because we performed a secondary analysis of medical chart data. We obtained approval for the review from the District Medical Officer (Ministry of Health & Sanitation).

Results

Description of Survivors

From the opening of the Survivor Clinic in October 2014 until November 13, 2015, a total of 124 survivors sought care at the clinic. Most (115 [92.7%]) previously had laboratory-confirmed EVD, and these 115 patients had attended the clinic a total of 621 times.

Most of the confirmed EVD survivors were women (70 [60.9%] of 115) and residents of Kenema District (109 [94.8%]), among whom most (96 [88.1%] of 109) were residents of Nongowa Chiefdom, from which most EVD cases in the District were reported during the outbreak. The median patient age was 28 (range 3 months to 70 years).

Patients attended the clinic a median of 5 times (range 1–21 times). Median time from EVD discharge to first attendance was 114 days (4–395 days) and from EVD discharge to any attendance was 261 days (4–504 days) (Figure 1).
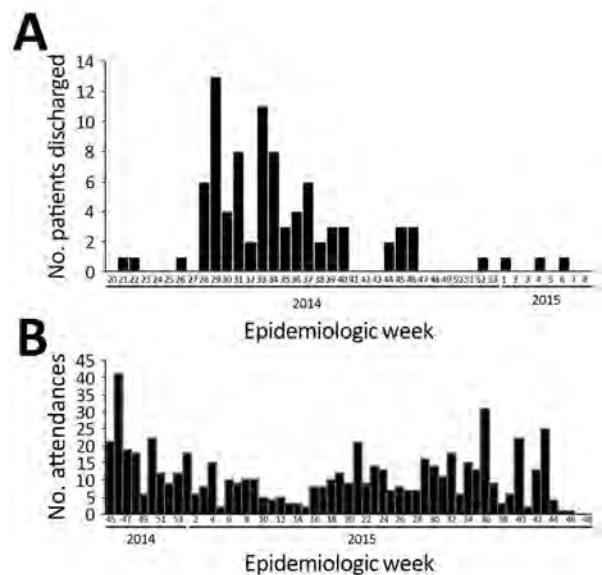
Figure 1. Hospital discharge and attendances for 115 survivors of laboratory-confirmed Ebola virus disease (EVD) attending the Survivor Clinic at Kenema Government Hospital (KGH), Kenema, Sierra Leone), 2014–2015. A) Discharge from KGH after initial EVD diagnosis for the 88 (76.5%) survivors for whom data were available on date of EVD discharge. B) Dates of the 621 attendances at KGH during convalescence by the 115 EVD survivors.

Signs and Symptoms

Of the 621 attendances by the 115 survivors, the most commonly reported symptoms were headache (63.1% [95% CI 59.2%–67.0%]), fever (61.7% [95% CI 57.7%–65.5%]), and myalgia (43.3% [95% CI 39.4%–47.3%]). Joint pain was the fifth most common symptom (30.1% [95% CI 26.5%–33.9%]). At the 238 attendances for which patients did not have fever, the most commonly reported symptoms were headache (50.4%), myalgia (43.7%), and joint pain (31.1%) (Figure 2, panel A), but the relative frequencies
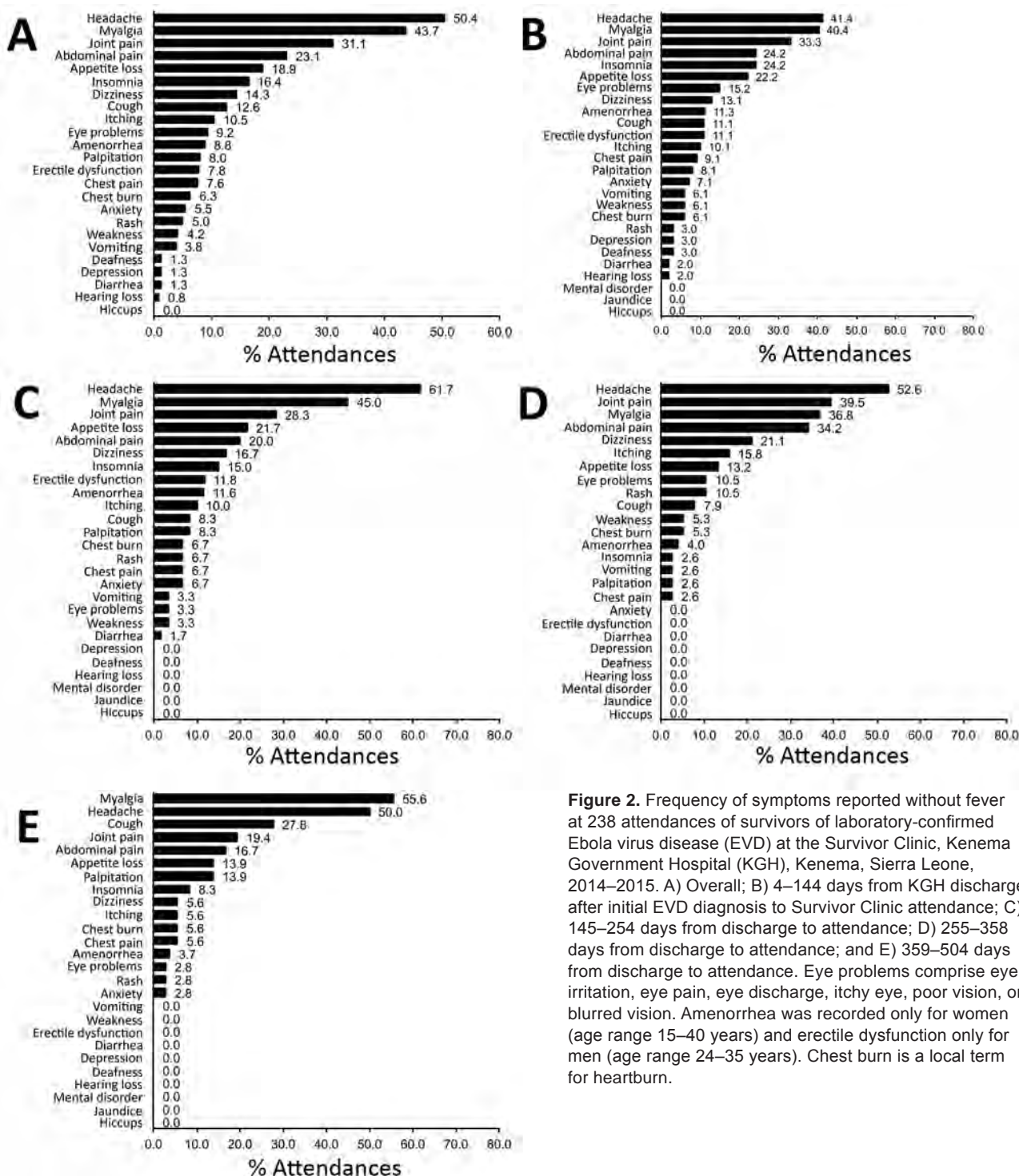
Figure 2. Frequency of symptoms reported without fever at 238 attendances of survivors of laboratory-confirmed Ebola virus disease (EVD) at the Survivor Clinic, Kenema Government Hospital (KGH), Kenema, Sierra Leone, 2014–2015. A) Overall; B) 4–144 days from KGH discharge after initial EVD diagnosis to Survivor Clinic attendance; C) 145–254 days from discharge to attendance; D) 255–358 days from discharge to attendance; and E) 359–504 days from discharge to attendance. Eye problems comprise eye irritation, eye pain, eye discharge, itchy eye, poor vision, or blurred vision. Amenorrhea was recorded only for women (age range 15–40 years) and erectile dysfunction only for men (age range 24–35 years). Chest burn is a local term for heartburn.

changed with increasing time since EVD discharge (Figure 2, panels B–E).

At least 1 eye problem was reported at 51 attendances; the most common of these were blurred vision (37.3%), poor vision (29.4%), and itchy eyes (25.5%). When we considered time since EVD discharge, eye problems were most commonly reported within the first quartile of follow-up (4–144 days after discharge; Figure 2, panels B–D). However, these eye problems also were reported much later; the median time from EVD discharge to attendance with an eye problem was 146 days (range 35–380 days).

Referrals to mental health counselling were reported only at 4 attendances by 4 unique survivors, of whom 2 were reported with depression: 1 occurred 146 days and the other 172 days after EVD discharge. However, based on feedback from clinic staff, these mental health referrals were not consistently reported in the medical charts; thus, this figure underestimates the rate of referrals.

Of the 115 survivors, 33 (28.7%) were <18 years of age. Compared with attendances by survivors ≥18 years of age (n = 489), attendances by survivors <18 years of age (n = 131) (age was missing for 1 survivor) were significantly more likely to be reported with fever (72.5% vs. 58.7%, p = 0.015), coughing (42.8% vs. 29.5%, p = 0.015), rash (14.5% vs. 2.7%, p<0.001), and vomiting (16.8% vs. 6.3%, p = 0.002) but less likely to be reported with myalgia (30.5% vs. 46.8%, p = 0.001) and insomnia (7.6% vs. 16.2%, p = 0.009). Survivors <18 years of age were also less likely than those ≥18 years of age to have been reported with eye problems (5.3% vs. 9.0%), but this finding was not statistically significant (p = 0.255). We found no other statistically significant differences in symptoms between survivors <18 years and ≥18 years of age, and all associations remained similar in magnitude and significance after adjustment for time since discharge (data not shown).

When comparing attendances reported with (n = 383) or without (n = 238) fever (Figure 3; Table), we found that cough (p<0.001), headache (p<0.001), vomiting (p = 0.010), and appetite loss (p = 0.028) were significantly more likely to be reported with fever. Itching (p = 0.028), blurred vision (p = 0.034), poor vision (p = 0.043), chest pain (p = 0.044), and anxiety (p = 0.014) were significantly less likely to be reported with fever. Fever was also significantly less likely to be reported at attendances closer to the date of EVD discharge (4–144 days [p<0.001] and 145–254 days [p<0.001] vs. 359–504 days after EVD discharge). Similarly, survivors 29–35 (p = 0.025) and 36–70 years of age (p = 0.010) were significantly less likely than survivors <18 years of age to have fever.

When we determined the frequency of symptoms for the 115 survivors over their entire attendance history (Figure 4), we found the most commonly reported symptoms were headache (93.9% [95% CI 87.9%–97.5%]), fever (93.0% [95% CI 86.8%–96.9%]), and myalgia (77.4% [95% CI 68.7%–84.7%]); eye problems were reported by 37 (32.2% [95% CI 23.8%–41.5%]) survivors. All but 8 survivors were reported as having had fever at least once, so frequencies of symptoms stratified by the presence or absence of fever are not presented.

Morbidity

Of the 621 attendances by the 115 survivors, the most commonly listed differential diagnoses were malaria (52.8% [95% CI 48.8%–56.8%]), musculoskeletal pain (36.1% [95% CI 32.3%–40.0%]), and respiratory tract infections (23.2% [95% CI 19.9%–26.7%]) (Figure 5). However, malaria was not routinely confirmed through diagnostic testing and often was diagnosed on the basis of clinical presentation.

Figure 3. Frequency of symptoms reported at 621 attendances with and without reported fever by 115 survivors of laboratory-confirmed Ebola virus disease at the Survivor Clinic, Kenema Government Hospital, Kenema, Sierra Leone, 2014–2015. Fever was recorded for 61.7% total attendances. Amenorrhea was recorded only for women (age range 15–40 years) and erectile dysfunction only for men (age range 24–35 years). Chest burn is a local term for heartburn. p values are from univariate generalized estimating equations logistic regression analysis. *p<0.01; †p<0.05.

For 45 (7.2%) attendances, musculoskeletal pain was given as the differential diagnosis either solely or in the presence of 1 of the following: abscess, diarrhea, erectile dysfunction, arthritis/sore throat, insomnia, "post-Ebola weakness," scabies, or ulcer. For the 31 attendances for which only musculoskeletal pain was diagnosed in the absence of fever, the median time from EVD discharge was 231 days (range 43–464 days).

Discussion

Our comprehensive review of the medical records of patients attending the Survivor Clinic at KGH revealed that many experienced potential EVD-related sequelae >1 year after hospital discharge. The most commonly reported sequelae at afebrile presentation were headache, myalgia, joint pain, and abdominal pain, which is largely consistent with reports from survivors of the 2013–15 West Africa

Table. Characteristics reported at 621 attendances by 115 survivors of laboratory-confirmed Ebola virus disease attending the Survivor Clinic at Kenema Government Hospital, Sierra Leone, 2014–2015*

Characteristic	Overall†	Fever‡		p value§
		Yes	No	
Sex				
M	187 (30.7) [27.1–34.4]	123 (30.2)	64 (25.1)	0.309
F	423 (69.3) [65.6–72.9]	284 (69.8)	191 (74.9)	Ref
Duration since discharge, d				
4–144	131 (21.1) [18.1–24.5]	61 (14.8)	107 (42.1)	<0.001
145–254	134 (21.6) [18.5–25.0]	96 (23.4)	69 (27.2)	<0.001
255–358	221 (35.6) [31.9–39.5]	125 (30.4)	41 (16.1)	0.321
359–504	134 (21.6) [18.5–25.0]	129 (31.4)	37 (14.6)	Ref
Age, y				
<18	153 (25.0) [21.7–28.5]	109 (26.4)	39 (15.1)	Ref
18–28	148 (24.1) [20.9–27.7]	86 (20.8)	52 (20.1)	0.178
29–35	153 (25.0) [21.7–28.5]	142 (34.4)	110 (42.5)	0.025
36–70	159 (25.9) [22.6–29.6]	76 (18.4)	58 (22.4)	0.010
Median (range)	30.0 (0.25–70.00)	30.0 (0.25–70.00)	33.0 (3–70.00)	0.001
Signs and symptoms¶				
Joint pain	187 (30.1) [26.6–33.8]	113 (29.5)	74 (31.1)	0.627
Myalgia	269 (43.3) [39.5–47.3]	165 (43.1)	104 (43.7)	0.874
Cough	201 (32.4) [28.8–36.2]	171 (44.7)	30 (12.6)	<0.001
Weakness	37 (6.0) [4.3–8.1]	27 (7.1)	10 (4.2)	0.097
Dizziness	89 (14.3) [11.8–17.3]	55 (14.4)	34 (14.3)	0.915
Rash	32 (5.2) [3.7–7.2]	20 (5.2)	12 (5.0)	0.903
Itching	49 (7.9) [6.0–10.3]	24 (6.3)	25 (10.5)	0.028
Blurred vision	19 (3.1) [2.0–4.8]	8 (2.1)	11 (4.6)	0.034
Vision loss	0	0	0	NA
Eye discharge	12 (1.9) [1.1–3.4]	5 (1.3)	7 (2.9)	0.097
Poor vision	15 (2.4) [1.5–4.0]	6 (1.6)	9 (3.8)	0.043
Eye pain	10 (1.6) [0.9–3.0]	7 (1.8)	3 (1.3)	0.837
Eye itch	13 (2.1) [1.2–3.6]	6 (1.6)	7 (2.9)	0.113
Eye irritation	9 (1.4) [0.8–2.8]	8 (2.1)	1 (0.4)	0.119
Eye problems	51 (8.2) [6.3–10.7]	29 (7.6)	22 (9.2)	0.205
Headache	392 (63.1) [59.2–66.8]	272 (71.0)	120 (50.4)	<0.001
Diarrhea	17 (2.7) [1.7–4.4]	14 (3.7)	3 (1.3)	0.116
Vomiting	53 (8.5) [6.6–11.0]	44 (11.5)	9 (3.8)	0.010
Insomnia	89 (14.3) [11.8–17.3]	50 (13.1)	39 (16.4)	0.370
Chest burn	35 (5.6) [4.1–7.8]	20 (5.2)	15 (6.3)	0.562
Chest pain	31 (5.0) [3.5–7.0]	13 (3.4)	18 (7.6)	0.044
Abdominal pain	157 (25.3) [22.0–28.9]	102 (26.6)	55 (23.1)	0.620
Appetite loss	149 (24.0) [20.8–27.5]	104 (27.2)	45 (18.9)	0.028
Depression	6 (1.0) [0.4–2.1]	3 (0.8)	3 (1.3)	0.759
Anxiety	20 (3.2) [2.1–4.9]	7 (1.8)	13 (5.5)	0.014
Mental disorder	2 (0.3) [0.1–1.3]	2 (0.5)	0	NA
Palpitation	42 (6.8) [5.0–9.0]	23 (6.0)	19 (8.0)	0.223
Jaundice	1 (0.2) [0.0–1.1]	1 (0.3)	0	NA
Hiccups	0	0	0	NA
Deafness	4 (0.6) [0.2–1.7]	1 (0.3)	3 (1.3)	0.218
Hearing loss	3 (0.5) [0.2–1.5]	1 (0.3)	2 (0.8)	0.350
Amenorrhea	31 (7.3) [5.2–10.2]	16 (6.3)	15 (8.8)	0.222
Erectile dysfunction	6 (3.2) [1.4–7.0]	1 (0.8)	5 (7.8)	0.047

*Within each cross-tabulation, because of missing data, the sum of the component cells may be less than the total number of attendances. Where a zero cell was reported in the cross-tabulation with fever, no p value is reported. NA, not applicable; Ref, reference category.
†All values are no. (%) [95% CI] unless indicated otherwise indicated.
‡All values are no. (%) unless otherwise indicated.
§p values are from univariate generalized estimating equations logistic regression analysis.

Figure 4. Frequency of symptoms reported by 115 survivors of laboratory-confirmed Ebola virus disease attending the Survivor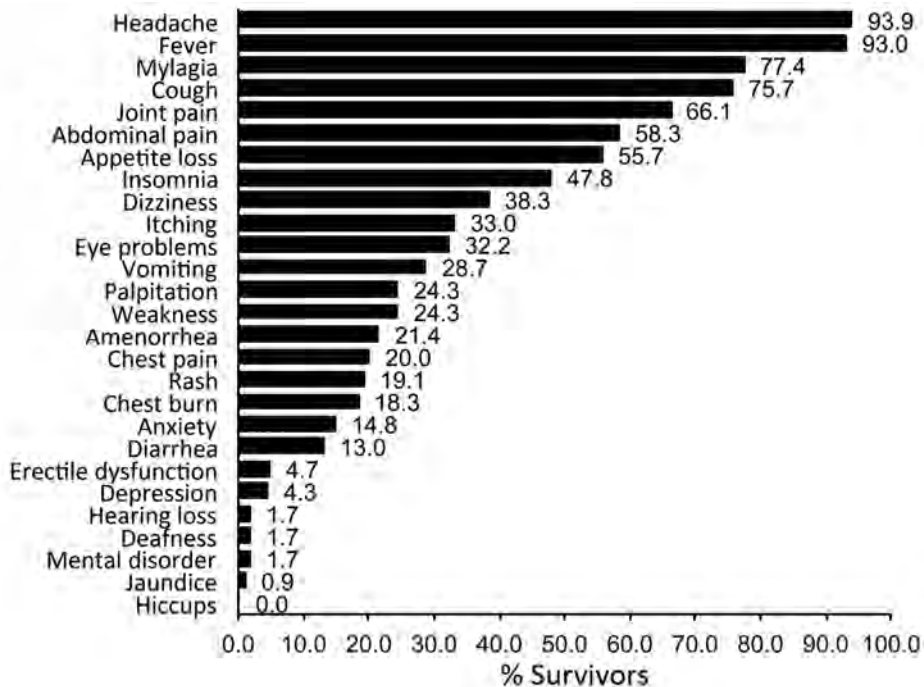 Clinic, Kenema Government Hospital, Sierra Leone, 2014–2015. Eye problems comprise eye irritation, eye pain, eye discharge, itchy eye, poor vision, or blurred vision. Amenorrhea was recorded only for women (age range 15–40 years) and erectile dysfunction only for men (age range 24–35 years). Chest burn is a local term for heartburn.

outbreak (*5,8,14,15*). A retrospective cohort study of EVD survivors and their contacts in Uganda highlighted that long-term sequelae persist for >2 years (*10*), but similar evidence is lacking from the West Africa EVD outbreak, which was caused by a different virus species (Zaire).

Nanyonga et al. reported frequent sequelae at least 4 months after discharge among survivors in Kenema and, because their survey was based on a cross-sectional study of a small convenience sample of survivors, recommended a more systematic assessment of sequelae (*5*). Accordingly, our report is based on an analysis of a longitudinal dataset of all attendances at the KGH Survivor Clinic over a longer period of follow up; thus, it contributes to the understanding of the patterns of sequelae within this cohort. Our findings are consistent with those of the survey by Nanyonga et al. (*5*), which reported joint pain, headache, and myalgia as the most common sequelae, and those of another review of data from medical consultations at a survivor clinic in Freetown, Sierra Leone; in the case of the latter, arthralgia, fatigue, and abdominal pain were the 3 most common complaints (*15*). In the Freetown report, data were reported from presentations earlier in the course of convalescence than in our review, and no distinction was made on the basis of afebrile or febrile status, but the authors were able to evaluate risk factors for uveitis.

New ocular symptoms were reported from >55% of persons attending survivor clinics in Port Loko and Freetown (Sierra Leone), and the incidence of uveitis was high (*6,15*). In comparison, in our analysis, the frequency of eye problems was lower, but this finding may be due to

underreporting because survivors were not assessed by ophthalmologists at the KGH Survivor Clinic. Also, although eye problems were documented in the medical charts, the frequency of uveitis could not be determined because slit-lamp examinations were not provided at the KGH clinic. In our chart review, >90% of the survivors were reported as having had fever at least once since being discharged; although no information is available about the etiology, transient fevers have been reported in a few survivors months after recovery, suggesting that fever might be an underreported sequela of EVD (*11*). Although reports of EVD recrudescence (*16*) are limited, given the high frequency of febrile illness among the survivors at KGH, further research is needed to assess the persistence of Ebola virus in immunologically protected body sites to inform guidelines on retesting.

Our findings suggest that survivors' needs vary with age. Survivors <18 years of age sought care for complaints that differed from those of adults. This finding was reported previously in Freetown; that report, despite using a different age cutoff, also found that persons <16 years of age were more likely than those ≥16 years of age to have rash and were less likely to have insomnia (*15*).

This medical chart review captured data on all attendances by all confirmed EVD survivors attending the Survivor Clinic. We cannot definitively state that symptoms such as myalgia and joint pain did not result from infection with other pathogens endemic to the community, but we were able to assess the frequency of symptoms in the absence of fever. Itching, blurred vision, poor vision, chest
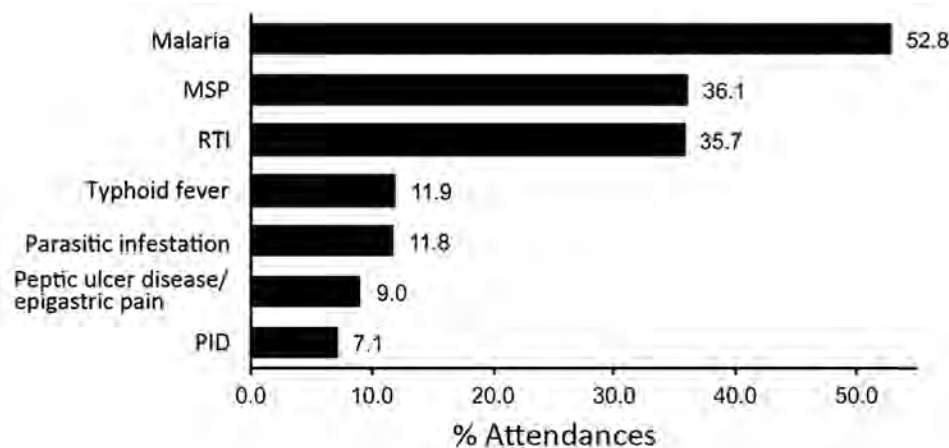
Figure 5. Diseases and conditions with at least a 5% frequency diagnosed at 621 attendances by 115 survivors of laboratory-confirmed Ebola virus disease at the Survivor Clinic at Kenema Government Hospital, Kenema, Sierra Leone, 2014–2015. MSP, musculoskeletal pain; PID, pelvic inflammatory disease (women only); RTI, respiratory tract infection.

pain, and anxiety were more likely to be reported among attendees without fever than with fever. Furthermore, survivors attended the clinic up to 464 days after EVD discharge with the sole complaint or musculoskeletal pain, providing evidence of the persistence of this sequela >1 year after EVD discharge.

Malaria was diagnosed at ≈50% of attendances, but because we restricted our review to EVD survivors, we cannot infer a higher incidence of malaria or of other illnesses in the absence of a comparison group, especially given the estimated increased malaria incidence during the EVD outbreak (*17*). Because many diseases were diagnosed without laboratory confirmation, illnesses might have some degree of misclassification. Most patients attending the KGH Survivor Clinic resided in Nongowa Chiefdom, where KGH is located; EVD survivors from other chiefdoms might be less likely to attend KGH because of the cost and difficulty of transport.

Our review has some limitations because the medical charts did not consistently document the number or outcome of any pregnancies in female survivors or referrals to mental health counseling. Counseling sessions were performed at the Survivor Clinic, so they might have been performed as part of the clinic visit without being documented in the medical chart. Additionally, because our review considered only live survivors, we do not have information about deaths during convalescence due to EVD (*15*), EVD-related sequelae, or non-EVD causes. We were able to assess only the presence of signs and symptoms, and no information was available about their severity. Because our exploratory analysis was to assess the association between each of 33 signs and symptoms with febrile presentation, some associations we found might have been due to chance. Last, our review used only data contained within the medical charts; thus, we have no information about the Ebola virus viral load of survivors while they had acute EVD. Although recently published reports suggest

that higher viral loads are associated with specific sequelae, such as headache or uveitis, we could not assess this (*6,14*).

Given the large cohort of EVD survivors after the West Africa outbreak, a more universal assessment of possible sequelae should be performed in other districts of Sierra Leone. Using district-level registers of survivors, a prospective age, sex and district-matched cohort study could quantify the risk for EVD sequelae and other illnesses. Because this outbreak has ended in the 3 most affected countries and because the health needs of survivors are complex and not yet fully understood (*11*), the risk exists that sustained essential services might not be available for EVD survivors in the region. Rapid chart reviews at survivor clinics should be repeated at regular intervals to review the persistence of sequelae and the incidence of illness, which can, in turn, be used to prioritize service delivery.

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EID SPOTLIGHT TOPIC

Ebola, previously known as Ebola hemorrhagic fever, is a rare and deadly disease caused by infection with one of the Ebola virus strains. Ebola can cause disease in humans and nonhuman primates (monkeys, gorillas, and chimpanzees).

Ebola is caused by infection with a virus of the family *Filoviridae*, genus *Ebolavirus*. There are five identified Ebola virus species, four of which are known to cause disease in humans. Ebola viruses are found in several African countries; they were first discovered in 1976 near the Ebola River in what is now the Democratic Republic of the Congo. Before the current outbreak, Ebola had appeared sporadically in Africa.

The natural reservoir host of Ebola virus remains unknown. However, on the basis of evidence and the nature of similar viruses, researchers believe that the virus is animal-borne and that bats are the most likely reservoir. Four of the five virus strains occur in an animal host native to Africa.

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Cost-effectiveness of Increasing Access to Contraception during the Zika Virus Outbreak, Puerto Rico, 2016

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We modeled the potential cost-effectiveness of increasing access to contraception in Puerto Rico during a Zika virus outbreak. The intervention is projected to cost an additional $33.5 million in family planning services and is likely to be cost-saving for the healthcare system overall. It could reduce Zika virus–related costs by $65.2 million ($2.8 million from less Zika virus testing and monitoring and $62.3 million from avoided costs of Zika virus–associated microcephaly [ZAM]). The estimates are influenced by the contraception methods used, the frequency of ZAM, and the lifetime incremental cost of ZAM. Accounting for unwanted pregnancies that are prevented, irrespective of Zika virus infection, an additional $40.4 million in medical costs would be avoided through the intervention. Increasing contraceptive access for women who want to delay or avoid pregnancy in Puerto Rico during a Zika virus outbreak can substantially reduce the number of cases of ZAM and healthcare costs.

Zika virus infection during pregnancy can cause microcephaly with severe brain damage in the fetus (referred to here as Zika virus–associated microcephaly [ZAM]) and is linked to pregnancy loss and to problems in infants, including eye defects, hearing loss, and impaired growth (*1*). Zika virus is a flavivirus transmitted primarily by infected *Aedes* species mosquitos (*2*). Zika virus can also be sexually transmitted (*3*). Puerto Rico

has the largest number of Zika virus disease cases in the United States and its territories (*4*) and, based on extrapolations from the experiences of other countries with Zika virus outbreaks, will probably experience large numbers of Zika virus–exposed pregnancies (*5*).

A primary strategy to reduce Zika virus–associated adverse pregnancy outcomes is to assist women who want to delay or avoid pregnancy. An estimated 65% of pregnancies in Puerto Rico are unintended (unwanted or mistimed), compared with 45% in the continental United States (*2,6*). Women in Puerto Rico face multiple barriers to contraceptive use, including high out-of-pocket costs, a shortage of contraceptive supplies, lack of education about options, and a limited number of family planning delivery sites (*2*).

In response to the Zika virus outbreak, the Centers for Disease Control and Prevention and other federal and local partners are seeking to improve access to contraception for women in Puerto Rico who desire it but encounter barriers to accessing the full range of contraception methods, including long-acting reversible contraceptives (LARCs). The objective of this analysis was to estimate the potential cost-effectiveness of increasing access to contraception in Puerto Rico during the 2016 Zika virus outbreak.

Methods

We constructed a decision tree cost-effectiveness model for a target population of 163,000 women who at the time of the intervention are sexually active with a male partner, fertile, not desiring pregnancy within the next 12 months, and not using permanent contraception methods (e.g., tubal ligation and vasectomy) (online Technical Appendix Table and Figure 1, http://wwwnc.cdc.gov/EID/article/23/1/16-1322-Techapp1.pdf). In the no intervention scenario, no changes in contraceptive use distributions from the status quo are expected to occur. In the intervention scenario, women in Puerto Rico are assumed to

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have same-day access to contraception methods, including LARC, with no out-of-pocket costs. In addition, healthcare providers would be trained to provide client-centered contraceptive counseling and outreach so that women have the information they need to make an informed choice on the contraception method that is best for them. The model specifies contraceptive method use distribution, unintended pregnancy events, and the frequency of ZAM (online Technical Appendix Figure 1).

We assumed an intervention in place throughout a year-long Zika virus outbreak in Puerto Rico. We evaluated the costs and outcomes of increased access to contraception compared with no intervention (i.e., status quo). Output measures included numbers of ZAM cases prevented, including stillbirths, elective terminations, and live-born infants, and healthy life years (HLY) gained. Economic benefits of the intervention included avoided costs from ZAM cases prevented and costs avoided for monitoring for Zika virus–exposed pregnancies and infants born from Zika virus–infected mothers. In addition, the avoided cost of prenatal, delivery, postpartum, and neonatal care associated with avoided unwanted pregnancies was considered an economic benefit. In cost-effectiveness analyses, if total avoided cost exceeds the cost of an intervention that improves health, the intervention is considered cost-saving. For scenarios with positive net costs, we reported the incremental cost-effectiveness ratio (ICER), which is the net cost per HLY gained in comparison to the status quo.

Independent of Zika virus–exposed pregnancies and ZAM, unintended pregnancy is associated with adverse maternal and child health outcomes. Because roughly 60% of unintended pregnancies are classified as mistimed, which might result in a delayed rather than avoided pregnancy, with the same costs occurring later (7), we only estimated avoided medical costs from prevention of the 40% of unintended pregnancies presumed to be not desired at a later time irrespective of Zika virus infection. However, we included all ZAM cases prevented during the intervention period.

Contraception Use with and without the Intervention

We estimated the inputs for the decision-tree model and their sources (Table 1, http://wwwnc.cdc.gov/EID/article/23/1/16-1322-T1.htm). In the no intervention scenario, we took the distribution of women in the target population by use of different types of reversible contraceptives (or no use) from a 2002 survey administered in Puerto Rico and adjusted it to reflect the 36% decrease in fertility rates in Puerto Rico during 2002–2015 (8,23,24).

For the main intervention scenario, we assumed that 50% of no contraception users, 60% of less-effective contraceptive method users, and 100% of moderately effective contraceptive method users would visit a healthcare provider during the intervention period and be counseled about contraception use (Table 1). The first 2 percentages are roughly twice the percentages of women reported in the 2011–2013 US National Survey on Family Growth to have received contraceptive services (contraception or counseling) within the past year because we assumed that, during the Zika virus outbreak, more women and providers would discuss contraception; virtually all moderately effective method users were assumed to see providers to obtain contraceptive prescriptions.

For the main scenario, we also assumed, optimistically, that 50% of women in the target population who receive contraceptive services during the Zika virus outbreak would be willing to change to a more effective contraceptive method, evenly divided between moderately effective and highly effective methods. We applied data from the Contraceptive CHOICE Project (67% of participants used LARC and 33% used moderately effective methods) (9) to the 40% of women assumed to not want to be pregnant; we assumed 20% of other women not intending pregnancy would use LARC. We further assumed that 30% of moderately effective contraception users would also choose to use condoms (dual-method use) under the intervention, based on a study reporting dual-method use among persons at risk for HIV (25).

Epidemiologic Model Input Parameters

We calculated method-specific annual pregnancy rates by applying failure rates of contraception methods under typical use (10), in combination with information on estimated numbers of unintended pregnancies, to adjust for other factors influencing pregnancy risk (19). We estimated the proportion of fetal losses among unintended pregnancies from data for the Caribbean region, including Puerto Rico (12), and calculated the proportion of induced abortion among unintended pregnancies from a survey conducted in Puerto Rico in 2001 (the latest year for which data were available) (11). We assumed that the distribution of fetal loss and induced abortions in unintended pregnancies unaffected by ZAM would not be altered by the Zika virus outbreak or the intervention.

For adverse pregnancy and birth outcomes associated with Zika virus, we only considered ZAM and associated brain anomalies, including live births, stillbirths, and terminations attributable to prenatal diagnosis. Although Zika virus can cause brain lesions and dysfunction in fetuses and newborns who do not have microcephaly (26), we lacked the data to model their prevalence and cost. In the main analysis, we assumed 58 cases of ZAM per 10,000 live births (range 32–86/10,000) based on a modeling study that considered data from other mosquitoborne illnesses in Puerto Rico and Zika virus outbreaks in other locations (5). We assumed a pregnancy loss rate of 35% among Zika

virus–exposed fetuses with diagnosed birth defects based on cases in the US Zika Pregnancy Registry as of July 21, 2016 (*14*).

A summary measure of population health impact is healthy life expectancy at birth. We projected gains in HLY by multiplying total cases of ZAM prevented by 30.0, which is the average number of quality-adjusted life-years at birth in the United States for an infant without severe microcephaly (*15*) and the estimated loss in disability-adjusted life years from microcephaly (*27*). We multiplied 30.0 by the sum of live births and fetal losses associated with ZAM to calculate gains in HLY. We included fetal losses in the HLY calculations because in the absence of ZAM those pregnancies would have resulted in live births, with the same healthy life expectancy as other children (*15*).

Cost Parameters

We conducted the analysis from a healthcare system perspective that includes direct medically related costs regardless of payer. We used payments from private insurance because payments from Medicaid might underestimate the cost of healthcare (*28*). Intervention costs included program costs of training providers, patient educational materials, outreach/media campaigns on the availability of contraceptives services, and program coordination and the incremental costs of family planning services. The latter comprised the costs of contraception methods and related office visits and services (e.g., insertion and removal of LARC for new method users resulting from the intervention and the cost of more intensive counseling for all women receiving contraceptive services during the intervention). We took the 1-year costs for contraception methods from the literature (*16,29*) and based the other program costs on the estimated costs for a pilot program planned to increase access to contraception in Puerto Rico as part of the current Zika virus outbreak response (*30*). We did not apply a discount rate to intervention costs because of the time horizon of 12 months.

Zika virus–related costs prevented by this intervention were in 2 parts: 1) costs for Zika virus testing and monitoring for Zika virus–exposed pregnancies and infants, and 2) costs of ZAM cases (Table 1). The cost estimates for testing and monitoring presumed 100% adherence by clinicians and patients to recommendations (*20–22*).

The lifetime cost per live-born infant with ZAM includes direct medical and nonmedical costs. ZAM is among the most severe types of microcephaly and is associated with loss of brain tissue volume, increased fluid spaces, and intracranial calcifications. All 3 cases of live-born infants with ZAM in French Polynesia demonstrated severe neurologic outcomes with delayed cognitive development (*26*). On the basis of expert opinion, infants with ZAM who survive the neonatal period would be expected to have neurologic dysfunction consistent with severe cerebral palsy within 1–2 years of birth.

As a proxy for the medical cost of ZAM, we used the estimated cost of treating infants with microcephaly associated with a diagnosis of symptomatic congenital cytomegalovirus (CMV). We used the MarketScan Commercial Database (Truven Health Analytics) with a sample of ≈100 million US residents covered by employer-sponsored insurance at any time during 2009–2014. We used average costs for 4 newborn infants with diagnoses of microcephaly and CMV who survived and were enrolled in a health plan for ≥3 years. For the direct nonmedical cost of ZAM, we used the estimated cost for supportive care for children with severe congenital brain injury, both paid care and unpaid care. The total lifetime cost for surviving infants with ZAM was estimated at $3.8 million per infant, taking into account infant and child mortality and discounting of costs in future years at a 3% rate per year; the sum of undiscounted costs for children who survive to adulthood might reach $10 million.

We determined the estimated non–Zika virus–related medical costs associated with women's prenatal care, labor and delivery, and postpartum care for pregnancies ending in live birth and neonatal care from a study of US commercial health plan expenditures (*17*). Estimates for costs associated with pregnancies ending in induced abortion were based on our analyses of commercial claims data (Table 1).

Sensitivity Analyses

Because many parameters used in the model are uncertain, we conducted sensitivity analyses on selected parameters, including different scenarios for the baseline and postintervention contraception use distributions in Puerto Rico. We tested alternate baseline contraception use distributions in Puerto Rico for women at risk for unintended pregnancy by using the actual distribution of method use reported in 2002 (*8*) and among women attending Title X clinics in Puerto Rico in 2014 (*31*). For the postintervention contraception use distribution, we tested scenarios assuming different proportions of women receiving contraceptive services from a healthcare provider, different levels of willingness to switch to a more effective method, and different shares of moderately effective and highly effective methods among switchers. Other parameters evaluated during sensitivity analysis included the incidence of ZAM during the Zika virus outbreak in Puerto Rico, percentage of pregnancies with ZAM terminated, the cost of caring for a live-born infant with microcephaly, and the cost of the intervention.

We conducted sensitivity analyses in which we altered selected assumptions. In one, we annualized the cost of LARC devices considering the expected duration of method use. In another, we adjusted observed data on US healthcare and supportive care costs to the generally lower levels

of prices in Puerto Rico market by applying conversion factors of ratios of healthcare spending per capita and wages of nurse assistants between the United States and Puerto Rico (*32,33*). We also conducted a probabilistic sensitivity analysis by using Monte Carlo simulation (10,000 draws) that assumed different distributions for all the parameters used in the model (Table 1). All analyses were conducted using TreeAge Pro 2016 software (TreeAge Software, Williamstown, MA, USA) and Excel 2013 (Microsoft, Redmond, WA, USA). All costs were adjusted to 2014 US dollars by using the health component of the Personal Consumption Expenditures price index (*34*).

Results

In the main scenario, we predict the intervention would prevent 25 cases of ZAM among unintended pregnancies avoided, of which 16 would have resulted in live births (Table 2). The incremental intervention cost of US $33.5 million (i.e., $206 per member of target population) relative to no intervention (status quo) is more than offset by $65.2 million in avoided Zika virus–associated costs, $2.8 million from extra testing and monitoring for pregnant

women and infants for Zika virus–exposed pregnancies avoided, and $62.3 million from ZAM cases prevented. The net savings from Zika virus–associated costs alone is $31.7 million.

The number of ZAM cases prevented and Zika virus–associated costs avoided are sensitive to the proportion of women receiving contraceptive services and the proportion of those women willing to switch to a more effective contraception method during the Zika virus outbreak (Figure; Table 3). If the proportions of women receiving contraception services are assumed to be the same as estimated for the continental United States in the National Survey of Family Growth for 2011–2013 (i.e., 21% among no contraception users, 33% among less-effective method users, and 97% among all moderately effective method users), 16 cases of ZAM are prevented, and the net savings is $15.4 million (Table 3). If 10% of women receiving contraceptive services switch to a more effective method, 6 cases of ZAM are prevented, and net saving is $2.8 million. If the intervention only shifts users of moderately effective methods to a highly effective method (no change in non-use or use of less-effective methods), 7 ZAM cases are prevented,

Table 2. Zika virus–associated microcephaly cases and costs, as well as additional costs associated with unwanted pregnancies, with and without intervention to increase access to contraception to women during the Zika virus outbreak, Puerto Rico, 2016, in main scenario*†‡

Parameter	Without intervention	With intervention	Difference
Prevention of ZAM and Zika virus–associated cost			
Total no. ZAM cases	99	74	−25
No. pregnancy terminations	28	21	−7
No. stillbirths	7	5	−2
No. live births	64	48	−16
Cost of family planning services (under intervention also includes program cost)	$38,269,679	$71,738,133	$33,468,454
Total Zika virus–associated cost	$256,578,162	$191,422,342	−$65,155,820
Costs of extra testing and monitoring for Zika virus during pregnancy and for infants exposed in utero during Zika virus outbreak§	$11,125,061	$8,303,158	−$2,821,903
Direct costs of ZAM¶	$245,453,101	$183,119,184	−$62,333,917
Pregnancy terminations	$139,343	$103,956	−$35,387
Stillbirths	$40,025	$29,861	−$10,165
Live births	$245,273,733	$182,985,368	−$62,288,366
Cost savings from Zika virus–associated cost avoided only#			−$31,687,366
Prevention of unwanted pregnancies			
No. of unwanted pregnancies**	11,995	8,949	−3,046
No. induced abortions	3,385	2,525	−860
No. spontaneous abortions and fetal deaths	1,679	1,253	−426
No. unwanted live births	6,856	5,117	−1,739
Medical cost for unwanted pregnancy	$159,074,573	$118,722,504	−$40,352,069
Net cost savings from avoiding both Zika virus–associated cost and unwanted pregnancy cost††			−$72,039,435

*ZAM, Zika virus–associated microcephaly.
†The numbers in the columns and rows might not exactly match because of rounding.
‡Target population size: 163,000 women who do not intend to become pregnant during Zika virus outbreak. Women of reproductive age in Puerto Rico who are sexually active with a male partner, fertile, not desiring pregnancy, and not using permanent contraception methods (e.g., tubal ligation and vasectomy).
§Only including cost of testing for Zika virus and monitoring for exposed infants without ZAM; testing costs for infants with ZAM are included in the direct costs of ZAM.
¶From healthcare system perspective, includes direct medical and medical-related costs, including supportive care for persons with ZAM, even if the cost might not be paid by healthcare payers or delivered by healthcare providers.
#Total Zika virus–associated cost avoided (absolute value) minus the additional cost of family planning service under intervention compared with no intervention.
**Unwanted pregnancies which are not desired in the future (assuming 60% of unintended pregnancies are mistimed), irrespective of Zika virus infection
††Absolute value of net medical cost for unwanted pregnancy plus absolute value of net cost savings from Zika virus–associated costs avoided.
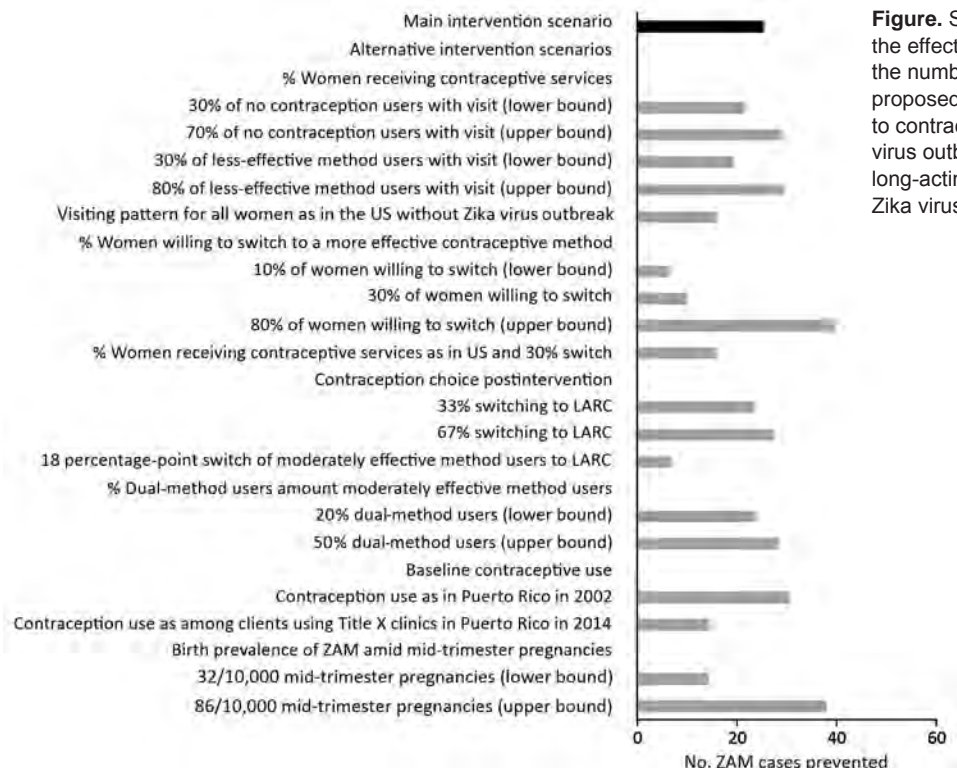
Figure. Sensitivity analysis indicating the effect of changes of assumptions on the number of ZAM cases prevented in a proposed intervention to increase access to contraception to women during the Zika virus outbreak, Puerto Rico, 2016. LARC, long-acting reversible contraceptive; ZAM, Zika virus–associated microcephaly.

with an ICER of $24,608/HLY gained. Increasing the proportion of dual-method users increases the number of cases of ZAM prevented and net savings attributable to higher contraception effectiveness. The results are also sensitive to the prevalence of ZAM among mid-trimester pregnancies, the percentage of ZAM cases resulting in live-born infants, lifetime cost per live-born infant with ZAM, and the intervention cost. If we adjust US cost estimates for lower prices in Puerto Rico while keeping intervention costs at US prices, net savings are $1.7 million. In all but 1 of the scenarios tested, the intervention is cost-saving.

A probabilistic sensitivity analysis scatter graph shows that most of the model simulations result in ICERs in the lower right quadrant with lower costs and better health outcomes (online Technical Appendix Figure 2). Specifically, the intervention is cost-saving in 92.11% of the 10,000 iterations, and in 98.10% of the iterations, the intervention has an ICER of <$20,000/HLY gained.

The intervention is also predicted to prevent $40.4 million in medical costs from unwanted pregnancies avoided in the main scenario (Table 2). In many sensitivity analyses, the cost avoided from these unwanted pregnancies prevented alone is greater than the intervention cost. The larger the numbers of no contraception users and less-effective method users receiving contraceptive services and willing to switch to more effective methods, the greater the magnitude of cost savings from unwanted pregnancies avoided (Table 3).

Discussion

The results of our modeling analysis suggest that increasing access to effective contraception in the context of the 2016 Zika virus outbreak for women in Puerto Rico who do not intend to become pregnant could proportionally reduce the number of unintended pregnancies and cases of ZAM by 25%. The intervention is cost-saving (negative net cost) when considering the benefits from preventing ZAM and avoiding Zika virus–exposed pregnancy costs in the main scenarios and in most of the scenarios we tested. In scenarios in which the intervention is not cost-saving, it is still cost-effective relative to accepted cost-effectiveness thresholds (*35*). The World Health Organization suggests that interventions that cost <3 times the gross domestic product per capita per HLY (equivalent to $150,000 in the United States and $60,000 in Puerto Rico) are cost-effective and those costing less than gross domestic product per capita are highly cost-effective (*36*). When considering additional benefits from preventing unintended pregnancies not desired at a later time, the intervention is cost-saving in all scenarios. Previous studies have shown that expanding access to contraception, especially LARC, is cost-saving (*16,37,38*). Likewise, our findings suggest that this intervention could be cost-saving or cost-effective within the context of a public health emergency response.

Our study has several limitations. First, we project the effects of a hypothetical intervention in place in

Table 3. Sensitivity analyses indicating the number of ZAM cases prevented and Zika virus–associated costs avoided in proposed intervention to increase access to contraception to women during Zika virus outbreak, Puerto Rico, 2016*

Parameter	No. ZAM cases prevented	Incremental intervention cost, millions	Zika virus–associated cost avoided, millions	Total incremental cost,† millions	Cost per HLY gained	Additional cost avoided from UP, millions
Main scenario	25	$33.5	$65.2	−$31.7	CS	$40.4
% Women receiving contraceptive services from healthcare provider; main scenario, 50% of no method users, 60% of less-effective method users, and 100% of moderately effective method users						
30% of no method users‡	22	$32.4	$55.8	−$23.5	CS	$34.6
70% of no method users	29	$34.6	$74.5	−$39.9	CS	$46.1
30% of less-effective method users	19	$26.0	$50.0	−$24.0	CS	$31.0
80% of less-effective method users	29	$38.5	$75.2	−$36.8	CS	$46.6
% Women receiving contraceptive services as in NSFG 2011–2013§	16	$25.2	$40.6	−$15.4	CS	$25.1
% Women willing to change to more effective method;¶ main scenario value: 50%						
10%	6	$13.0	$15.8	−$2.8	CS	$9.7
30%	16	$23.2	$40.5	−$17.3	CS	$25.0
80%	39	$48.8	$102.2	−$53.3	CS	$63.3
% Women receiving contraceptive services from healthcare provider as in NSFG 2011–2013 with 30% of them willing to change to a new method	10	$18.2	$25.7	−$7.6	CS	$15.9
Use of highly effective methods among switchers; main value 50%						
67%	27	$38.4	$69.9	−$31.5	CS	$43.3
33%	23	$28.5	$60.4	−$31.8	CS	$37.4
Contraception switching pattern reported in Colorado Family Planning Initiative#	7	$21.8	$17.0	$4.8	$24,608	$10.5
Dual-method use; 30% of moderately effective method users in main scenario						
20% of moderately effective users	24	33.1	61.3	−$28.2	CS	$38.0
50% of moderately effective users	28	34.1	−72.9	−$38.7	CS	$45.1
Contraception use distribution at baseline						
As reported in 2002 BRFSS survey**	30	33.6	−78.4	−$44.8	CS	$48.6
As in Title X clinics in 2014††	14	$30.1	$36.7	−$6.6	CS	$22.7
Rate of ZAM among all live-born infants; main scenario value 58/10,000						
32/10,000	14	$33.5	$37.5	−$4.0	CS	$40.4
86/10,000	38	$33.5	$96.3	−$62.8	CS	$40.3
Lifetime costs for microcephaly; main scenario value $3.8 million						
$1.9 million	25	$33.5	$33.5	0	CN‡‡	$40.4
$2.2 million	25	$33.5	$39.5	−$6.1	CS	$40.4
$5.5 million	25	$33.5	$93.5	−$60.0	CS	$40.4
Termination of pregnancy with ZAM						
20%	25	$33.5	$72.8	−$39.3	CS	$40.4
50%	25	$33.5	$44.1	−$10.6	CS	$40.3
Cost of the program other than providing the contraception at no cost to patients; main scenario value $39/person						
$0/person	25	$27.1	$65.2	−$38.0	CS	$40.4
$100/person	25	$43.4	$65.2	−$21.8	CS	$40.4
Annualized LARC device cost	25	$17.5	$65.2	−$47.7	CS	$40.4
Puerto Rico costs§§	25	$30.8	$32.5	−$1.7	CS	$14.4
Discount rate						
0%	25	$33.5	$105.4	−$72.0	CS	$40.4
5%	25	$33.5	$52.9	−$19.4	CS	$40.4

*BRFSS, Behavioral Risk Factor Surveillance System; CN, cost-neutral; CS, cost-saving; HLY, healthy life years; LARC, long-acting reversible contraceptive; NSFG, National Survey of Family Growth; UP, unwanted pregnancy; ZAM, Zika virus–associated microcephaly.
†Total incremental cost is the additional cost of contraception minus Zika virus–associated cost avoided.
‡30% of no contraception users, 60% of less-effective contraceptive method users, 100% of moderately effective contraceptive method users seeking contraceptive services from healthcare provider during the Zika virus outbreak.
§Based on NSFG 2011–2013, among women of reproductive age who are sexually active, did not intend to become pregnant, and were not using permanent contraceptive methods, 21% of no contraception users, 33% of less-effective contraceptive method users, 97% of moderately effective contraceptive method users, and 94% of dual-method users had at least 1 contraceptive service visit in the last 12 months (in total 50%).
¶Based on Title X Family Planning annual report for 2007–2015 in Colorado, 30% of clients who visited Title X clinics switched to a new method.
#Eighteen percentage points of users of moderately effective methods are assumed to switch to highly effective methods, of whom 21% were dual-method users.
**Contraception distribution in Puerto Rico in 2002 15.9% no method, 41.6% less-effective methods, 40.2% moderately effective methods, and 2.4% highly effective methods.
††In 2014, in Title X clinics in Puerto Rico, 20% of women at risk for unintended pregnancy used less-effective methods, 77% used moderately effective methods, and 2% used highly effective methods.
‡‡Intervention cost equals to the medical savings from ZAM cases prevented.
§§Conversion factor of 0.36 applied to pregnancy and ZAM medical costs based on the ratio of per capita medical expenditure in Puerto Rico and in the United States in 2012 as in Portela et al. 2015 (32); conversion factor of 0.72 applied to costs of supportive care for live-born infants with ZAM, based on the ratio of annual salary for assistant nurses in Puerto Rico and in the United States (33).

Puerto Rico during the 2016 Zika virus outbreak. However, the qualitative results would apply in future outbreaks. Second, the baseline contraception use distribution is based on a 2002 survey; the current distribution in Puerto Rico might be different. Third, uncertainty exists about the effect of the proposed intervention on postintervention contraceptive use distribution; however, the sensitivity analyses indicate that different distributions of LARC types among switchers does not have a substantial influence on the results. Fourth, our study assumes that women have full access to healthcare providers. In areas with limited access to providers, the effectiveness of the intervention might be lower, although Puerto Rico has a similar ratio of physicians to population as the United States as a whole (39), and despite a loss of physicians in recent years, Puerto Rico has a network of providers, federally qualified health clinics, and Title X providers in rural and urban areas. Fifth, the distribution of outcomes of unintended pregnancies in Puerto Rico is uncertain. We lack data on miscarriage and induced abortion rates in Puerto Rico and so did not have sufficient data to model uncertainty in these parameters. The rates of stillbirth and pregnancy termination among pregnancies with ZAM in Puerto Rico are also unknown. Our assumed percentage of live births among pregnancies with recognized ZAM (65%) compares with a 38% rate reported in French Polynesia during the 2013 Zika virus outbreak (11). Sixth, pregnancy intentions and use of contraception among women in Puerto Rico might differ during the Zika virus outbreak compared to preoutbreak periods. Seventh, our analysis does not consider possibly higher rates of fetal loss and induced abortion among women infected by Zika virus during early pregnancy or brain abnormalities or conditions related to Zika virus not involving microcephaly. Eighth, the assumed Zika virus testing costs assume 100% adherence to recommended testing practices; the actual cost savings taking nonadherence into account would be lower. Ninth, the cost estimates of ZAM cases in live-born infants do not include costs of managing mental health conditions among parents of affected infants. Tenth, using private insurance payments might overstate the healthcare cost of treating ZAM. However, if the cost of ZAM exceeds $1.9 million, the intervention is still cost-saving. Finally, if efforts to prevent transmission of Zika virus in Puerto Rico are effective, the rate of infection in pregnancy and the incidence of ZAM relative to that projected could be reduced.

Despite its limitations, our study has several strengths. First, the study is based on the most current available information. Second, the contraception scenarios are based on real-world programs and have resulted from consultation with subject matter experts. Third, expenditure data from a large sample of US residents with commercial health insurance were used to calculate the potential medical cost of ZAM on the basis of combinations of diagnostic codes for virus-associated microcephaly, although costs might be lower for similar children with public insurance. Finally, sensitivity analyses give consistent results indicating expected net cost savings associated with an intervention that would increase access to contraception in response to the Zika virus outbreak in Puerto Rico.

Zika virus can cause devastating birth defects, and infants born with ZAM and their families will require lifelong support. Avoiding unintended pregnancies is a critical intervention to mitigate the effects of ZAM. Efforts to prevent adverse Zika virus–related pregnancy outcomes in Puerto Rico are especially important because of limited resources (40). Our analyses suggest that increasing access to a full range of contraception among women in Puerto Rico who want to delay or avoid becoming pregnant during a Zika virus outbreak would be a cost-saving strategy to reduce the effects of ZAM. The magnitude of cost savings is even greater when considering the avoided cost of unwanted pregnancies prevented.

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- Antibodies against MERS Coronavirus in Dromedary Camels, Kenya, 1992–2013
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- Shelter Dogs as Sentinels for *Trypanosoma cruzi* Transmission across Texas, USA
- Natural Intrauterine Infection with Schmallenberg Virus in Malformed Newborn Calves
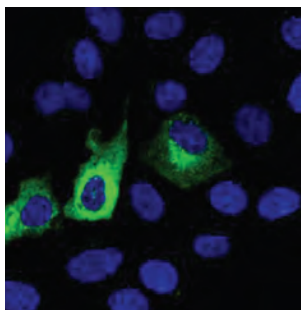
- Role of Migratory Birds in Spreading Crimean-Congo Hemorrhagic Fever, Turkey
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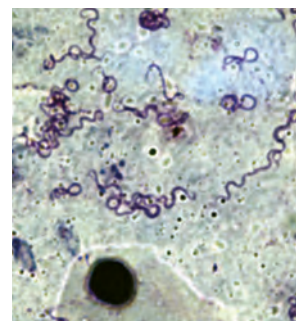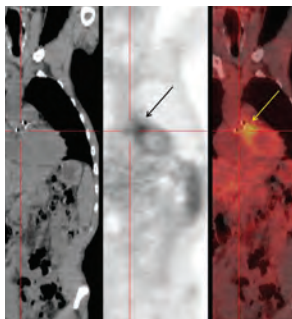
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- Detection of East/Central/South African Genotype of Chikungunya Virus in Myanmar, 2010
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EMERGING INFECTIOUS DISEASES

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Host-Associated Absence of Human Puumala Virus Infections in Northern and Eastern Germany

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Human hantavirus disease cases, caused by Puumala virus (PUUV), are mainly recorded in western and southern areas of Germany. This bank vole reservoir survey confirmed PUUV presence in these regions but its absence in northern and eastern regions. PUUV occurrence is associated with the presence of the Western bank vole phylogroup.

Puumala virus (PUUV) causes most hantavirus disease cases in Central and Western Europe and is the only human pathogenic hantavirus in Fennoscandia (*1*). The human infection is characterized by a mild-to-moderate form of hemorrhagic fever with renal syndrome designated nephropathia epidemica (NE), with a case fatality rate of <0.1%. The only virus reservoir in Central and Western Europe is the bank vole, *Myodes glareolus* (*1*).

PUUV causes most human hantavirus infections in Germany, with an incidence of 10.31 cases/100,000 inhabitants (*2*). Human disease reports fluctuate temporally with peaks in the years 2007, 2010, and 2012, but reports also show a heterogeneous spatial distribution (*2,3*). Generally and during outbreak years, the highest numbers of cases occurred in the western and southern parts of Germany, whereas in the northern and eastern parts of the country only a few cases were recorded (Figure 1, panel A).

Molecular analyses of bank voles from endemic regions detected the presence of PUUV at 30 sites in Germany (Figure 1, panel A) and resulted in the definition of several PUUV sublineages of the Central European (CE) clade (*3,8*). In addition, an 8-year monitoring study on the bank vole populations in a PUUV-endemic region of north-

western Germany indicated the long-term presence of particular PUUV strains (*4*).

To evaluate potential reasons for the almost total absence of human PUUV infections in northern and eastern Germany, we investigated bank voles from these regions and from PUUV-endemic regions in the western and southern parts of Germany for the presence of PUUV and typed the voles to major evolutionary lineages on the basis of *cytochrome b* gene sequences.

The Study

A total of 1,774 bank voles were collected by partners of the network Rodent-borne Pathogens (*3,5,6,9–11*) at sites in PUUV-endemic regions of western and southern Germany and sites in the eastern and northern parts of Germany (Figure 1, panel A; online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/23/1/16-0224-Techapp1.pdf). Chest cavity lavage samples of voles were investigated by IgG ELISA using a recombinant nucleocapsid protein of PUUV (*6*). For molecular PUUV detection, RNA was isolated from lung or heart tissue by using a QIAzol Lysis Reagent (QIAGEN, Hilden, Germany) extraction protocol. The RNA samples were subjected to small (S) segment reverse transcription PCR (RT-PCR) with primer pair Pu342F and Pu1102R (*6*), and the resulting cDNAs were sequenced. RNA samples were also subjected to a novel PUUV S segment–specific real-time RT-PCR with primers PUUV S-broad-F (5′-AACCCGCCATGAACAA-CAAC-3′) and PUUV S-broad-R (5′-TGCTGACACT-GTTTGTTGCC-3′) and fluorescence reporter probe PUUV S-broad (5′-6-FAM-GGAAATGGACCCAGATGACGT-BHQ-1-3′) (for further details see footnote of online Technical Appendix Table).

First, serologic investigation of 1,758 chest cavity lavage samples indicated 99 seropositive voles exclusively originating from the endemic regions in southern and western Germany (Figure 1, panel A; online Technical Appendix Table). This analysis failed to detect any antibody-positive animals within the 1,210 bank voles of this panel originating from the eastern and northern parts of Germany.

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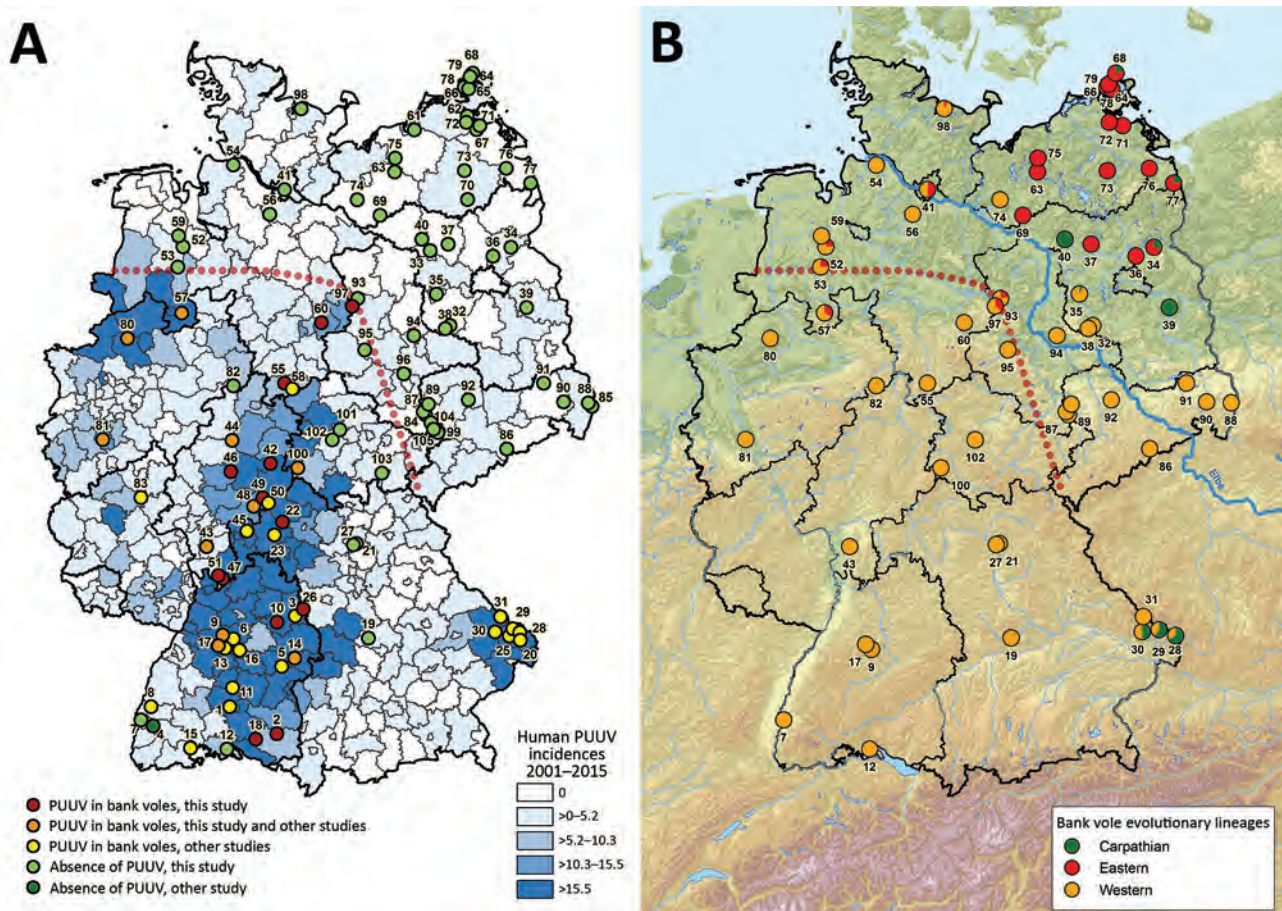
Figure 1. Geographic distribution of Puumala virus (PUUV)–positive and PUUV-negative bank voles in Germany (A) and assignment of bank voles to the evolutionary lineages Western, Eastern, and Carpathian (B). The coloration of the map in panel A was generated on the basis of the human PUUV incidence per district (*2*). PUUV detection in previous studies was extracted from (*3–7*). The identification of the bank vole evolutionary lineages shown in panel B was determined by using partial *cytochrome b* gene sequences (see Figure 2). The red dotted line illustrates the hypothetical current edge of the range of PUUV-positive bank voles.

Subsequent conventional PUUV RT-PCR analysis of RNA samples from 440 voles (comprising 86 seropositive and 334 seronegative voles, 9 with equivocal results, and 11 not investigated because of the lack of chest cavity lavage samples) revealed 79 positive and 361 negative samples (online Technical Appendix Table). All RT-PCR–positive samples again only originated from the PUUV-endemic regions. A final real-time RT-PCR investigation of 364 RNA samples, 34 being positive and 329 being negative by conventional RT-PCR, confirmed the results of the conventional RT-PCR analysis.

Including results of previously published studies (*3,4,7*), PUUV seroprevalence in the endemic regions showed an average of 23.9% and varied between 4.6% and 66.7% (online Technical Appendix Table). According to the serologic and RT-PCR data, a PUUV-endemic region can be identified spanning the western and southern parts of Germany (Figure 1, panel A, below the dotted red line). In this study, the easternmost PUUV-positive sites were located in Saxony-Anhalt

(site 97), Lower Saxony (site 60), and Thuringia (site 100) (*7*). The northernmost sites were located in Lower Saxony (sites 57 and 60) and Saxony-Anhalt (site 97). Nucleotide sequence determination and subsequent phylogenetic analysis showed that all PUUV sequences belong to the CE PUUV clade, which is divergent from other European PUUV lineages (online Technical Appendix Figure).

To test for a potential association between PUUV distribution in the reservoir and evolutionary bank vole lineages, we isolated mitochondrial DNA from 383 selected voles by using the GeneMATRIX Tissue DNA Purification Kit (Roboklon, Potsdam, Germany) according to manufacturer's guidelines. The *cytochrome b* PCR was performed and used for determination of the bank vole evolutionary lineages as described previously (*12*).

The *cytochrome b*–based typing revealed the presence of the bank vole Western, Eastern, and Carpathian evolutionary lineages (Figure 2). Most of the territory of

Germany was inhabited by the Western evolutionary lineage, with its northern and eastern borders located close to the Elbe River (Figure 1, panel B). The distribution of the Eastern lineage ranged over almost the entire northern part of Germany, with partial sympatric occurrence of the Carpathian lineage in the northeast (sites 34, 68,

77) and the Western lineage in the central and northwest (sites 41, 52, 53, 57, 93, 97, 98). The Carpathian lineage was additionally located in the southeastern part of Germany (sites 28–30).

A comparison of the distribution of PUUV and the bank vole evolutionary lineages indicates an association
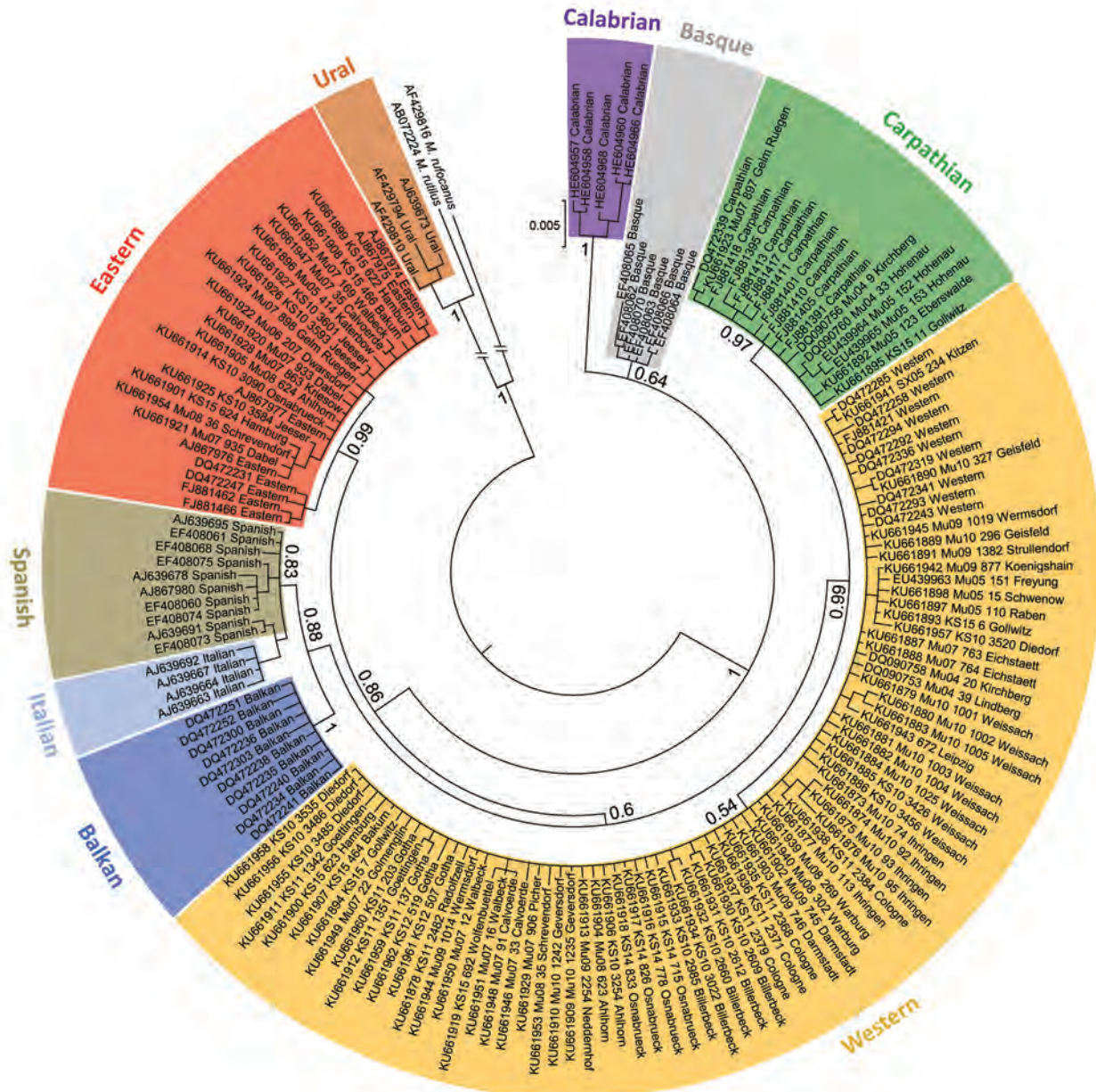
Figure 2. Phylogenetic relationships of European bank vole lineages. Sequences are categorized on the basis of mitochondrial *cytochrome b* gene sequences and shown as a maximum clade credibility phylogenetic tree with posterior probabilities displayed for major nodes. Novel sequences are labeled with individual code and trapping site (online Technical Appendix Table, http://wwwnc.cdc. gov/EID/article/23/1/16-0224-Techapp1.pdf). Additional published sequences are included as references for bank vole evolutionary lineages, labeled with GenBank accession number followed by lineage indication. Phylogenetic analyses were performed with MrBayes version 3.2.2 (https://sourceforge.net/projects/mrbayes/files/mrbayes/) on the CIPRES platform for 166 *cytochrome b* sequences of 843-bp length. A mixed nucleotide substitution matrix was specified in 4 independent runs of 10^7 generations for the data set. A burn-in fraction of 25% was discarded and samples were recorded every 10^3 generations. *Cytochrome b* sequences of *M. rutilus* and *M. rufocanus* voles were used as outgroups.

of PUUV with the Western evolutionary lineage (Figure 1; online Technical Appendix Table). This finding is in line with the detection of PUUV in Belgium and France and the exclusive occurrence of the Western evolutionary lineage in the PUUV-endemic regions of these countries (8,13,14). In the Bavarian Forest, the district Osnabrück (site 57), and at the easternmost distribution range in Walbeck (site 97), PUUV infections were also detected in sympatric bank voles of the Carpathian (n = 6) and Eastern (n = 7; n = 1) lineages, respectively.

Conclusions

The occurrence of PUUV in Germany (and Belgium and France) is preferentially associated with the presence of the Western evolutionary lineage of the bank vole, but the virus was also detected in sympatric animals of the Eastern or Carpathian lineage. Future studies will have to determine if the current distribution of PUUV can be explained by the postglacial colonization of Germany by bank voles of the Western evolutionary lineage from western refugia through southern Germany (13–15).

The observed limited geographic distribution of PUUV in bank voles has important implications for public health measures and development of early warning modules for hantavirus outbreaks. These public health measures of monitoring local bank voles for PUUV strains (4) should be expanded to evaluate for further northeastern expansion.

Dr. Drewes is currently a postdoctoral researcher at the Friedrich-Loeffler-Institut in Greifswald-Insel Riems, Germany. His research interests include epidemiology and molecular evolution of hantaviruses in association with their corresponding hosts.

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Norovirus Infection in Harbor Porpoises

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A norovirus was detected in harbor porpoises, a previously unknown host for norovirus. This norovirus had low similarity to any known norovirus. Viral RNA was detected primarily in intestinal tissue, and specific serum antibodies were detected in 8 (24%) of 34 harbor porpoises from the North Sea.

Noroviruses have been detected in humans, cats, dogs, pigs, sheep, cattle, sea lions, rodents, and bats (*1–4*). In humans, norovirus is a leading cause of gastroenteritis (*1*). Seven different norovirus genogroups have been described for the norovirus genus (*1*) that can be further subdivided in ≈30 genotypes. Noroviruses comprise a single-strand positive-sense RNA genome that is divided into 3 open reading frames (ORFs). Recombination among different genotypes is frequently observed for human noroviruses, most commonly near the junction of ORF1 and ORF2, leading to a recommendation for multilocus genotyping (*5*). Surprisingly, recombinant human noroviruses regularly contain previously undetected ORF1 sequences, which raises questions about the reservoirs of these viruses.

Noroviruses can spread through the fecal–oral route, and sewage contamination in coastal environments can result in contamination of shellfish, such as oysters. Oysters filter several liters of seawater daily and contain histo-blood group antigens resembling those of humans. These antigens can be specifically bound by noroviruses, resulting in bioaccumulation (*6*); as a result, eating oysters is linked to foodborne norovirus outbreaks in humans (*7*). This mode of transmission, however, could expose humans to viruses from other animal reservoirs, such as marine mammals, and vice versa.

The Study

A juvenile male harbor porpoise (*Phocoena phocoena*) ≈10.5 months of age was found alive on the coast of the Netherlands on May 10, 2012, and was transported to the SOS Dolphin Foundation (Harderwijk, the Netherlands) for rehabilitation (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/23/1/16-1081-Techapp1.pdf). Important clinical signs at the rehabilitation center were anorexia, labored breathing, and disorientation. The animal showed no evidence of gastrointestinal disease, such as vomiting or diarrhea. Eight days after arrival in the rehabilitation center, the animal was euthanized because of the severity of clinical signs, and necropsy was performed according to standard procedures (*8*). The main pathology findings were bronchopneumonia associated with lungworm infection and encephalitis and hepatitis of unknown cause. The intestine did not show significant lesions macroscopically; microscopically, the enterocytes at the luminal surface of the intestine had sloughed into the lumen as a result of freeze–thaw artifact. The cells lining the intestinal crypts consisted of a mixture of enterocytes and mucus cells. The proportion of mucus cells increased progressively toward the end of the intestine. The lamina propria was infiltrated diffusely with a moderate number of lymphocytes, plasma cells, and eosinophils. This infiltrate was considered normal for this species.

In the frame of a research program focusing on the identification of new viruses in possible reservoir hosts, we collected fecal material and performed random PCR in combination with 454-sequencing as described previously (*9*). This analysis resulted in 5,774 reads, of which 88 reads were most closely related to the norovirus genus, as determined by blastn and blastx analysis (*10*). Other reads that were most similar to viral genomes were most closely related to a coronavirus (16 reads [35%–94% nt identity]), salmon calicivirus (3 reads [11%–69% nt identity]), and porcine anello virus (1 read [91% nt identity], 2 reads [36% amino acid identity]). The harbor porpoise norovirus (HPNV) sequence was confirmed by Sanger sequencing (6,293 nt; GenBank accession no. KP987888) by using specific primers and comprising 3 ORFs, the partial ORF1 encoding the putative polyprotein, ORF2 encoding viral protein (VP) 1, and a partial ORF3 encoding VP2 (Figure 1, panel A). Phylogenetic analysis revealed that the HPNV RNA-dependent RNA-polymerase encoded by ORF1 clustered together with human genogroup I (GI)
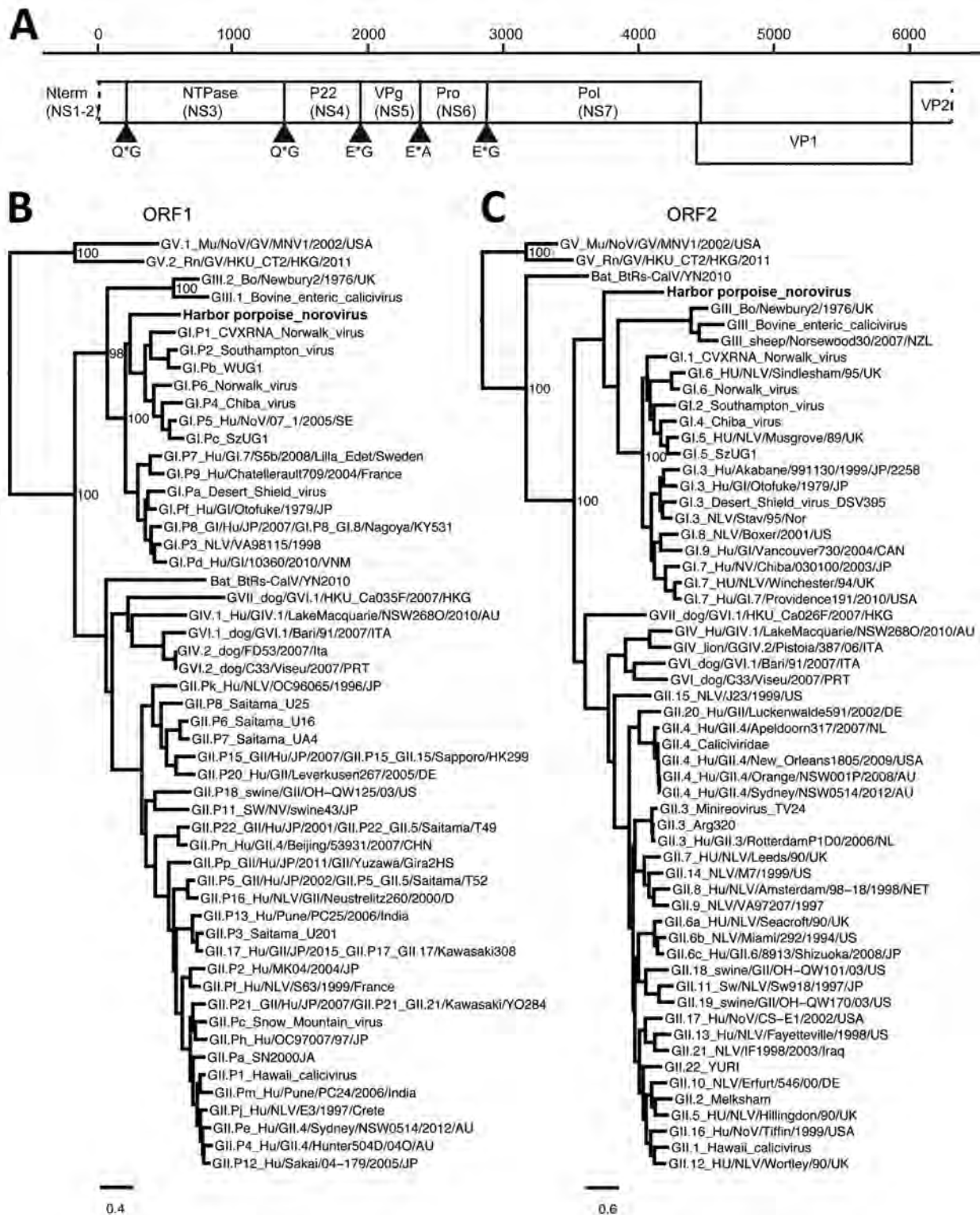
Figure 1. Genetic characterization of harbor porpoise norovirus. A) Genome organization of harbor porpoise norovirus. The putative cleavage sites are shown with arrowheads. B, C) Maximum-likelihood trees of the RNA-dependent RNA-polymerase (B) and ORF 2 (C) were inferred by PhyML 3.0 software (http://www.atgc-montpellier.fr/phyml/) by using the general time reversible nucleotide substitution model. Selected bootstrap values >70 are depicted. Scale bars indicate nucleotide substitutions per site. NS, nonstructural; NTPase, nucleoside triphosphatase; ORF, open reading frame; P, protein; Pol, polymerase; Pro, protease; VP, viral protein.

sequences (Figure 1, panel B), whereas VP1 clustered near strains belonging to human GI and bovine GIII (Figure 1, panel C).

To determine the tissue tropism of HPNV, we extracted RNA from formalin-fixed paraffin-embedded (FFPE) tissues collected from the HPNV-positive animal for histopathology. Tissues from all main organs and lymph nodes were tested for HPNV RNA by real-time PCR (online Technical Appendix). Only the intestinal tissue and the FFPE material for immunohistochemical analysis (containing a mixture of tissues) were positive for norovirus with cycle threshold values of 30.5 and 37.4, respectively.

We conducted in situ hybridization to determine the cellular tropism of HPNV, as described previously (Figure 2) (12). HPNV-specific transcripts were detected in the cells in the intestine, indicating that this virus replicates in the intestinal tract. Sequential slides stained with hematoxylin and eosin or pankeratin showed that positive cells corresponded with enterocytes that had sloughed into the intestinal lumen because of freeze–thaw artifact, although we cannot exclude the possibility that other cell types were present (Figure 2).

To estimate the percentage of norovirus-infected harbor porpoises in our dataset, we extracted RNA from FFPE porpoise intestinal tissues collected from 48 animals during a 10-year period. Including the animal in which we detected HPNV, 10% (5/49) of the animals were positive for norovirus (cycle threshold <37) (Table 1), with primers designed to detect HPNV. Macroscopic and microscopic examination of the intestine of these animals showed no pathologic differences from harbor porpoises without norovirus infection.

To detect norovirus-specific antibodies in porpoise serum, we developed an ELISA based on HPNV VP1 and subsequently screened 34 harbor porpoise serum samples collected during 2006–2015 in the Netherlands (online
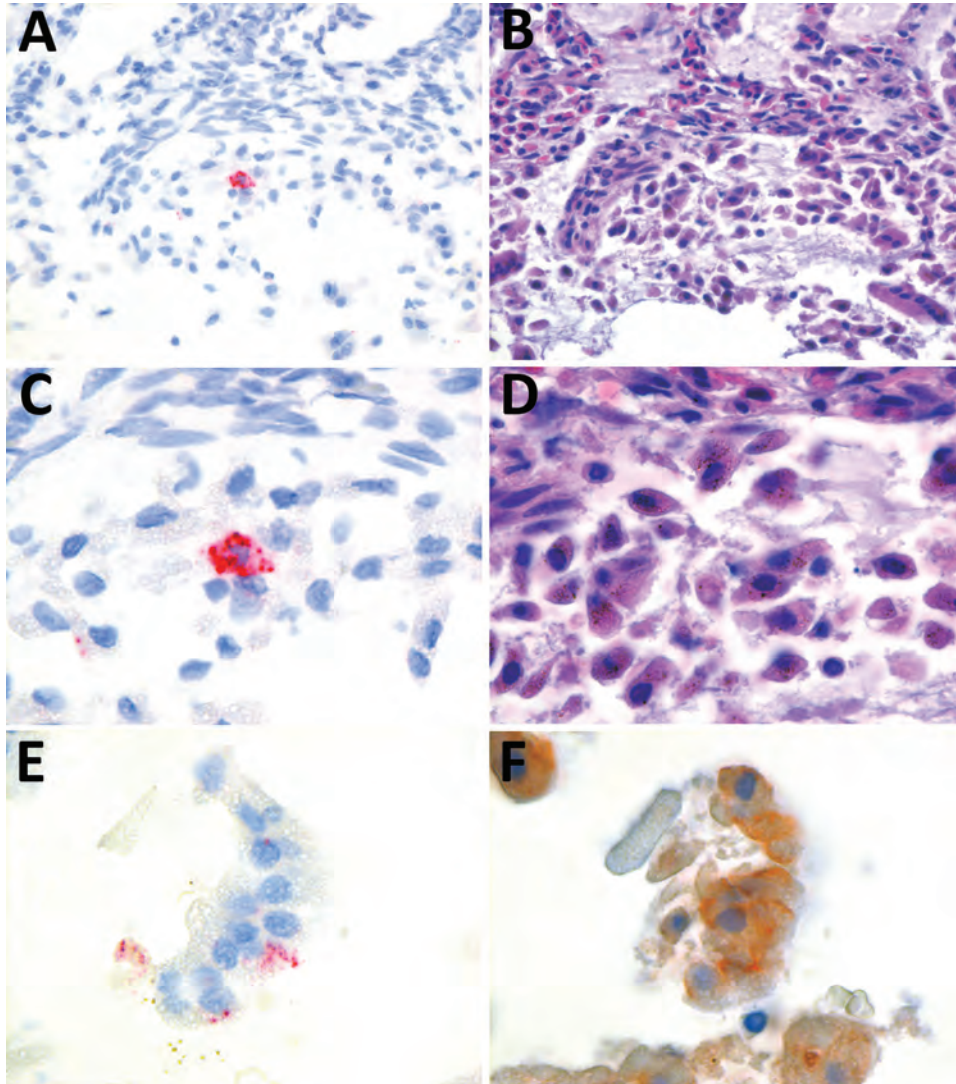
Figure 2. Detection of harbor porpoise norovirus transcripts in intestinal tissue of a harbor porpoise (*Phocoena phocoena*) using in situ hybridization with probes designed by Advanced Cell Diagnostics (Hayward, CA, USA), based on the 6,293 nt of harbor porpoise norovirus (A, C, E; original magnification ×40, ×100, ×100, respectively). Consecutive slides were stained with hematoxylin and eosin (B, D; original magnification ×40, ×100, respectively) and pankeratin (F, original magnification ×100), as described previously (11).

Table 1. Formalin-fixed paraffin-embedded tissues subjected to reverse transcription PCR in a study of norovirus in 48 harbor porpoises (*Phocoena phocoena*), the Netherlands

Year	Tested samples, no.	Positive samples, no. (%)	Cycle threshold
2006	12	0	–
2007	4	1 (25)	33.8
2008	3	0	–
2009	1	0	–
2010	4	0	–
2011	10	1 (10)	33.8
2012	6	3 (50)	30.6, 36.8, 36.4
2013	4	0	–
2014	3	0	–
2015	2	0	–

Technical Appendix). Samples from 8 (24%) harbor porpoises were positive for norovirus antibodies (Table 2). This dataset included samples from 2 harbor porpoises that were positive for norovirus RNA; however, their serum samples were negative for norovirus-specific antibodies.

Conclusions

Similar to human noroviruses, HPNV replicates in the intestine. B cells and enterocytes support human norovirus replication in vitro (*13*,*14*). We detected HPNV in cells corresponding to enterocytes, and it will be interesting to determine whether these viruses share receptor use with other noroviruses. In humans, norovirus infections are self-limiting in healthy persons but can result in illness and death in high-risk groups (*4*). Because the harbor porpoise in which we detected HPNV did not exhibit clinical signs of gastrointestinal disease, norovirus infection probably was not a major factor in the death of this animal.

Remarkably, the HPNV displayed 99% sequence homology to a short (300-nt) norovirus VP1 sequence detected in oysters (*15*). These oysters had been sampled because they were associated with a foodborne gastroenteritis outbreak in Ireland in 2012 (*15*). Oyster samples were collected from the restaurant where the outbreak occurred and from their harvesting area. Strains belonging to genotypes GI.1, GI.4, GII.4, GII.3, GII.1, GII.6, GI.2, GII.7, GI.11, and the strain that was homologous to HPNV were detected, although the HPNV-like strain was detected only in

Table 2. Prevalence of norovirus-specific antibodies in harbor porpoises (*Phocoena phocoena*), the Netherlands

Year	No. samples tested	Positive samples, no. (%)	ELISA titer
2006	12	1 (8)	≥160
2007	4	1 (25)	20
2008	2	0	
2009	1	1 (100)	20
2010	5	2 (40)	40, 80
2011	5	3 (60)	20, 40, 40
2012	1	0	
2013	0	0	
2014	1	0	
2015	1	0	

oysters from the harvesting area. In the patients, only GI.4, GI.2, GI.6, GII.1, and GII.7 strains were detected, but the fact that noroviruses infecting marine mammals closely related to human noroviruses have been found infecting harbor porpoises and contaminating oysters raises the question of whether HPNV could infect humans through contamination of oysters or other shellfish.

On the basis of our findings that norovirus infections might be a common infection in harbor porpoises from the southern North Sea and the detection of a norovirus in a sea lion (*3*), it is not unlikely that noroviruses are common in other marine mammals as well. The high genetic diversity within this genus complicates detection of new noroviruses. The discovery of HPNV and the recent discovery of noroviruses in bats highlight that much still remains to be discovered about animal reservoirs of noroviruses and triggers questions about the zoonotic potential of these viruses.

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December 2015: Zoonotic Infections
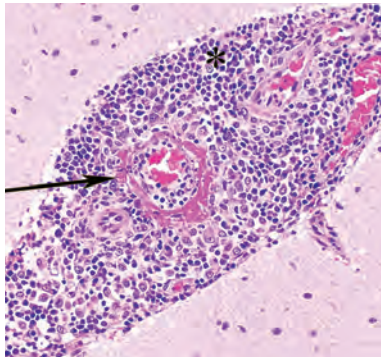
- Identifying and Reducing Remaining Stocks of Rinderpest Virus
- Opportunistic Pulmonary *Bordetella hinzii* Infection after Avian Exposure
- Zoonotic Leprosy in the Southeastern United States
- Infection Risk for Persons Exposed to Highly Pathogenic Avian Influenza A H5 Virus–Infected Birds, United States, December 2014–March 2015
- High Prevalence of Intermediate *Leptospira* spp. DNA in Febrile Humans From Urban and Rural Ecuador

- Biological Warfare Plan in the 17th Century—the Siege of Candia, 1648–1669
- Influenza A(H6N1) Virus in Dogs, Taiwan
- Methicillin-Resistant *Staphylococcus aureus* Prevalence among Captive Chimpanzees, Texas, USA, 2012
- Novel *Waddlia* Intracellular Bacterium in *Artibeus intermedius* Fruit Bats, Mexico
- Tembusu-Related Flavivirus in Ducks, Thailand
- Japanese Macaques (*Macaca fuscata*) as Natural Reservoir of *Bartonella quintana*
- *Onchocerca lupi* Nematode in a Cat, Europe

- Increased Number of Human Cases of Influenza Virus A(H5N1) Infection, Egypt, 2014–15
- Replication Capacity of Avian Influenza A(H9N2) Virus in Pet Birds, Chickens, and Mammals, Bangladesh
- Hendra Virus Infection in Dog, Australia, 2013
- No Evidence of Gouléako and Herbert Virus Infections in Pigs, Côte d'Ivoire and Ghana
- Aquatic Bird Bornavirus 1 in Wild Geese, Denmark
- Vectorborne Transmission of *Leishmania infantum* from Hounds, United States
- Porcine Deltacoronavirus in Mainland China

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Reconstruction of Zika Virus Introduction in Brazil

**Kate Zinszer, Kathryn Morrison,
John S. Brownstein, Fatima Marinho,
Alexandre F. Santos, Elaine O. Nsoesie**

We estimated the speed of Zika virus introduction in Brazil by using confirmed cases at the municipal level. Our models indicate a southward pattern of introduction starting from the northeastern coast and a pattern of movement toward the western border with an average speed of spread of 42 km/day or 15,367 km/year.

Autochthonous transmission of Zika virus has been confirmed in 67 countries worldwide and in 46 countries or territories in the Americas (*1,2*). It is believed that Zika virus was introduced into the Americas through Easter Island in 2014, after an outbreak in French Polynesia (*3,4*). Despite the rapid spread of Zika virus across the Americas and global concerns regarding its effects on fetuses, little is known about the pattern of spread. The risk for local transmission in unaffected regions is unknown but potentially serious where competent Zika virus vectors are present (*5*) and also given the additional complexities of sexual transmission and population mobility (*3,6*).

Knowledge of the direction and speed of movement of a disease is invaluable for public health response planning, including timing and placement of interventions. We estimated the speed of Zika virus spread in Brazil by using data on confirmed cases of Zika virus disease at the municipal level and applying an approach used in estimating the speed of Ebola spread across parts of West Africa (*7*).

The Study

Confirmed cases of Zika virus disease were obtained from the Brazil Ministry of Health. Additional reports were also extracted from ProMED mail (*8*) and HealthMap (*9*). We performed the analysis by using 3 dates: 1) date of case registration in the surveillance system of the Brazilian Ministry of Health (model 1); 2) earliest of either date of symptom onset (if available) or registration date (model 2); and 3) earliest of either case registration date, date of

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symptom onset, or date of case report by other sources (model 3). Surface trend analysis was used to interpolated a continuous estimate of disease spread speed in magnitude and direction (*10*) by using available spatial and temporal information. Time of dispersal was calculated from the start of the epidemic for each model (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/23/1/16-1274-Techapp1.pdf).

Data provided by the Brazilian Ministry of Health on May 31, 2016, indicated that Zika had been confirmed in 316 of 5,564 municipalities in 26 states; 6 additional municipalities were identified from other reporting sources. Contour maps of interpolated temporal trends (Figure 1) indicate a trend of spread into southern and western Brazil, and initial outbreak reports originated from municipalities along the northeastern coast. On the basis of confirmed cases, the earliest location of spread was the northeastern coastal area between the states of Paraíba, Ceará, Bahía, Alagoas, and Rio Grande do Norte. There were also earlier dates of self-reported symptom onset in the northwestern state of Amazonas (January 1, 2015), the west-central state of Matto Grosso (January 4, 2015), and the southeastern coastal state of Rio de Janeiro (January 1, 2015).

Contour maps (Figure 1) indicate slight differences in patterns of dispersion between the models. Model 1 indicates the strongest trend of a southward spread from the northeastern coast toward the populous southeastern coastal states of Rio de Janeiro, Espírito Santo, and São Paulo; the estimated time of dispersal was 22 weeks (Figure 1, panel A). In addition to west to east spread of Zika in southern Brazil, there was a pattern of movement west toward Bolivia.

The dispersal trend for model 2 was more varied but also indicated spread to the southeastern coastal states of Rio de Janeiro, Espírito Santo, and São Paulo (Figure 1, panel B). This model also suggests an initial spread north from the earliest reports in the northeastern region and a spread west toward Bolivia. The model estimates a north to south diffusion of ≈27 weeks. Model 3 suggests a strong southward spread originating from the northeastern coast toward the southeastern coastal states (approximate dispersal time of 29 weeks) and toward the western border and northwestern state of Amazonas (Figure 1, panel C).

Overall, the average speed of diffusion was 42.1 km/day or 15,367 km/year. The minimum speed across all 3 models was 6.9 km/day, and the maximum speed was 634.1 km/day (Figure 2). Municipalities in northeastern and northern regions had the slowest speeds, and municipalities in the west-

Figure 1. Contour surface trends and directional vectors for reconstructing Zika introduction in Brazil. A) Date of case registration (model 1); B) earliest date between date of symptom onset (if available) and date of registration (model 2); C) earliest date between date of case registration, date of symptom onset, and date of case reporting by other sources (model 3). Each contour line represents a 1-day period, and contour lines farther apart show that the disease spread rapidly through an area, whereas lines close together show slower progression in an area. Arrows indicate direction of Zika spread. Magnitude of speed and direction should be interpreted cautiously near the edges of the study area. Estimates of speed are subject to edge effects, which indicates that estimates are less stable because they are based on fewer data (not as many neighboring values).

central and southeastern regions had the highest speeds. This finding was caused by proximity of cases in time and space. More cases occurred closer in time and over larger areas in southern, southeastern, and west-central regions, which resulted in faster rates of case introduction.

All models were consistent in agreement that Zika dispersal in Brazil followed a general pattern of southward spread toward the populous coastal states (average speed of introduction of 42 km/day), which could be explained by multiple introductory cases into different areas probably caused by movement of viremic persons. We estimate that it took ≈5–6 months for Zika to spread from the northeastern coast to the southeastern coast and western border of Brazil. These findings are supported by the first report of local transmission of Zika virus in Paraguay in late November 2015 (*11*) and in Bolivia in January 2016 (*12*), 7 months after the first registered case in Brazil.

Limitations of this analysis include quality and timeliness of surveillance data that provided the basis for this study. Symptom onset date is subject to error because it is based on self-report, and earlier introductions of Zika in some municipalities might not have been captured by the Ministry of Health surveillance system and supplementary data sources, given the mild and generic nature of Zika

symptoms and the high proportion of asymptomatic persons (*3*). The northern region of Brazil had a major dengue outbreak in early 2015, and given symptom similarities between dengue and Zika, it is probable that some suspected dengue cases were in fact early cases of Zika.

Sporadic geographically disparate cases were recorded in various parts of Brazil, which increased the uncertainty associated with speed analysis. These cases, such as those in northwestern Brazil, increased uncertainty in direction and speed estimates, which are also related to edge effects. Edge effects occurred along the boundary of the study area, which in this study were constructed by using fewer data points and are therefore less stable. This effect is shown with directional arrows pointing toward earlier areas of spread versus toward later areas of spread (Figure 1, panels B, C).

Conclusions

The arrival and rapid spread of Zika virus in the Americas resembles that of chikungunya virus, which was introduced into Saint Martin in the Caribbean in 2013 (*13*,*14*). Increased knowledge of the speed of spread and direction of Zika spread can help in understanding its possible future directions and pace at which it travels, which would be essential

for targeted mosquito control interventions, public health messages, and travel advisories. Future work will investigate underlying causes for the southward and westward spread in Brazil by incorporating mobility data and seasonal events, such as movement of persons between northeastern and southeastern regions for vacations, which could have driven the spatial transmission pattern. Furthermore, multicountry analysis is needed to understand continental spatial and

temporal patterns of dispersion of Zika virus and co-circulating viruses, such as chikungunya virus.

Dr. Zinszer is a postdoctoral fellow in the Informatics Program, Boston Children's Hospital, Boston, Massachusetts. Her primary research interests include understanding patterns and determinants of emergence and spread of infectious diseases.
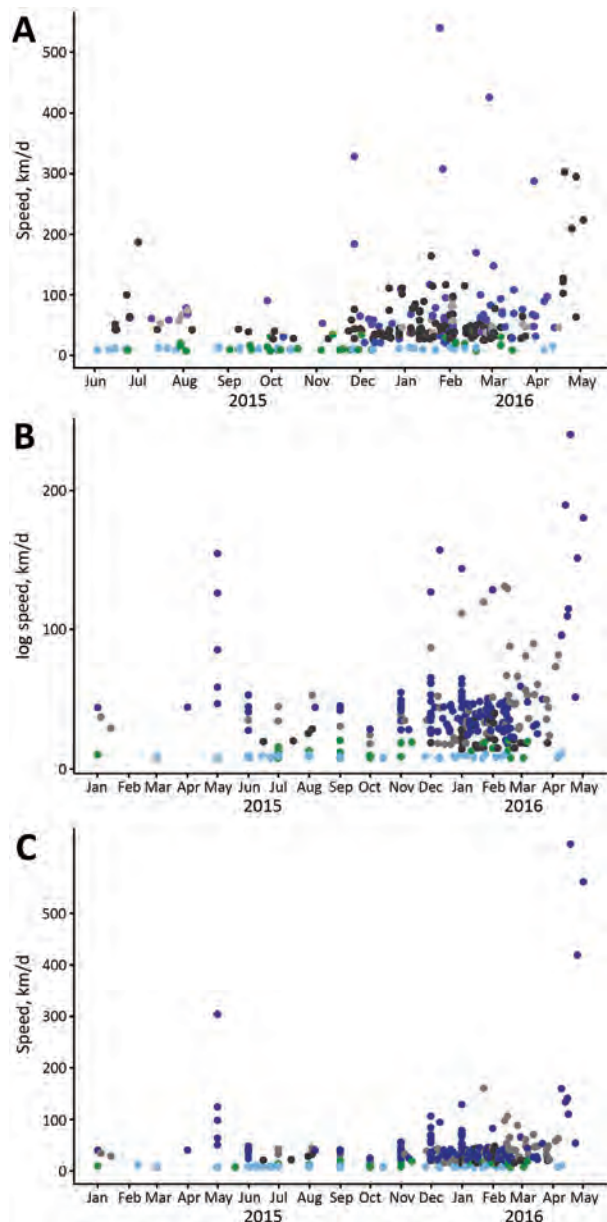
Figure 2. Speed or log speed (km/d) of Zika introduction into municipalities in Brazil. A) June 2015–May 2016; B) January 2015–May 2016; C) January 2015–May 2016. Municipalities are classified by region. Gray circles indicate central–western region, green circles indicate northern region, light blue circles indicate northeastern region, black circles indicate southern region, and dark blue circles indicate southeastern region.

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Acute Respiratory Disease in US Army Trainees 3 Years after Reintroduction of Adenovirus Vaccine[1]

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Joel C. Gaydos, Anthony W. Hawksworth,
Nikki N. Jordan

The 1999 cessation of vaccination against adenovirus types 4 and 7 among US Army trainees resulted in reemergence of acute respiratory disease (ARD) outbreaks. The 2011 implementation of a replacement vaccine led to dramatic and sustained decreases in ARD cases, supporting continuation of vaccination in this population at high risk for ARD.

In the past, febrile acute respiratory disease (ARD) was a major cause of illness among military members, especially those in initial entry training (IET [basic training]), a physically and mentally demanding 6- to 12-week program (1–5). Most cases were caused by infection with adenovirus types 4 and 7; 80% of trainees became infected and 20% were hospitalized (5).

Routine use of oral adenovirus type 4 and 7 (AdV-4 and -7) vaccine began in 1971 and eventually included year-round vaccination, resulting in plummeting ARD rates (1). In 1994, the sole vaccine manufacturer stopped production. The last doses were shipped in 1996 and administered only during winter until stocks were depleted in 1999; ARD rates subsequently increased at IET sites (3,4).

When the stock of AdV-4 and -7 vaccine was depleted, the Army's Acute Respiratory Disease Surveillance Program (ARD-SP), partnering with the Naval Health Research Center (San Diego, CA, USA) Febrile Respiratory Illness (NHRC FRI) Surveillance Program, demonstrated substantial increases in ARD cases, specifically adenovirus-associated ARD. These cases cost ≈$10–$26 million each year in medical care and lost recruit time (5). In addition, a threat existed for the emergence of other adenovirus types that could cause severe and fatal disease (5). In March 2011, a new, 2-tablet, live, enteric-coated oral AdV-4 and -7 vaccine was licensed by the US Food and Drug Administration for use in US military members. Administration of the vaccine to trainees early in their IET

program began in November 2011 and reached full coverage of all trainees by year's end. In 2014, NHRC reported that, after 2 years of AdV-4 and -7 vaccine use, a dramatic decline was seen in febrile ARD cases in training centers across the military services, and no indication was seen of a serious, sustained emergence of a new adenovirus threat (5). We report the ARD-SP and NHRC FRI data for the US Army IET population during the first 3 years after reintroduction of AdV-4 and -7 vaccine, looking at variations in ARD rates at 4 Army IET sites and at adenovirus types identified in trainees with ARD.

The Study

In 1966, the ARD-SP, then called the Adenovirus Surveillance Program, was implemented to monitor ARD and evaluate the new AdV-4 and -7 vaccine at IET sites (2). In 1996, partly in response to increasing ARD cases, the NHRC initiated the FRI Surveillance Program to assess febrile respiratory illness rates, etiologies, and trends across military training installations (5). ARD-SP captured all ARD cases, and NHRC FRI collected respiratory specimens from a convenience sample of the ARD-SP cohort. This subset was tested for respiratory pathogens, including adenoviruses. Together, the ARD-SP, operated by the Army Public Health Center (Aberdeen Proving Ground, MD), and the NHRC FRI program have provided coordinated surveillance of respiratory pathogens for Army IET populations.

We studied aggregate data from 2010–2014 from the ARD-SP and NHRC FRI programs. The Army Public Health Center collected weekly ARD-SP data from the Army's 4 IET sites (Fort Benning, GA; Fort Jackson, SC; Fort Leonard Wood, MO; and Fort Sill, OK). ARD case criteria were oral temperature ≥100.5°F, recent sign or symptom of acute respiratory tract inflammation, and having a limitation in training or removal from duty. We determined ARD rates for each IET installation and for the total Army IET population using the equation (ARD cases/all trainees) × 100 trainee weeks, and used SPSS version 21 (SPSS, Inc., Chicago, IL, USA) for analyses.

Table. Rate of ARD cases and percent change by year at 4 US Army initial entry training sites, 2010–2014*

| Year | Average ARD rate (% change), by training site† | | | | |
	Fort Benning	Fort Jackson	Fort Sill	Fort Leonard Wood	Overall†
2010	0.29	0.67	0.20	0.43	0.43
2011	0.18 (−38)	0.44 (−34)	0.75 (+275)	0.35 (−19)	0.39 (−9)
2012	0.06 (−79)	0.08 (−88)	0.19 (−5)	0.08 (−81)	0.09 (−79)
2013	0.06 (−79)	0.09 (−87)	0.13 (−35)	0.08 (−81)	0.08 (−81)
2014	0.04 (−86)	0.07 (−90)	0.08 (−60)	0.05 (−88)	0.06 (−86)

*The adenovirus vaccine program was reintroduced in November 2011 and rate changes were studied through 2014. ARD, acute respiratory disease.
†ARD rate = (ARD cases/all trainees) × 100 trainee weeks. % change calculated from last full year before vaccination (i.e., 2010).

An ongoing program of year-round administration of AdV-4 and -7 vaccine at Army IET sites began in November 2011. Overall, ARD rates decreased in November 2011 (9% from 2010 rates) and each subsequent year through 2014 (Table). However, in 2011, Fort Sill experienced an increase over 2010 ARD rates before rates dropped substantially in 2012 (0.75 cases/100 trainee weeks in 2011 vs. 0.19 cases/100 trainee weeks in 2012) and remained low through 2014 (Figure 1; Table). All 4 sites experienced similar declines from 2010 to 2014, ranging from 60% to 90% (Table). The combined mean ARD rate for 2010 was 7 times higher than that for 2014 (0.43 cases/100 trainee weeks vs. 0.06 cases/100 trainee weeks, respectively).

Prior to implementation of the new vaccine in 2011, adenovirus type 4 was the predominant type at all training sites (86%), followed by types 3 (7%) and 7 (5%). After the reintroduction of adenovirus vaccine, most (71%) adenovirus-positive specimens from 2012–2014 were positive for adenovirus types 1 and 2 (Figure 2). However, appearances of adenovirus types 1 and 2 were small in scale and scattered over place and time.

Conclusions

Reintroduction of AdV-4 and -7 vaccine had a profound effect at all Army IET sites; the combined ARD rate decreased from 0.43 cases/100 trainee weeks in 2010 to 0.06 cases/100 trainee weeks in 2014 (p<0.001), and adenovirus
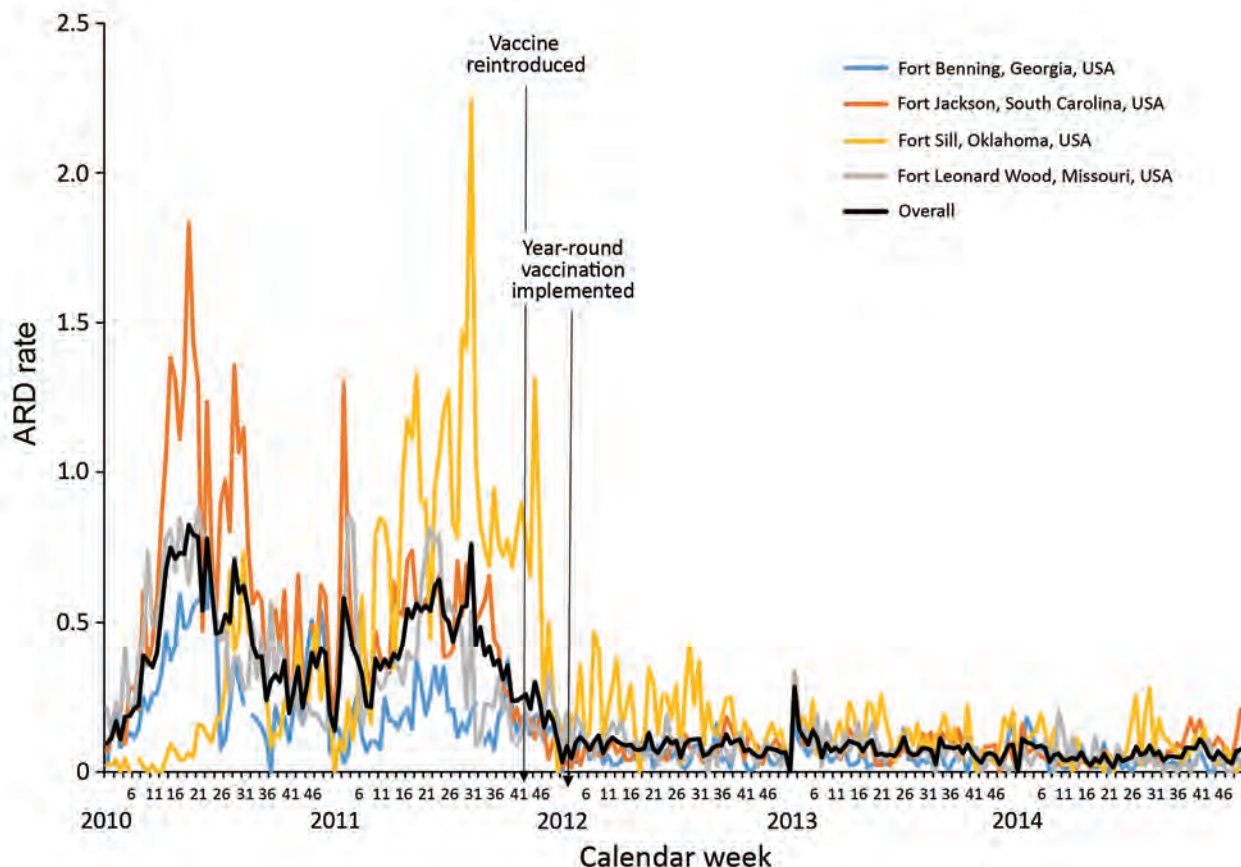
Figure 1. Weekly acute respiratory disease (ARD) rates by US Army initial entry training site, 2010–2014. ARD rate = (ARD cases/all trainees) × 100 trainee weeks.
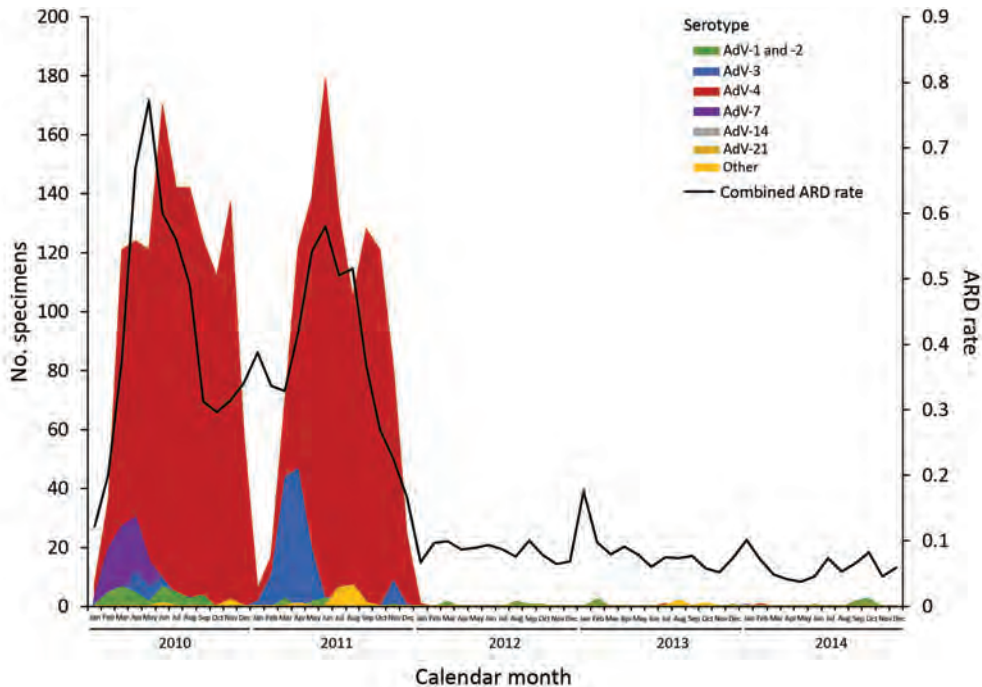
Figure 2. Adenovirus serotype distribution and acute respiratory disease (ARD) rate for all US Army initial entry training sites, by month, 2010–2014. ARD rate = (ARD cases/all trainees) × 100 trainee weeks.

was identified only sporadically in ill trainees. Although a low level of ARD activity, caused by many different agents, has persisted since reestablishment of the vaccine program, the vaccine has effectively controlled the major cause of ARD at IET sites at the low cost of $150/trainee (*5*).

The increased average ARD rate for Fort Sill during 2011 was likely an anomaly associated with a lapse in the military's long-standing routine use of benzathine penicillin G prophylaxis for group A β-hemolytic streptococcus infections coupled with a surveillance artifact introduced when Fort Sill made enhancements to their ARD surveillance program (*2,6*). After benzathine penicillin G prophylaxis was reintroduced, ARD rates substantially decreased in 2012, mirroring reductions observed at other IET sites after adenovirus vaccine administration.

Vaccination administration has multiple benefits. A study of US Air Force trainees with acute respiratory illness found decreased severity of systemic symptoms and reduced fever and heart rate in those who became ill after the vaccine was reintroduced (*7*). In addition, we observed an overall decrease in ARD rates and suppression of nearly all adenovirus types. Since introduction of the vaccine in 1971, many have suggested that this vaccine may have an effect on reducing ARD caused by agents other than adenovirus types 4 and 7. Recent studies have shown the AdV-4 and -7 vaccine to have a potentially broader effect, as demonstrated by decreased rates of overall febrile illness among trainees and other service members (*8–10*). This effect could be due to activation of innate immunity and heterotypic antibody response (*11,12*).

The overall observed reduction in ARD among Army IET trainees translates to substantial cost savings by reducing the probability of severe illness or death and lost training time. During the 1999–2010 lapse in adenovirus vaccine coverage, 8 adenovirus-infected service members died (*13*). Estimates showed each infection costs ≈$3,838, and each year the vaccine prevents 1 death, 1,100–2,700 hospitalizations, and 13,000 febrile infections among military recruits. Vaccination costs $150 per person, providing a net savings of ≈$20 million (*5*). Phase 3 safety studies of the vaccine established an excellent safety profile (*14*). Surveillance safety data since 2011 should be released soon and are expected to be consistent with the Phase 3 data.

The AdV-4 and -7 vaccine may have applications beyond the US military. Adenovirus outbreaks have occurred in non–US military populations and facilities where close contact and suboptimal hygiene may be present (e.g., militaries of other countries, dormitories, and healthcare facilities). In addition to the US military, populations in those and similar settings may benefit from AdV-4 and -7 vaccine (*15*).

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Prolonged Detection of Zika Virus in Vaginal Secretions and Whole Blood

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Infection with Zika virus is an emerging public health crisis. We observed prolonged detection of virus RNA in vaginal mucosal swab specimens and whole blood for a US traveler with acute Zika virus infection who had visited Honduras. These findings advance understanding of Zika virus infection and provide data for additional testing strategies.

Zika virus is a rapidly emerging mosquitoborne virus (*1*). In May 2015, Brazil reported autochthonous transmission of Zika virus (*2*). Over the course of 1 year, Zika virus spread to >50 countries and territories throughout the Americas (*3*). With the now confirmed link of Zika virus infection during pregnancy leading to fetal microcephaly (*4*) and reported cases transmitted by sexual contact (*5*), it is vital to understand the natural history of infection. We report an acute case of Zika virus infection in a traveler returning from Honduras to the United States and results from serial specimens collected for >11 weeks. These new data might serve as a potential guide for public health policy.

The Study

This study was reviewed and approved by the Baylor College of Medicine Institutional Review Board (H-30533). A previously healthy, nonpregnant, 26 year-old non-Hispanic white woman returned to the United States from Tegucigalpa, Honduras, during mid-May 2016. Five days after her return (day 0), signs and symptoms consistent with Zika virus infection developed, beginning with rash and subsequent fever, headache, and conjunctivitis (Table). Fever and rash continued through day 5 and day 6, respectively. By day 15, desquamation was noted on the palms of both hands and soles of both feet. By day 17, all symptoms had resolved.

Serial specimens were longitudinally collected for >11 weeks. The first specimens were collected on day 0, two hours after onset of rash and 2 h before development of fever. All remaining specimens were collected at 3, 8, 14, 21, 28, 35, 42, 53, 64, and 81 days after onset of illness. Specimens included serum, whole blood (EDTA anticoagulated), urine, saliva, and vaginal mucosa swabs. The patient was not menstruating when vaginal swab specimens were collected.

RNA was extracted from serum, whole blood, and urine samples by using the QIAamp MinElute Virus Spin Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Oral and vaginal mucosal swab specimens were collected by using the BBL CultureSwab Collection and Transport System (Becton Dickinson, Franklin Lakes, NJ, USA). Specimens were incubated in 250 µL of AL/carrier RNA lysis buffer for 10 min at room temperature; 200 µL of phosphate-buffered saline was added before RNA extraction.

Eluted RNA from all samples was tested in a quantitative reverse transcription quantitative PCR (qRT-PCR) that included a TaqMan Fast Virus 1-Step Master Mix (ThermoFisher Scientific, Foster City, CA, USA) and a TaqMan ZIKV 1107 assay (*6*) with appropriate positive and negative controls. We detected Zika virus RNA in serum up to day 8 after onset of illness and in body fluids up to day 14; whole blood samples remained positive up to day 81 (Figure). Results of qRT-PCR of saliva were negative after day 8, and results for urine and vaginal swab specimens did not become negative until after day 14. We tested a day 0 serum sample for dengue virus and chikungunya virus RNA by using TaqMan assays (*7,8*); all results were negative.

Virus isolations were performed for Vero cells in complete Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal bovine serum. Cells were infected with day 0 serum samples (or mock-infected with cell culture medium) and observed for cytopathic effects. Cell culture supernatants were sampled 13 days after cell culture infection, and RNA was extracted and tested for Zika virus RNA. Supernatant was collected on day 14, and viral titer was 8.5×10^5 PFU/mL by plaque assay. Attempts to isolate virus from the day 64 erythrocyte fraction showed no evidence of cytopathic effects, and first and second passages were negative by qRT-PCR. Because the day 81 whole blood specimen was still positive by qRT-PCR, we used ficoll to separate peripheral blood mononuclear cells

Table. Timeline of acute signs and symptoms and clinical progression/resolution for a 26-year-old woman infected with Zika virus who returned from Honduras to the United States

Day after illness onset	Signs and symptoms and clinical progression
0	Red, mottled, flat rash on stomach, back, and neck with pruritic progression over 24 h. Approximately 4 hours after first appearance of the rash, the patient became febrile (temperature 101.7°F), fatigued, and a headache developed.
1	Rash spread to the legs and upper arms, continuing to appear flat, mottled, and became pruritic. Fatigue persisted, along with headache with light sensitivity, myalgias (particularly in the back and shoulders), and nausea with anorexia. The patient reported that her eyes were painful to open, but upon examination, the eyes appeared normal with no redness or swelling.
3	The rash continued to progress to the entire body, but with decreasing pruritus and increasing papular appearance. Conjunctivitis and cervical adenopathy were noted on physician examination, and the patient reported increased myalgias and dysphagia, with development of painful vesicles throughout the oral mucosa.
5	Fever resolved
6	Rash and sore throat resolved
15	Desquamation was noted on palms of both hands and soles of both feet
17	Resolution of all signs and symptoms

and erythrocytes and found that erythrocytes were the only fraction positive for Zika virus RNA. The partial sequence of the virus we isolated was submitted to GenBank under accession no. KX928077.

On day 8, plasma was evaluated by using an ELISA (*9*) to assess IgM and IgG binding to Zika virus envelope protein (Zika Virus Envelope Recombinant Protein, #R01635; Meridian Life Sciences, Memphis, TN, USA); positive results were obtained. Plasma-neutralizing antibodies against Zika virus were detected (50% focus reduction neutralization test titer 1:1,438), but neutralization of dengue virus serotypes 1–4 was not detected. These findings indicated a robust Zika virus–specific humoral response.

Conclusions

Given recent concerns regarding the ongoing epidemic of Zika virus disease, there is an urgent need to document the natural history of infection and assess transmission risk through nonvector routes. We had the unique opportunity to prospectively monitor the clinical and virologic course of Zika virus infection in a patient starting on day 0.

We detected viral shedding in vaginal secretions up to day 14. Only 1 human study reported Zika virus RNA in cervical mucous up to day 11 after onset of signs and symptoms (*10*). These findings are supported by recent results for 2 animal models. Zika virus (Asian lineage strain) RNA was detected in vaginal swab specimens obtained on days 1 and 7 postinfection of nonpregnant female rhesus

macaques (*11*). Zika virus replication was also detected in vaginal mucosa of mice (*12*).

We could not determine whether positive results by qRT-PCR indicated replicating virus. With the recent finding of possible female-to-male virus transmission (*5*), infectious virus might be present in the vaginal canal and could serve as a risk for sexual or intrapartum transmission.

We detected viral RNA in serum up to 8 days and in whole blood up to 81 days after onset of illness. Diagnosis of infection currently relies mostly on PCR detection of Zika virus in serum. With concerns for Zika virus infection during pregnancy, screening of whole blood might be more sensitive in identifying infected patients, particularly if an asymptomatic patient has traveled from an area where exposure is a concern, had high-risk sexual contact, or is convalescing and PCR for a serum sample would probably yield a negative result.

Our observation is further supported by another recent study that found whole blood samples positive for Zika virus by PCR up to 2 months postinfection (*13*). In our study, we confirmed that a positive result was attributed to the erythrocyte component of whole blood, similar to what has been found in studies of West Nile virus (*14,15*). One study found that West Nile virus adheres to erythrocytes and could infect Vero cells (*14*). Although we did not observe infectious virus associated with erythrocyte positivity for Zika virus at day 64, this finding is still of concern and requires further investigation. Because the last whole blood sample collected on day 81 was positive for Zika virus RNA, follow-up testing will continue to define the longevity of viremia in whole blood.

In conclusion, this case study advances understanding of the natural history of Zika virus. It provides new findings, including detection of Zika virus RNA in vaginal
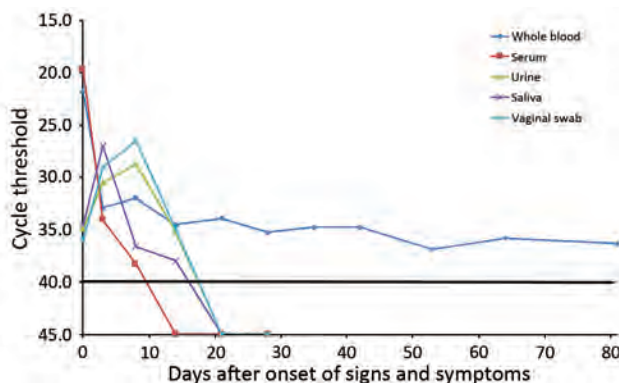
Figure. Quantitative reverse transcription PCR cycle threshold values over time (days after onset of illness) for whole blood, serum, urine, saliva, and vaginal mucosal swab specimens obtained from a 26-year-old woman infected with Zika virus who returned from Honduras to the United States. A cutoff value for a negative result was established at a cycle threshold of 40 (black horizontal line).

secretions up to day 14 and in erythrocytes up to day 81, the longest reported duration of detection in this sample type. A desquamating rash developed on the hands and feet of the patient, which we presume was related to her infection. To our knowledge, this finding has not been previously described.

Additional studies involving larger cohorts of acutely ill Zika virus–infected patients tested over a longer period would solidify our understanding of the natural history of infection, duration of viral detection, and clinical outcomes. These studies will enable further development of evidence-based policies regarding diagnosis and clinical management of Zika virus–infected patients.

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Frequent Transmission of Gonorrhea in Men Who Have Sex with Men

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The rate of gonorrhea is much higher in men who have sex with men than in heterosexuals. Because of unique behavioral characteristics, asymptomatic sites of infection, mainly the pharynx, are principal drivers of gonorrhea prevalence in men who have sex with men. On the basis of this observation, we call for interventions.

The rates of sexually transmitted infections are rising rapidly in men who have sex with men (MSM) (*1*). Gonorrhea is of particular concern because rising rates will increase the probability of antimicrobial drug resistance (*2*). In response, the Centers for Disease Control and Prevention has recommended reducing the prevalence of gonorrhea as a key strategy to mitigate against antimicrobial resistance (*2*). However, reducing prevalence requires understanding why gonorrhea is so common in MSM. We suggest that specific sexual practices of MSM result in them having a high prevalence of asymptomatic infection in particular anatomic sites and that these infections are the primary drivers of transmission (*3*).

In heterosexuals, the primary sites of gonorrheal infection are the urethra in men and cervix in women (*4*). Most heterosexual men with urethral infection become symptomatic and quickly seek healthcare (after a few days) (*5*). About half of women are asymptomatic, and thus they take longer to seek healthcare than men (*5,6*).

In MSM, 3 sites are commonly infected: pharynx, rectum, and urethra (*7*). In a Seattle clinic, the proportion of MSM with pharyngeal gonorrhea was 6.5%, rectal gonorrhea 9.7%, and urethral gonorrhea 5.5% (*7*). Almost all urethral infections were symptomatic (96%), but most pharyngeal and rectal infections were asymptomatic. Most pharyngeal or rectal infections (58%) were not associated with urethral infection (*7*).

An additional factor favoring the persistence of gonorrhea-infected sites in MSM is their lower rate of partner notification compared with heterosexuals (*8*). This behavior

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creates a scenario in which men with pharyngeal or rectal gonorrhea often go untreated, even if they transmit an infection to the urethra of a sex partner. This longer duration of infectiousness translates into a higher reproductive rate for gonorrhea in MSM compared with heterosexuals, independent of the number of sexual partners. Determining the key drivers of the reproductive rate for gonorrhea in MSM involves characterizing transmission between anatomic sites, which requires quantifying the site-specific sexual practices of MSM. Studies assessing the most recent sexual acts among MSM show that most have kissed (75%), practiced mutual masturbation (64%), or had oral sex (77%) (*9*); oro-anal sex (25%) and penile-anal sex (35%) are less common (*9*). In contrast, in heterosexuals, penile-vaginal sex occurs in 95% of most recent sexual acts; therefore, most sexual acts between heterosexuals in which gonorrhea transmission occurs will lead to symptomatic infections that prompt them to seek treatment (*9,10*).

One behavior that may be important for transmitting gonorrhea that has not been well studied is kissing (*11*). Kissing has not been asked about in any national sex surveys and only occasionally in clinical sexually transmitted infection studies (*9*). We were unable to find any published studies on kissing partners in which sex did not occur (termed kissing-only partners) either in heterosexuals or MSM, besides the data we recently presented (*3*). We surveyed 1,151 MSM attending our clinic in 2016 and found a mean of 3.7 kissing-only partners and a mean of 4.5 kissing and sex partners in the previous 3 months (*3*) (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/23/1/16-1205-Techapp1.pdf). Kissing-only partners were much more common among younger MSM, who are at substantially higher risk for gonorrhea than older MSM (*3,12*). The reason for this preponderance of gonorrhea in young MSM is currently unknown but is consistent with and could be explained by kissing being an important transmission route.

We determined what we consider to be the accepted transmission routes for gonorrhea by anatomic site in MSM (Figure, panel A), although one should acknowledge that no studies have reported site-specific gonorrhea transmission between MSM partners. Major textbooks and published studies indicate the penis is key to gonorrhea transmission between men (Figure, panel A) (*4*). Studies suggest that urethral infection is largely acquired from unprotected anal sex, with perhaps one third of cases acquired by receiving oral sex (online Technical Appendix Table).
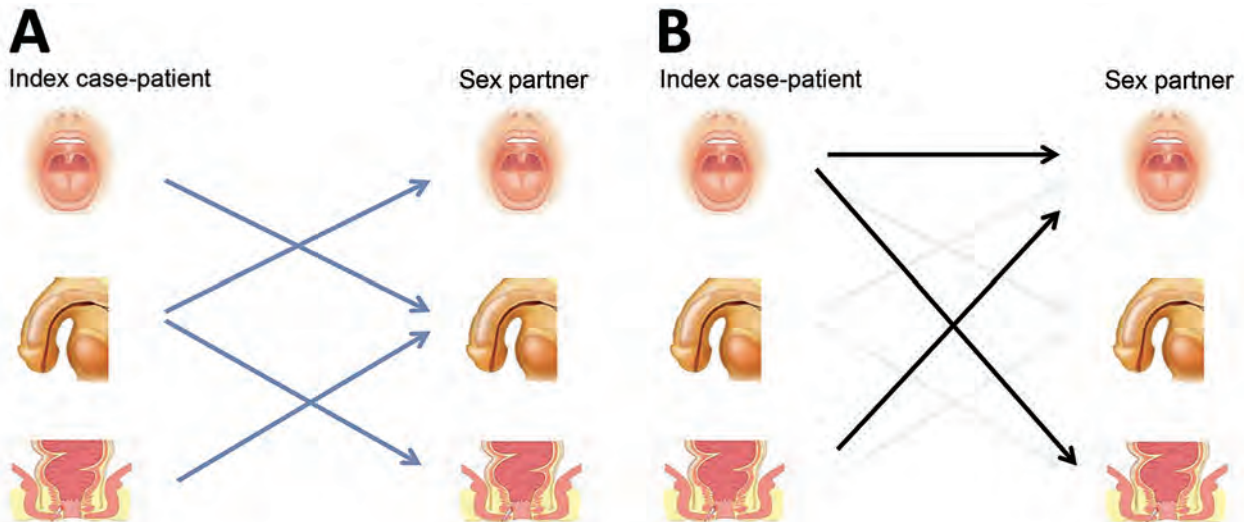
Figure. Traditional and proposed transmission models for gonorrhea in men who have sex with men (MSM). A) Generally accepted transmission routes (arrows) for gonorrhea between sites in MSM from an infected index case-patient to an uninfected sexual partner. B) Additional proposed transmission routes (dark arrows) compared with accepted transmission routes (light arrows). MSM, men who have sex with men.

Relatively little research has been done on gonorrhea transmission not involving the penis. Some observational studies support the potential transmission of gonorrhea between the pharynx and rectum, although this is not consistently described as a route of transmission in major textbooks (*4*). Studies have shown that receptive oro-anal sex has been associated with rectal infection, and oro-anal sex has been associated with pharyngeal gonorrhea (online Technical Appendix Table).

We propose new models of gonorrhea transmission: throat-to-throat transmission through kissing and throat-to-anus transmission (and vice versa) through oro-anal sex (Figure 1, panel B). We propose that transmission to the penis occurs but contributes little to the reproductive rate because it is present there a short time relative to the other anatomic sites.

Unfortunately, there are few studies on gonorrhea transmission between the throats of sex partners to support or refute our suggestion. We did, however, find case reports of transmission through kissing from >40 years ago, and kissing is a well-recognized transmission route for other *Neisseria* species (*11,13*). In a matched, case–control study of 15- to 19-year-olds, intimate kissing with multiple partners was associated with an odds ratio of 3.7 for meningococcal disease (*13*). One of the few cohort studies in MSM to ask about kissing showed it to be significantly associated with pharyngeal gonorrhea (online Technical Appendix Table), but few studies have examined kissing behavior.

The frequent detection of gonorrhea in the saliva of men with pharyngeal infection suggests saliva likely plays a role in gonorrhea transmission (*14*). Saliva is central to oral sex, oro-anal sex, and even penile-anal sex; saliva is commonly used as lubricant (*14*).

Transmission models for gonorrhea in MSM should be consistent with current site-specific prevalence and incidence. We estimated prevalence and incidence of pharyngeal and anal gonorrhea from 3,034 MSM attending a Seattle clinic on the basis of site-specific duration data (online Technical Appendix Figure 2) (*7,15*). The incidence of urethral gonorrhea was ≈5.5/100 person-years, and we estimated the prevalence among MSM to be low (0.24%) because the infections are often of short duration due to their treatable and symptomatic nature. It is difficult to see how, even with frequent changes in sex partners, the estimated incidence of pharyngeal infection (26/100 person-years) could arise from urethral infection, given its low prevalence.

There are several implications if our model of transmission is correct. First, a preventive approach using condoms will not work because, unlike heterosexuals, the penis is not responsible for most gonorrhea transmission among MSM. Second, the screening that is advocated annually for MSM would need to be much more frequent to reduce the disease reproductive rate. MSM taking pre-exposure prophylaxis for HIV are screened every 3 months; this screening frequency might be sufficient to reduce gonorrhea prevalence. Third, our model suggests reducing pharyngeal duration and transmissibility is needed for gonorrhea control, and we call for suggestions of interventions that might achieve this. One approach we are investigating is an antibacterial mouthwash (clinical trial no. ACTRN12616000247471), following up some of our earlier data.

Finally, it is possible that the rapidly rising rates of syphilis in MSM may share similarities with gonorrhea transmission. Syphilis is also uncommon in heterosexuals and more likely to be asymptomatic in MSM with anal

infection. When interventions are being tested for their effects on gonorrhea transmission, investigators might consider including syphilis as an outcome.

C.K.F. was responsible for the initial hypothesis. This was developed further in discussions with E.F.P.C., L.Z., and J.S.H. All authors contributed significantly to the scientific content of this paper and approved of the final draft.

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Sequence Analysis of Toxin Gene–Bearing *Corynebacterium diphtheriae* Strains, Australia

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By conducting a molecular characterization of *Corynebacterium diphtheriae* strains in Australia, we identified novel sequences, nonfunctional toxin genes, and 5 recent cases of toxigenic cutaneous diphtheria. These findings highlight the importance of extrapharyngeal infections for toxin gene–bearing (functional or not) and non–toxin gene–bearing *C. diphtheriae* strains. Continued surveillance is recommended.

Pharyngeal diphtheria caused by toxigenic *Corynebacterium diphtheriae* is well-controlled in Australia due to a vaccine administered as part of the national immunization program. Rare cases of cutaneous and pharyngeal diphtheria have been reported in the country; however, the disease remains endemic in other regions of the world, and the potential for cases among travelers and their contacts remains (*1–4*). Historical data suggest that cutaneous diphtheria could be more contagious than respiratory diphtheria because environmental contamination from the skin is more common (*5*).

Detection of diphtheria toxin genes in either *C. ulcerans* or *C. diphtheriae* is notifiable in Queensland, regardless of the site of infection (*6*). Extrapharyngeal disease, such as cutaneous infection or endocarditis, caused by either toxigenic or nontoxigenic strains can be clinically notable, however, and is not prevented by vaccination (*4*). Our reference laboratory (Queensland Health Forensic and Scientific Services, Brisbane) receives isolates of *C. diphtheriae* and *C. ulcerans* from clinical laboratories in Queensland and surrounding areas for toxin gene testing. PCR is used to test for the presence of the toxin gene, which encodes for both subunits of the AB exotoxin. However, the functionality of the gene is not routinely examined (*7,8*). Previous studies in the United Kingdom and Russia have reported nonsense mutations in the toxin gene; those strains are described as nontoxigenic toxin gene–bearing (NTTB) (*9,10*). We aimed to identify potential mutations in

the toxin gene in a selection of isolates in Australia, as well as describe the recent epidemiology of *C. diphtheriae* isolations in the local area after the annual number of isolates referred to the laboratory had increased 10-fold from 2012 (n = 9) through 2015 (n = 108).

During the 2-year period from July 1, 2013, until June 30, 2015, a total of 136 isolates of *C. diphtheriae* were referred to our laboratory for toxin gene screening; these isolates included 2 that were second isolations from patients, 2 and 3 months after the initial specimens were collected. Primary identification by diagnostic referring laboratories was confirmed by the presence of *dtxR* (*11*). We did not determine patient vaccination status, biotype of isolates, presence of co-infecting organisms, and antimicrobial susceptibility and treatment as part of this study.

Of the 136 isolates we received, 129 (95%) were from cutaneous wound swab specimens; 93 (72%) of 129 wounds were located on the lower limbs. Six isolates were respiratory system–associated, including 1 from the ear swab specimen of a patient with otitis media. Four isolates, including 1 nontoxigenic isolate from a blood culture, were from hospitalized patients, with the remainder presumed not to be. How this systemic case developed clinically is unknown. In most cases (71%), travel history or evidence on how the infection was acquired was not provided; however, when such information was given, tropical travel locations and injuries involving seawater or coral were typically noted. Isolates were collected from patients in both urban and rural areas.

Five of the 136 isolates had both A and B subunits of diphtheria toxin (*tox*), detected by multiplex PCR, all of which appeared to be functional by sequence analysis (*7*). These 5 isolates were obtained from lower limb wound specimens from patients with a history of travel in a tropical travel area. Whole-genome sequencing with the Ion Torrent platform (Life Technologies, Grand Island, NY, USA) was performed on the 5 isolates with toxin genes detected by PCR and 1 historical isolate. We de novo assembled reads in Geneious R7 (Biomatters, Auckland, New Zealand) and used Ridom Seqsphere+ (Ridom GmbH, G Würzburg, Germany) to extrapolate in silico multilocus sequence typing (MLST) results and the sequences of diphtheria toxin repressor (*dtxR*) gene and *tox* genes. We also sequenced and analyzed the *tox* genes of an additional 8 historical *C. diphtheriae* isolates from our culture collection using methods

described (*9,12*). MLST results for 3 historical isolates known to be related to each other were determined as described (*13*).

We analyzed a total of 14 *tox*-positive isolates (Table). All of those with MLST results had unique sequence types, except for the 3 known linked historical isolates. Novel sequences for *dtxR* and *tox* were submitted to GenBank (accession nos. KU869770–5). The novel *dtxR* sequences contained silent mutations and the novel frame-shift, missense, and/or nonsense mutations of the novel *tox* sequences. We predicted that 2 historical isolates would have nonfunctional *tox* genes, with single nucleotide deletions at positions 55 and 226 in fragment A, causing frame-shift mutations and premature stop codons at aa 38 and aa 92, respectively. These strains were isolated in 2006 or earlier and are considered to be NTTB strains. One of these strains has previously been reported as toxigenic; however, *tox* functionality was not assessed in that study by either sequence analysis or Elek testing (*14*).

The introduction of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as a routine identification tool in clinical microbiology laboratories has likely been a factor responsible for the continued increase in referral of isolates to our laboratory in recent years, possibly in addition to increased awareness after the fatal case of respiratory diphtheria in Australia in 2011. The 5 recent cases of functional toxin gene–

bearing cutaneous *C. diphtheriae* infection more likely reflect an increase in testing cutaneous isolates rather than a true increase in incidence. Extrapharyngeal infections, particularly cutaneous, with both toxigenic and nontoxigenic strains are more common in this geographic region than is classical pharyngeal diphtheria, and their incidence is likely to have been historically underestimated. Repeat isolates from the same patient months after previous isolation reflect the chronic nature of cutaneous infection. This observation is also supported by most patients receiving care through outpatient settings. Any difference in the severity of disease caused by strains included this study is unknown, although we presume that functional toxin gene–bearing strains cause more severe disease.

Because of the theoretical possibility that NTTB stains and non–toxin gene–bearing strains could gain functional toxin expression by spontaneous mutation reversion or homologous recombination between different corynebacteriophages, these strains should be considered *tox* gene reservoirs (*9*). These strains also can cause systemic infections, as the blood culture isolate included in this study demonstrates. The genetic variation among the 5 recent functional toxin gene–bearing isolates indicates the absence of a particular circulating clone in the area. We recommend continued surveillance of *C. diphtheriae* and identification of NTTB strains.

Table. Analysis of tox gene–positive *Corynebacterium* diphtheria isolates, Australia*

Strain no.	GenBank accession no. dtxR gene	tox gene	Predicted diphtheria toxin peptide sequence	MLST	Year isolated	Site	Patient travel history	Clinical note
WM960461361	Detected, NP	KX702990	Complete	NP	Unknown (1996 or prior)	Unknown	Unknown	Historical isolate
WM960431373	Detected, NP	KX702991	Complete	NP	Unknown (1996 or prior)	Unknown	Unknown	Historical isolate
WM00M102	Detected, NP	KX702992	Complete	NP	Unknown (2000 or prior)	Unknown	Unknown	Historical isolate
WM00M103	Detected, NP	KX702993	Truncated	NP	Unknown (2000 or prior)	Unknown	Unknown	Historical isolate
2006M0083	Detected, NP	KX702994	Complete	NP	2005	Lower limb	Indonesia	Coral cut
2006M2336	KU869770	KU869773	Truncated	ST379	2006	Lower limb	Indonesia	Coral cut
2011M2688	Detected, NP	KX702995	Complete	ST125	2011	Throat	No travel	Contact of carrier
2011M2777	Detected, NP	KX702996	Complete	ST125	2011	Throat	Papua New Guinea	Carrier
2011M2861	Detected, NP	KX702997	Complete	ST125	2011	Throat	No travel	Contact of case
2013M7922	KX702987	KU869774	Complete	ST381	2013	Lower limb	Papua New Guinea	Wound
2014M5840	KU869772	KX702998	Complete	ST243	2014	Lower limb	Cambodia	Wound
2014M7492	KX702988	KX702999	Complete	ST382	2014	Lower limb	Indonesia	Surf injury
2014M8143	KX702989	KU869775	Complete	ST120	2014	Lower limb	Unknown	Coral cut
2015M2871	KU869771	KX703000	Complete	ST380	2015	Lower limb	Solomon Islands	Wound

*MLST, multilocus sequence typing; NP, sequencing not performed.

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Media Messages and Perception of Risk for Ebola Virus Infection, United States

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Thomas A. Burke, Lainie Rutkow

News media have been blamed for sensationalizing Ebola in the United States, causing unnecessary alarm. To investigate this issue, we analyzed US-focused news stories about Ebola virus disease during July 1–November 30, 2014. We found frequent use of risk-elevating messages, which may have contributed to increased public concern.

The 2014–15 outbreak of Ebola virus disease (EVD) generated much news media coverage and highlighted the role of news media with regard to providing information about risks to the public (*1–3*). Research shows that the news media can influence knowledge and perceptions about a topic (*4–6*). The way risks are discussed and communicated (often through news coverage) can also affect how risk is perceived (*7–9*). Our objective was to analyze the volume and content of messages promoted in US news media with regard to risk for EVD and to examine how these messages relate to risk-perception theory.

The Study

Using established methods, we analyzed EVD coverage from 12 news sources (9 print, 3 television) published July 1–November 30, 2014 (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/23/1/16-0589-Techapp1.pdf). News media stories were collected through searches of LexisNexis, ProQuest, and NewsBank online archives by using the term "Ebola." The search yielded 2,989 news stories, which were reviewed to determine if they met inclusion criteria (focus on US-associated EVD). The 374 stories that did not place EVD in a US context were included in our analysis of news volume only. The final sample for content analysis included 1,262 news stories and opinion pieces from print and television sources.

Our coding instrument contained 9 risk-elevating messages with characteristics that could increase perception of risk and 5 risk-minimizing messages with characteristics that could decrease perception of risk (online Technical Appendix Tables 2–4), developed according to the risk perception framework of Slovic (*7*). To assess interrater reliability, we coded a random sample of 15% of news stories. Most items met conventional standards for adequate reliability; κ values were $\geq$0.69 (*10*). For 4 items, κ values were slightly below this threshold but raw percentage agreement was high (90%–94%); therefore, these items were also included (online Technical Appendix Table 3). We assessed news story content about the EVD outbreak by calculating the proportion of stories that mentioned each EVD-associated message over the study period.

The volume of US-focused news coverage of the EVD outbreak peaked slightly after the arrival (August 2, 2014) of the first patient transported to the United States for treatment and increased much more after a case was diagnosed in Dallas, Texas, USA, on September 30, 2014 (Figure). Overall, 96% of print and television news stories that covered EVD in the context of the United States included $\geq$1 risk-elevating messages, 55% of stories contained $\geq$1 risk-minimizing messages, and 53% contained both message types. The most common risk-elevating messages (72%) concerned foreigners or travelers bringing Ebola virus to the United States. The most frequent risk-minimizing messages (32%) described scientific knowledge about EVD (Table).

Our analysis of news volume suggested that diagnosis of the first EVD case in Dallas and subsequent cases diagnosed in the United States were influential time points in the escalation of EVD outbreak news coverage, although internationally, the outbreak had reached historic levels months earlier. As noted elsewhere (*1,11*), the volume of EVD news was largely reduced after the US midterm elections. This reduction may reflect inclusion of EVD as a campaign issue late in the election cycle or may reflect lack of newly diagnosed cases in the United States.

The high frequency of risk-elevating messages in news coverage may have contributed to increased public concern about EVD in the United States, which was greater than the situation warranted. Consumers of news media would have been exposed to risk-elevating messages more often than risk-minimizing messages, potentially increasing their perception of risk for EVD. Risk messages of both types were more frequently included in television news than in print news, potentially leading to differences in perceived

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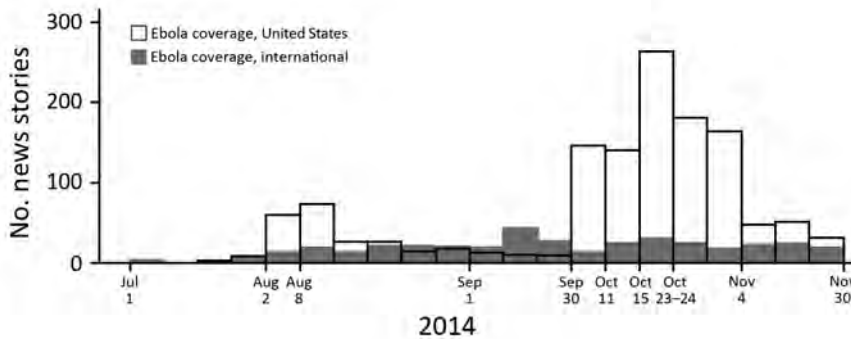
Figure. News coverage of Ebola virus disease comparing stories about Ebola in the United States and internationally, July–November 2014. Aug 2, first Ebola virus disease patient arrives in the United States; Aug 8, World Health Organization declares public health emergency of international concern; Sep 30, first case diagnosed in Dallas, Texas, USA; Oct 11, first case in healthcare worker; Oct 15, second case in healthcare worker; Oct 23–24, case diagnosed in New York, USA, and nurse quarantined; Nov 4, US midterm elections.

EVD risk among consumers of different news types. Although many factors can alter a message's effectiveness, frequency of exposure to risk-related messages can alter public perception and contribute to social amplification of risk; even when coverage is balanced, reassuring messages may be less able to counter messages that increase perception of risk (6,9). However, several messages that were seen significantly more frequently in liberal news sources (defined in Table) may have been associated with increasing awareness of specific issues, such as medical countermeasure development efforts and large-scale growth of the EVD epidemic.

The news media have been blamed for sensationalizing the EVD outbreak in the United States and unnecessarily alarming the public (3). Although the volume of news coverage may have influenced public attention, the content of analyzed news stories does not necessarily suggest that news media were reporting news about EVD in a hyperbolic or irresponsible manner. Comparison of opposing messages, such as the ability to stop transmission or the outbreak in the United States, which was more frequently mentioned than the inability to do so, suggests that some concerns may have resulted from the nature of the risk itself, rather than irresponsible news media coverage. Additionally, messages that were most inflammatory (e.g., science not understanding the disease, inability to stop Ebola in the United States, terrorism/use of Ebola as a bioweapon) were mentioned less frequently than nearly all other messages analyzed.

Although the methods used in this study do not allow for causal inference between news media coverage and public polling about EVD, comparison with public polling may provide useful context. EVD news volume roughly reflected changing levels of concern about EVD (1,12,13). News media coverage could have increased public concern, or public concern could have increased news coverage of risks. Despite widespread coverage of EVD, poll respondents were often misinformed about how the disease was spread; 85% of respondents indicated that a person was likely to get EVD via a sneeze or cough from a symptomatic

person, and 48% believed that transmission could occur before symptoms appeared (14). In our analysis, only 32% of news stories included scientific knowledge such as how the disease is spread. More in-depth and frequent coverage of the scientific aspects (and disease contagion pathways in particular) of a public health threat may prevent these types of misperceptions.

Our results should be considered in light of several limitations. First, the sample did not include all news types (e.g., talk radio, social media, local television, blogs) or international news sources. Furthermore, κ statistics for 4 items in the coding instrument were slightly below conventional reliability standards; however, these messages were either very common or rare, which can result in lower κ agreement (15). These items were thus included because of high raw percentage agreement. Although the process used to create and evaluate the coding instrument should have accounted for risk-elevating or risk-minimizing messages used frequently in coverage of EVD, some risk-related messages may have been unintentionally omitted and the imbalanced number of messages may have influenced our analysis of the overall frequency of message types. Furthermore, trends in news coverage may have been influenced by competing issues in the news cycle. Last, this study does not provide direct measurement of exposure to or influence of messages. Examination of competing messages within news stories and comparison of news sources such as blogs or international sources may be promising areas for future research.

Conclusions

The 2014–15 Ebola outbreak provides a useful case for studying emerging outbreaks and other public health emergencies. Certain risk messages about Ebola were used more frequently than others by US news media, which may have affected risk perception during the outbreak.

Acknowledgments

Table. Risk-related news media messages about Ebola virus disease, July–November 2014*

Messages	News stories with message, %						
	Print and TV, n = 1,262†	Ebola case/local controversy, n = 655‡	National, no Ebola case/ controversy, n = 607§	Conservative, n = 302¶	Liberal, n = 595#	Print, n = 1,109**	TV, n = 153††
That could increase perception of risk							
Lack of/limited availability of countermeasures to stop Ebola	17	13	21 (p<0.001)	11	19 (p<0.01)	17	20
Ebola causes deaths	66	64	68	70	65	66	65
Potential US outbreak/persons in the United States contracting Ebola	35	33	36	35	33	34	41
Inability to stop transmission/outbreak in the United States	7	4	9 (p<0.01)	4	6	6	7
Growth of the Ebola epidemic	23	17	30 (p<0.001)	14	26 (p<0.001)	21	36 (p<0.001)
Science does not understand Ebola (e.g., previous knowledge about the disease was wrong or expert advice was incorrect)	8	8	8	7	9	7	13 (p<0.05)
Ebola's potential use in terrorism or as a biologic weapon	1	1	1	1	1	1	1
Ebola has an incubation period	34	34	35	37	33	33	43 (p<0.05)
Foreigners or travelers bringing Ebola to the United States	72	71	74	72	70	71	79 (p<0.05)
That could decrease perception of risk							
Lower Ebola death rates in the United States	5	4	6	3	4	4	10 (p<0.001)
Ability to stop transmission/outbreak in the United States	20	16	24 (p<0.01)	24	17 (p<0.01)	18	30 (p<0.01)
Low risks related to Ebola (e.g., low risk of the disease coming to the United States, low risk of someone transmitting the disease, low risks of school children acquiring Ebola)	28	25	30	25	27	26	42 (p<0.001)
How to prevent spread of Ebola	12	12	13	12	10	11	20 (p<0.05)
Description of scientific knowledge about Ebola (e.g., transmission dynamics or other known aspects of the disease)	32	30	33	29	30	31	35

*Time frame selected to capture potential differences before and after key US Ebola events. χ^2 tests were used to test differences in the proportion of news stories mentioning each Ebola-related message in compared news sources.
†Sources included in all news stories: Atlanta Journal Constitution, Chicago Tribune, CNN Situation Room, Fort Worth Star-Telegram, Fox Special Report, NBC Nightly News, New York Daily News, New York Times, Orange County Register, Portland Press Herald, USA Today, and Washington Post.
‡New sources with an Ebola case or controversy in the locality: Atlanta Journal Constitution, Fort Worth Star-Telegram, New York Daily News, New York Times, and Portland Press Herald.
§Nationally produced new sources or those without an Ebola case or controversy in the locality: Chicago Tribune, CNN Situation Room, Fox Special Report, NBC Nightly News, Orange County Register, USA Today, and Washington Post.
¶Conservative news sources: Fort Worth Star-Telegram, Fox Special Report, and New York Daily News.
#Liberal news sources: Chicago Tribune, New York Times, and Washington Post.
**Print news sources: Atlanta Journal Constitution, Chicago Tribune, Fort Worth Star-Telegram, New York Daily News, New York Times, Orange County Register, Portland Press Herald, USA Today, and Washington Post.
††TV news sources: CNN Situation Room, Fox Special Report, and NBC Nightly News.

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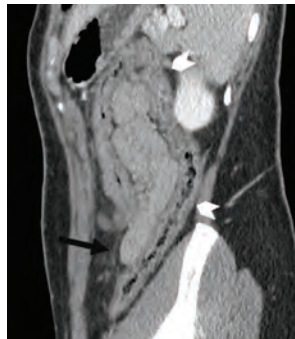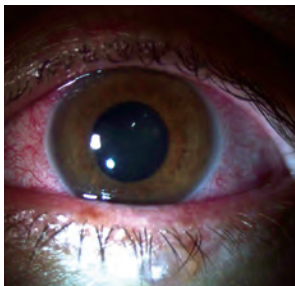America. PLoS One. 2015;10:e0129179. http://dx.doi.org/10.1371/journal.pone.0129179

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February 2016: Ebola

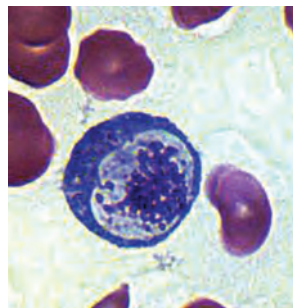**EMERGING INFECTIOUS DISEASES**

http://wwwnc.cdc.gov/eid/articles/issue/22/02/table-of-contents

Haemophilus influenzae Type b Invasive Disease in Amish Children, Missouri, USA, 2014

**Angela L. Myers, Mary Anne Jackson,
Lixin Zhang, Douglas S. Swanson,
Janet R. Gilsdorf**

During 5 months in 2014, three Amish children in Missouri, USA, were diagnosed with invasive *Haemophilus influenzae* type b infection. Two were rural neighbors infected with a genetically similar rare strain, sequence type 45. One child had recently traveled, raising the possibility of maintenance of this strain among unvaccinated carriers in Amish communities.

*H*aemophilus influenzae type b (Hib) vaccine was introduced in the United States in 1985, and since then, the incidence of invasive Hib infection among young children (<5 years of age) has decreased by 99%, from 46–100 cases/100,000 children to <1 case/100,000 children (*1–4*). However, small pockets of unimmunized and underimmunized children remain in this country and may continue to serve as potential reservoirs for disease. In a cluster of cases that occurred during 1999–2000 among Amish children in Pennsylvania, USA, 3 of 8 strains were genetically similar and identified by multilocus sequence typing (MLST) as sequence type (ST) 45, a previously unreported strain type in the United States (*5*). In 2014, we confirmed invasive Hib disease in 3 Amish children from 2 different communities in Missouri; 2 patients had disease caused by the ST45 strain that was implicated in the 1999–2000 Hib cluster in Pennsylvania (Table).

The Patients

Patient 1 was an unimmunized 13-month-old Amish boy from southwestern Missouri (community A) who had a fever (40°C) and refused to bear weight on his left leg. Blood and synovial fluid grew β-lactamase–negative Hib. After receiving parenteral ceftriaxone for 10 days, the patient was transitioned to oral amoxicillin to complete a 21-day course of antimicrobial therapy. At follow-up 2 weeks after hospital discharge, all signs and symptoms of infection had resolved.

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Patient 2 was an unimmunized 2-year-old Amish girl from rural northwestern Missouri (community B), 250 miles north of community A. She had a fever (39°C) and throat pain with drooling and difficulty breathing. She required immediate intubation for epiglottitis, and vancomycin and ceftriaxone were initiated. Blood cultures grew Hib, and a tracheal culture grew methicillin-susceptible *Staphylococcus aureus* and Hib. She suffered severe neurologic injury, and care was withdrawn per parent request. The patient subsequently died.

Patient 3 was an unimmunized 13-month-old Amish girl from northwestern Missouri (community B) and a neighbor of patient 2. She had a history of a fall from a wagon 4 weeks before hospital admission. She had persistent pain and limited range of motion of the right leg, which had become swollen 2 weeks before hospitalization. Magnetic resonance imaging at admission showed osteomyelitis of the right acetabulum, with dislocation of the right femoral head with necrosis, and extensive soft tissue and muscular abscesses around the proximal femur and into the right pelvis and lower abdominal retroperitoneum. Synovial fluid culture grew Hib. The patient underwent 3 operative washout procedures and placement of a spica cast. After 10 days of intravenous therapy with cefepime followed by ampicillin, she was transitioned to oral amoxicillin to complete a total of 6 weeks of therapy, after which she was fully recovered.

The Missouri State Health Department performed confirmatory serologic testing on all isolates, using antiserum for Hib capsular types a–f (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). We used PCR, as previously described (*6,7*), to test all *H. influenzae* strains in this cluster for specific capsule types a–f. We also used PCR to test the strains for capsule genes *bexA* and *bexB* (b capsule expression A and b capsule expression B) and for superoxide dismutase gene (*sodC*) to distinguish division I (*sodC−*) from division II (*sodC+*) *H. influenzae* (*8*). We performed MLST using the 7 standard MLST alleles. Whole-genome sequencing of all 3 *H. influenzae* strains was performed by Illumina (http://www.illumina.com/techniques/sequencing.html); the strain from patient 1 was sequenced a second time by PacBio (http://www.pacb.com/) (*9,10*). The 2 children from community B were infected with nearly identical Hib strains identified as ST45 (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/23/1/16-0593-Techapp1.pdf); the child from community A was infected

Table. Demographic and infection and characteristics for 3 Amish children infected with *Haemophilus influenzae* type b strains, Missouri, USA, 2014*

Patient no., community	Patient age, mo	Month of Hib diagnosis	Infection	Outcome	Capsule genes			MLST
					bexA/bexB	*sodC*		
1, A	13	Jan	Septic arthritis	Recovered	+/+	–		ST6
2, B	24	Feb	Epiglottitis	Died	+/+	–		ST45
3, B	13	May	Septic arthritis	Recovered	+/+	–		ST45

*None of the children had previous underlying conditions. *bexA*, b capsule expression A; *bexB*, b capsule expression B; community A, Amish community in southwestern Missouri; community B, Amish community in northwestern Missouri; MLST, multilocus sequence type; *sodC,* superoxide dismutase gene.

with a genetically different strain, ST6. The ST45 Hib strain is rarely reported and represents only 3 (0.5%) of 598 type b strains and 3 (0.15%) of 1,982 *H. influenzae* strains in the MLST database (*10*).

During December 1999–February 2000 in Pennsylvania, 7 cases of Hib infection were identified in children <3 years of age (*3*). Six of these cases occurred in Amish communities (*5*). Among the 7 Hib isolates, 2 were ST6 strains from 2 different communities, 3 were ST45 strains from 3 different communities, and 2 were ST44 strains (community source not available). We are not aware of subsequent ST45-related cases until now.

Although we found no epidemiologic link between the 2 Missouri children infected with Hib ST45 strains and Amish communities in Pennsylvania, patient 2 had traveled to Indiana and Wisconsin to visit family before the infection developed, raising the possibility of contact with a carrier of this rare strain among unvaccinated children in Amish communities. To enable evaluation of possible epidemiologic links, strain sequence typing should be considered for cases of invasive Hib disease, especially in the setting of underimmunized communities.

Conclusions

Since the implementation of Hib conjugate vaccination, the incidence of Hib disease in the United States has markedly declined. By reducing asymptomatic nasopharyngeal carriage, high rates of vaccination provide herd immunity protection for undervaccinated children. However, in underimmunized communities, relatively high prevalence rates of Hib carriage can serve as a reservoir for the organism (*3*), and our report illustrates that children from underimmunized communities remain at risk for serious Hib disease. Although now uncommon in the United States, Hib disease must be considered in the differential diagnosis of unimmunized and undervaccinated children with symptoms compatible with Hib infection.

Immunization is not forbidden by Amish religious teachings, but vaccination rates are generally low in many Amish communities (*3,11*). Surveys of Amish communities have identified fear of side effects, philosophical objections, and lack of priority as some reasons for vaccine hesitancy (*3,11*). Parents of one of our patients reported worry about vaccine side effects and preference for more natural healthcare as reasons for not immunizing their

children. Thoughtful, respectfully delivered public health education may help influence Amish parents to accept vaccines (*3,11*).

Although there was no known contact between patients 2 and 3, the local health department initiated a vaccination campaign within community B because the 2 Hib cases occurred within a 3-month period. Over the course of a year after these 2 cases in community B, the local health department provided vaccine to children <5 years of age on 4 separate occasions. Of the vaccine-eligible children (n = 40), 35 had completed the series as of July 2015, and the other 5 were progressing toward completion. A mass vaccination campaign was not undertaken in community A. However, the patient's siblings ultimately received Hib vaccine. Familiarity with the recent cases and education about Hib disease and vaccines likely influenced the generally successful Hib vaccine campaign in community B. Efforts to identify and appreciate obstacles to vaccine utilization among Amish and other undervaccinated communities aid health departments and clinicians in their efforts to improve community education and prevent infection.

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June 2015: Bacterial/Fungal Infections

- Sequence Type 4821 Clonal Complex Serogroup B *Neisseria meningitidis* in China, 1978–2013
- Estimated Deaths and Illnesses Averted During Fungal Meningitis Outbreak Associated with Contaminated Steroid Injections, United States, 2012–2013
- Global Burden of Invasive Nontyphoidal *Salmonella* Disease, 2010
- Dose-Response Relationship between Antimicrobial Drugs and Livestock-associated MRSA in Pig Farming
- Cost-effectiveness of Chlamydia Vaccination Programs for Young Women
- Hospitalization Frequency and Charges for Neurocysticercosis, United States, 2003–2012
- Additional Drug Resistance of Multidrug-Resistant Tuberculosis in Patients in 9 Countries
- Oral Cholera Vaccination Coverage, Barriers to Vaccination, and Adverse Events following Vaccination, Haiti, 2013
- *Mycobacterium bovis* in Panama, 2013
- Endemic Melioidosis in Residents of Desert Region after Atypically Intense Rainfall in Central Australia, 2011
- Invasion Dynamics of White-Nose Syndrome Fungus, Midwestern United States, 2012–2014
- *Coccidioides* Exposure and Coccidioidomycosis among Prison Employees, California, United States
- Prospective Multicenter International Surveillance of Azole Resistance in *Aspergillus fumigatus*
- Oligoarthritis Caused by *Borrelia bavariensis*, Austria, 2014
- European Rabbits as Reservoir for *Coxiella burnetii*
- Drug Resistance–Associated Mutations in *Mycoplasma genitalium* in Female Sex Workers, Japan
- Lack of Protection Against Ebola Virus from Chloroquine in Mice and Hamsters
- *Wohlfahrtiimonas chitiniclastica* Bacteremia Associated with Myiasis, United Kingdom
- Response to Dectection of New Delhi Metallo-β-Lactamase–Producing Bacteria, Brazil
- Histoplasmosis in Idaho and Montana, USA, 2012–2013

EMERGING INFECTIOUS DISEASES

http://wwwnc.cdc.gov/eid/articles/issue/21/06/table-of-contents

Meningitis Associated with Simultaneous Infection by Multiple Dengue Virus Serotypes in Children, Brazil

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Jonatas Santos Abrahão, Maria Rios,
Roney Santos Coimbra, Erna Geessien Kroon

To determine the causes of viral meningitis, we analyzed 22 cerebrospinal fluid samples collected during the 2014–2015 dengue epidemics in Brazil. We identified 3 serotypes of dengue virus (DENV-1, -2, and -3), as well as co-infection with 2 or 3 serotypes. We also detected the Asian II genotype of DENV-2.

Dengue is a disease of high incidence and a major public health problem worldwide (*1*). Approximately 2.5 billion persons live in dengue transmission risk areas, and 50 million dengue virus (DENV) infections occur annually. This disease is endemic in Brazil, with 4 DENV serotypes circulating; >1.6 million clinical cases were reported in 2015 (*2*).

DENV belongs to the family *Flaviviridae*, genus *Flavivirus*, and has 4 serotypes (DENV-1–4). These viruses are usually associated with a systemic and dynamic disease; the clinical conditions range from a nonspecific viral syndrome to severe disease. The most common symptoms are fever, rash, headache, nausea, vomiting, retro-orbital pain, and weakness (*3*). Neurologic manifestations have been increasingly reported; DENV could be considered an emergent etiologic agent of central nervous system (CNS) infection that causes encephalopathy, encephalitis, and meningitis (*1*). DENV infections of the CNS may or may not be associated with the classical systemic manifestations of dengue (*4*).

In dengue-endemic areas, co-circulation of different serotypes has been reported (*5*). Co-infection by different

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DENV serotypes has already been reported in patients and arthropods, but the effects on the disease and on the virus cycle has not been well established (*6,7*). The molecular diversity of DENV serotypes has been linked to different patterns of virulence. DENV-3 genotypes I and III, which circulate in Brazil, demonstrate distinct biological characteristics in mouse models (*8*). In 2014, the number of suspected dengue cases in Brazil was 589,107, with 58,177 in the state of Minas Gerais; in 2015, the number of cases increased to 1,649,008 in Brazil and 189,378 in Minas Gerais, with DENV-1 being the most frequently detected serotype (*2*). We report the detection of DENV-1, -2, and -3 co-infections in the CNS by reverse transcription PCR (RT-PCR) from cerebrospinal fluid (CSF) samples that tested negative for other classic neurotropic pathogens.

The Study

During the 2014–15 DENV epidemic in Minas Gerais, 22 CSF samples were collected from children suspected of having viral CNS infection who were hospitalized at the Hospital Infantil João Paulo II, Belo Horizonte, Minas Gerais, a reference children's hospital for all counties of the state. A presumptive diagnosis of CNS viral infection was given when the CSF of patients with clinical signs and symptoms of CNS infection had normal or slightly altered cytochemical parameters and tested negative for bacterial pathogens (*9*). The protocol for this study was approved by the hospital's scientific and ethical committee (no. 132/2009), and consent was obtained from parents or accompanying relatives.

The CSF samples tested negative for typical neurotropic viruses such as enteroviruses and human herpesviruses 1, 2, and 3. For DENV detection, RNA isolation and RT-PCR from 140 µL of CSF targeting the NS5 region was performed as described (*10*). The analyses showed that 7 samples (32%) were DENV positive. DENV-1 was detected in 1 sample (14.2% of positive samples); DENV-2 was detected in 3 samples (42.9%); DENV-3 was detected in 1 sample (14.2%); and DENV-4 was not detected in any samples. Co-infection with >1 DENV serotype was found in 2 CSF samples (28.6%); 1 sample was co-infected with DENV-2 and DENV-3 (sample from patient 571), and the other sample was

Table. Information and laboratory analyses during hospital admission of pediatric patients suspected of having viral CNS infection, Minas Gerais, Brazil*

Patient no.	Age, y/sex	Days hospitalized	Hemogram†			CSF‡				RT-PCR result	Hospital diagnosis	DENV IgM
			Hct	PLT	Leuk	Protein	Glucose	Leuk	PMN			
100	3/F	70	42.1	205	8.4	50	75	30	40	DENV-1	Dengue	+
557	9/F	6	38.1	206	17.4	30	70	28	29	DENV-1/2/3	Dengue	+
571	9/F	3	43.1	243	9.7	24	63	15	86	DENV-2/3	Dengue/viral meningitis	+
572	0.6/M	ND	35.2	365	8	11	54	2	4	DENV-3	Viral meningitis	–
575	0.9/F	5	23.3	522	10.7	97	65	176	1	DENV-2	Viral meningitis	–
577	6	8	39.1	230	9	35	62	42	10	DENV-2	Dengue	+
606	0.4/M	3	35	507	7.1	34	45	5	2	DENV-2	Acute otitis	–

*CNS, central nervous system; CSF, cerebrospinal fluid; DENV, dengue virus; Hct, hematocrit; leuk, leukocytes; ND, not done; PLT, platelets; PMN, polymorphonuclear neutrophils; +, positive; –, negative.
†Units: Hct, %; PLT, x 10^3/mL; leuk, × 10^3 cells/mm^3.
‡These laboratory analyses correspond to the first examination made during the acute phase of CNS infection. Units: protein, g/L; glucose, mmol/L; leuk, × 10^3 cells/mm^3; PMN, %.

triple infected with DENV-1, -2, and -3 (sample from patient 557) (Table).

Retrospective analysis of medical records provided us with information on evolution of the clinical condition of the 7 DENV-positive patients (Table). Patients 575 and 572 received a presumptive diagnosis of meningitis on the basis of clinical signs and symptoms, but they did not show the classical symptoms and signs of dengue fever. Thus, serologic testing for dengue was not requested by clinicians. Patients 100, 557, 571, and 577 were admitted to the hospital with suspected dengue fever. In addition, they demonstrated some neurologic alterations such as seizures; among those patients, only patient 571 showed signs of meningitis. Patient 571 had a predominance of polymorphonuclear neutrophils in the CSF. However, the leukocyte count was <400 cells/mm^3, and the bacterial culture was negative (3).

To confirm DENV as the etiologic agent of CNS infection, we used a 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) to directly sequence the amplified DNA. The nucleotide sequences were aligned and used to construct phylogenetic trees by using the neighbor-joining method (Figure). The Tamura-Nei statistical model implemented in MEGA 6.0 software (Arizona State University, Tempe, AZ, USA) was used with 1,000 bootstrap replicates. These sequences were deposited in GenBank (sequence and accession nos.: 557 CNS DENV-1, KU615569; 557 CNS DENV-2, KU726002; 557 CNS DENV-3, KU948725; 571 CNS DENV-2, KU948727; and 571 CNS DENV-3, KU948726).

The 3 DENV serotypes found in CSF co-infection have been classified into genotypes, as described (11). Phylogenetic analysis of the NS5 gene sequences demonstrated that isolate 557 CNS DENV-1 grouped with isolates of American/African genotype V (Figure, panel A), which has been reported in Southeast Asia (Singapore), South America, and Africa and is the genotype that circulates in Brazil (12).

The DENV-2 isolates from samples from patients 557 and 571 grouped with isolates of Asian II, which is a genotype that consists of viruses circulating in China, the Philippines, Sri Lanka, Taiwan, and Vietnam (13). The American/Asian genotype has been the only genotype previously identified in Brazil, although some variations have occurred within this genotype (12) (Figure, panel B). Singh et al. demonstrated that the Asian II and Cosmopolitan DENV-2 genotypes co-circulated in Nepal, with no differences in their replication rate in mosquito cells (14,15).

The DENV-3 nucleotide sequences of samples from patients 577 and 571 showed a close phylogenetic relationship with genotype III when compared with the nonstructural-5 gene sequences of other DENV-3 viruses circulating in different parts of the world (Figure, panel C). Previous studies have reported circulation of DENV-3 genotypes I and III in Brazil, and differences in the course of the infection between these 2 genotypes in a mouse model have been described. This study relates DENV-3 genotype III to CNS infection (8). DENV serotype co-infection has already been described in the literature from human blood samples and in *Aedes* mosquitoes (5–7). However, to our knowledge, DENV serotype co-infections have not previously been detected in the CNS.

Conclusions

DENV co-infection with other flaviviruses has been described, but not in relation to the different DENV serotypes involved in CNS infection. We identified 2 patients with meningitis: 1 was infected with 2 DENV serotypes and the other with 3 DENV serotypes. We also report the circulation of the DENV-2 genotype Asian II in Brazil, where the DENV-2 genotype American/Asian has been the most prevalent genotype since 1990 (13). When and where genotype Asian II started to circulate in Brazil is unclear.
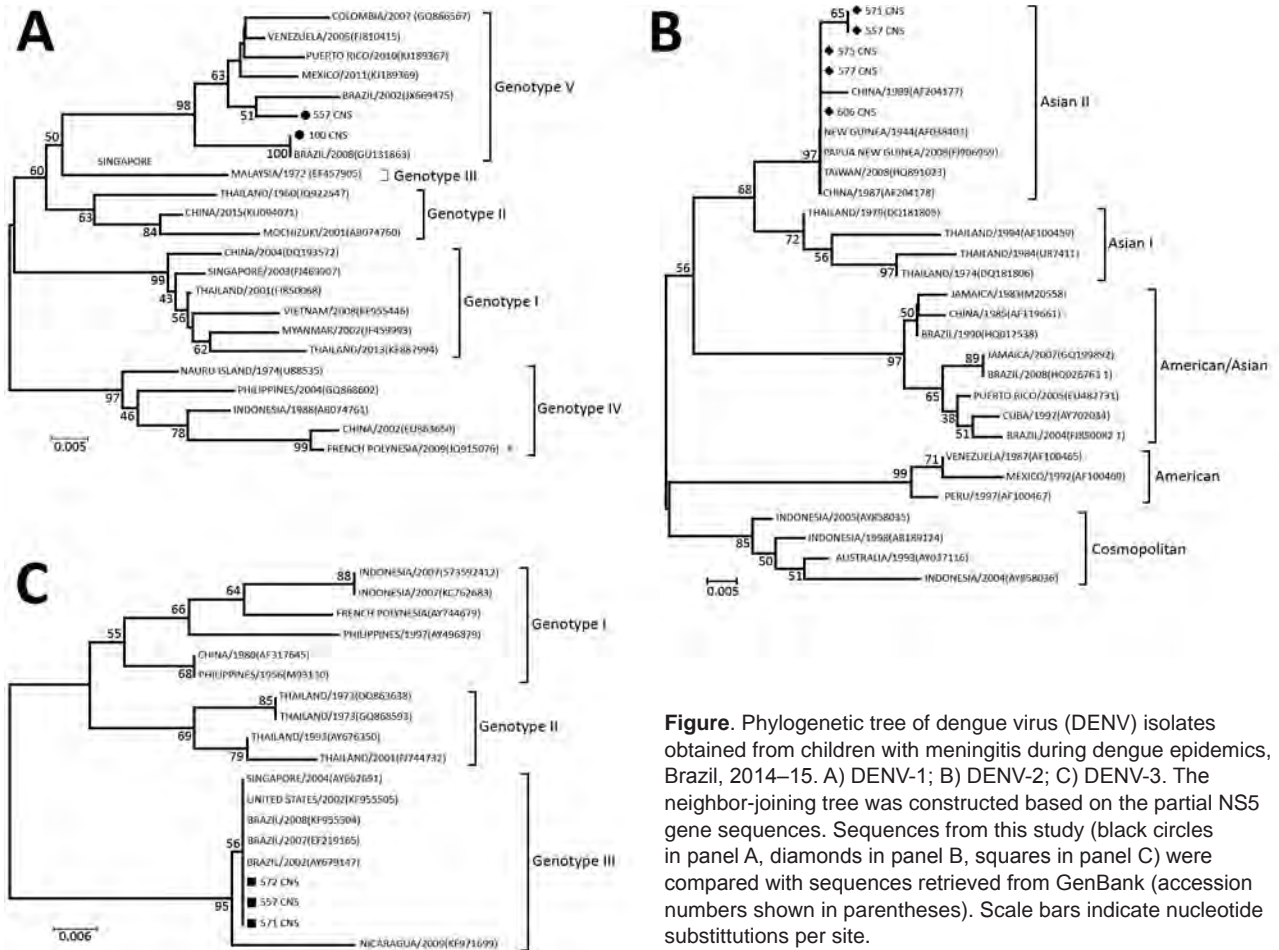
Figure. Phylogenetic tree of dengue virus (DENV) isolates obtained from children with meningitis during dengue epidemics, Brazil, 2014–15. A) DENV-1; B) DENV-2; C) DENV-3. The neighbor-joining tree was constructed based on the partial NS5 gene sequences. Sequences from this study (black circles in panel A, diamonds in panel B, squares in panel C) were compared with sequences retrieved from GenBank (accession numbers shown in parentheses). Scale bars indicate nucleotide substitutions per site.

Our results also suggest that CNS DENV infection can be preceded by classic dengue fever symptoms or can occur without any classic symptoms. Because the evolution of DENV infection and DENV serotype co-infection into a CNS infection is underreported, attempts to identify the serotypes and genotypes involved in this severe clinical manifestation should be undertaken to clarify the clinical relevance of cases of DENV serotype co-infections.

Acknowledgments

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May 2016: Vectorborne Diseases

- An Operational Framework for Insecticide Resistance Management Planning

- *Plasmodium falciparum* K76T pfcrt Gene Mutations and Parasite Population Structure, Haiti, 2006–2009

- Outbreak of Middle East Respiratory Syndrome at Tertiary Care Hospital, Jeddah, Saudi Arabia, 2014

- Differences in Genotype, Clinical Features, and Inflammatory Potential of *Borrelia burgdorferi* sensu stricto Strains from Europe and and the United States

- Expansion of Shiga Toxin–Producing *Escherichia coli* by Use of Bovine Antibiotic Growth Promoters

- Projecting Month of Birth for At-Risk Infants after Zika Virus Disease Outbreaks

- Genetic Characterization of Archived Bunyaviruses and Their Potential for Emergence in Australia

- *Plasmodium falciparum* In Vitro Resistance to Monodesethylamodiaquine, Dakar, Senegal, 2014

- *Rickettsia parkeri* Rickettsiosis, Arizona, USA

- Astrovirus MLB2, a New Gastroenteric Virus Associated with Meningitis and Disseminated Infection

- Spectrum of Viral Pathogens in Blood of Malaria-Free Ill Travelers Returning to Canada

- Expanded Geographic Distribution and Clinical Characteristics of *Ehrlichia ewingii* Infections, United States
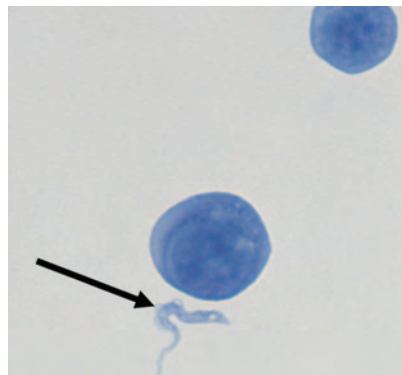
EMERGING INFECTIOUS DISEASES

http://wwwnc.cdc.gov/eid/articles/issue/22/05/table-of-contents

Travel-Related Tick-Borne Encephalitis, Israel, 2006–2014

Eyal Meltzer,[1] Yael Paran,[1] Yaniv Lustig, Shmuel Stienlauf, Miriam Weinberger, Eli Schwartz

During 2006–2014, four tick-borne encephalitis (TBE) cases occurred among Israeli travelers. We calculated TBE incidence at 321.0, 45.0, 13.2, and 7.5 cases/100,000 travelers/year of travel to Sweden, Switzerland, Austria, and Germany, respectively. TBE incidence among travelers to these destinations appears to justify TBE vaccination in accordance with World Health Organization recommendations.

Tick-borne encephalitis (TBE), an arboviral zoonosis transmitted mostly through the bite of *Ixodes* spp. ticks, is endemic to many popular tourist destinations in Europe (*1*). TBE vaccine is considered immunogenic and safe. It is highly effective in reducing TBE in disease-endemic areas such as Austria, the first country to include the vaccine in its national vaccine program. The World Health Organization (WHO), the US Centers for Disease Control and Prevention, and the Israeli Ministry of Health have published advice on TBE vaccination for travelers (*2–4*), suggesting limiting vaccination to travelers planning activities with high-risk exposures. However, the evidence base for these recommendations is not well established.

The most comprehensive study of travel-related TBE (*5*) comprised 38 travel-related cases of TBE and estimated an annual attack rate of 1 case/1.3–2 × 10⁶ travelers. However, the lack of an accurate denominator and absence of data on exposure time raise questions about its accuracy. We investigated the epidemiology of TBE in travelers from Israel and assessed the risk for travel-related TBE.

The Study

In Israel, viral encephalitis is a notifiable disease. Diagnostic tests for TBE in Israel have been available since 2006 and are performed only in the Israeli Central Virology Reference Laboratories (Tel Hashomer, Israel) of the Israeli Ministry of Health by using an indirect immunofluorescence

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commercial kit (Euroimmun AG, Lübeck, Germany) according to manufacturer instructions.

As numerator, we included all confirmed cases of TBE (according to criteria adopted by the European Commission in 2012 [*6*]). During 2006–2014, a total of 4 TBE cases were diagnosed in Israel: 1 case each acquired in Austria, Russia, and Sweden and 1 case acquired in either Germany or in Switzerland (Table 1; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/23/1/16-0888-Techapp1.pdf).

As denominator we used 2 sets of data. For the first, in emulation of Steffen et al. (*5*), we calculated TBE attack rate per 100,000 traveler entries during the study period, according to the number of Israeli tourists obtained from the United Nations World Tourism Organization database (Table 2). Cumulatively, 3,928,164 Israeli travelers had entered these countries during the study period. The combined attack rate for these countries (according to whether Germany or Switzerland was considered the place of acquisition of 1 case) ranged from 1 case per 837,528 to 1 case per 573,493 Israeli tourist entries.

Because the United Nations World Tourism Organization database lacks information about duration of stay, we also used data on the absolute number of nights stayed by Israeli travelers during the study period from the published tourism statistics of Switzerland, Germany, and Austria (*10–12*) (similar data for Russia were lacking); for Sweden, we obtained the number of overnight stays from Statistics Sweden (E. Meltzer, unpub. data). TBE incidence ranged from 1 case per 697,700 person-weeks (Germany) to 1 case per 16,270 person-weeks (Sweden), which is equivalent to an annual incidence of 7.5–321.0 cases/100,000 travelers per year of travel (Table 2).

Conclusions

TBE is endemic to some of the most popular tourist destinations in developed countries, raising questions about vaccination and advice to travelers. However, there is such a dearth of published research on the actual risk to travelers that TBE is termed a neglected disease in travel medicine (*13*). A 2016 study of travel-related TBE estimated an attack rate of 1 case per 1.3–2 × 10⁶ tourist-entries, concluding that vaccination should be offered only to travelers planning activities resulting in at-risk exposures (*5*). By using travel duration data, we found that the actual TBE incidence rate in travelers from Israel to

[1]These authors contributed equally to this article.

Table 1. Clinical, epidemiologic, and laboratory data for TBE cases, Israel*

Variable	Case-patient 1	Case-patient 2	Case-patient 3	Case-patient 4
Destination	Austria	Germany, Switzerland	Sweden	Russia
Probable area of exposure	Salzburgland	Baden-Wurttemberg	Northwest of Stockholm	Southwestern Siberia
Month of travel	2014 Jun	2012 Sep	2011 May–Aug	2010 Aug
Duration of travel, d	3	14	107	17
Duration of probable exposure to tick habitat	1 h	4 d	Undetermined	17
Recorded tick bite	Yes	No	No	No
Neurologic manifestations during acute phase	Diplopia → stupor, aphasia, quadriparesis	Dysphagia, dysgeusia, bilateral facial nerve paralysis	Meningismus, mild confusion, dysarthria → Lt facial nerve. paralysis	Acute confusion, stupor
Neurologic outcome at 6 mo	Complete motor recovery, difficulty in complex tasks, depression.	Complete recovery	Complete recovery	Complete recovery
TBE serology results†				
First serum sample	IgM positive, IgG positive	IgM positive, IgG negative	IgM positive, IgG positive	IgM positive, IgG positive
Convalescent-phase serum sample	IgM positive, IgG 10-fold increase	IgM positive, IgG seroconversion	IgM positive‡, IgG positive‡	ND

*ND, not done; TBE, tickborne encephalitis.
†West Nile virus was ruled out serologically in all cases.
‡Direct comparison between serum samples was not possible because initial sample was taken abroad.

Sweden, Austria, Germany, and Switzerland (Table 2) is at least as high as that of the local population (5/100,000 in Austria and <1/100,000 in Germany, for example [1]) and is higher than the WHO-recommended threshold for universal TBE vaccination (an annual incidence >5/100,000 population) (14).

Few other studies on the incidence of travel-related TBE have been published. These studies also suggest a substantial TBE risk to travelers that is similar in range to our results (Table 2).

Our study has several limitations. Calculating incidence rates when event numbers are small runs the risk for overestimation, according to the law of small numbers. However, by considering the total period of TBE test availability in Israel (9 years), rather than just the years in which cases were detected, we believe we avoided such overestimation. In addition, reporting of notifiable diseases might be incomplete, and clinicians' awareness of the need to consider TBE in a returning traveler from Europe or northern Asia probably is insufficient. Also, milder TBE cases might have been overlooked. These considerations also might have resulted in an underdetection of cases. Statistics on overnight stays might not include some travelers, such as expatriates in private residences or those staying with families. On the other hand, in such travel scenarios, persons with TBE might have been treated abroad and therefore missed. We believe the combined effect of these limitations is more likely to have led to an underestimation than an overestimation of TBE risk for travelers. Indeed, our calculations included all Israeli tourists traveling in all seasons, whereas as shown in the 4 cases reported here as well (Table 1), TBE risk is seasonal: were only summer tourists considered, incidence rates of travel-related TBE probably would have been even higher.

Table 2. Calculated incidence of travel-related TBE for selected countries, Europe*

Study (source country, years of study [reference])	Country of travel	Population studied	TBE incidence		
			Cases/tourist entries	Cases/person-weeks	Cases/100,000 travel-years
Incidence of TBE disease					
This study (Israel, 2006–2014)	Germany	Travelers	1/1,634,192	1/697,700	7.5
	Austria	Travelers	1/732,160	1/393,556	13.2
	Switzerland	Travelers	1/ 578,052	1/199,102	45.0
	Sweden	Travelers	1/128,642	1/16,270	321.0
	Russia	Travelers	1/855,118	NA	NA
Reusken et al. (Netherlands [7])	Austria	Travelers	NA	1/1,380,952	3.8
Incidence of TBE seroconversion					
Sanchez et al. (USA [8])	Bosnia-Herzegovina	Military personnel	NA	1/12,501	416.0, 8.3†
McNeil et al. (USA [9])	Germany	Military personnel	NA	1/4,775	1,088.9, 21.8†

*NA, not available; TBE, tick-borne encephalitis.
†Estimated incidence of clinical disease if 98% of infections are subclinical.

It is interesting to compare the data presented here on TBE to another travel-related, vaccine-preventable flavivirus: Japanese encephalitis (JE). The risk for JE among travelers is considered low: $<1/1 \times 10^6$ travelers staying 1 month (which equals an incidence lower than 1.2 cases/100,000 travelers/year of travel) (*2*). Our findings, as well as those of previous reports, suggest that the risk posed by TBE far exceeds that posed by JE to travelers. Only 1 case of JE in an Israeli traveler has been reported in >2 decades (*15*), whereas at least 4 TBE cases were diagnosed during the past 9 years. Similar to JE, TBE had been documented to cause severe and fatal illness among travelers, but whereas JE vaccines for travelers are promoted by most government advisory boards in developed countries, very little is done regarding TBE vaccination. This difference probably underlies the fact that in Israel, for example, during 2012–2014, a total of 46,773 JE vaccine doses were distributed, whereas only 960 TBE vaccine doses were sold during the same period (E. Schwartz, unpub. data).

In conclusion, actual incidence of TBE among Israeli travelers to Austria, Germany, Sweden, and Switzerland appears to be higher than the threshold recommended by WHO for universal TBE vaccination. Vaccination should be recommended to all travelers with potential exposure to tick habitat in these destinations. However, TBE vaccine appears to be greatly underutilized among Israeli travelers.

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Increased Invasive Pneumococcal Disease, North East England, UK

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Since April 2014, invasive pneumococcal disease incidence has increased substantially across North East England, United Kingdom, reversing the decline that followed the 2006 introduction of pneumococcal conjugate vaccines. Significant increases occurred in serotypes exclusive to the 23-valent polysaccharide vaccine and in nonvaccine serotypes. Public health strategies for tackling persistent disease should adapt.

The UK routine immunization program includes 2 vaccines against pneumococcal disease (*1*). The 7-valent pneumococcal conjugate vaccine (PCV7), introduced in 2006 and replaced by the 13-valent pneumococcal conjugate vaccine (PCV13) in 2010, is given to infants 2, 4, and 12 months of age (*1*). The 23-valent pneumococcal polysaccharide vaccine (PPV23) has been recommended for persons in clinically defined risk groups ≥2 years of age since 1992 and for all persons ≥65 years of age since 2003 (*1*). National coverage of PCV at 12 months reached 90% by epidemiologic year (April 1–March 31, indicated by slashes in year ranges) 2008/2009 and remains >93% (*2*). Since 2009/2010, coverage in North East England (NEE) has been >95% (*2*). By 2007/2008, PPV coverage in England and NEE reached 70% among all persons ≥65 years of age and remained there through March 31, 2016 (*3*).

Invasive pneumococcal disease (IPD) incidence in NEE declined significantly after the introduction of PCV7 and subsequently PCV13 among persons in vaccinated and nonvaccinated age groups, consistent with other countries and the United Kingdom (*4–8*). This decline coincided with emergence of less frequent nonvaccine type (NVT) serotypes, reinforcing the need for continued IPD surveillance (*4–8*). Using enhanced surveillance data for April 1, 2006, through March 31, 2016, we detected increased IPD incidence in NEE.

The Study

In April 2006, the NEE Invasive Pneumococcal Disease Enhanced Surveillance System was established (*4*) and gathered data from microbiology services, hospitals and primary care clinicians, and the Public Health England Respiratory and Vaccine Preventable Bacteria Reference Unit (*9*). We compared IPD incidence during the 2015/2016 epidemiologic year with that from previous epidemiologic years and with the average annual incidence during the 3 epidemiologic years covering April 1, 2011–March 31, 2014. We analyzed IPD incidence across all cases combined and cases stratified by vaccine serotype subgroups: PCV7/PCV13 serotypes (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F); PPV23-exclusive serotypes (2, 8, 9N, 10A, 11A, 12F, 15B [including 15B/C], 17F, 20, 22F, 33F); and NVT serotypes (*1*). We examined IPD incidence trends by specific serotype during April 2013–March 2016 by using incidence rate ratios (IRRs), estimated by using negative binomial regression (with counts per calendar quarter, robust standard errors, and offset with the natural logarithm of the NEE population [*10*]).

For each epidemiologic year spanning April 1, 2011–March 31, 2014, an average of 211 IPD cases (8.1 cases/100,000 population) were reported. In contrast, during 2015/2016, a total of 298 cases (11.4/100,000) were reported. This incidence was significantly greater than that for 2014/2015 (230 cases, 8.8/100,000; IRR 1.30, 95% CI 1.09–1.55, p = 0.003); significantly greater than the average during the 3 epidemiologic years spanning 2011–2014 (IRR 1.40, 95% CI 1.17–1.68, p<0.001); and similar to 2006/2007 (11.91/100,000; IRR 0.96, 95% CI 0.81–1.12, p = 0.577) (Table 1). A similar trend occurred among patients 5–64 years of age (2015/2016 vs. 2014/2015 IRR 1.32, 95% CI 1.01–1.73, p = 0.036; 2015/2016 vs. 2011–2014 IRR 1.43, 95% CI 1.09–1.88, p = 0.008; 2015/2016 vs. 2006/2007 IRR 0.97, 95% CI 0.76–1.24, p = 0.796) and patients ≥65 years of age (2015/2016 vs. 2014/2015 IRR 1.25, 95% CI 0.98–1.61, p = 0.067; 2015/2016 vs. 2011–2014 IRR 1.40, 95% CI 1.08–1.82, p = 0.008; 2015/2016 vs. 2006/2007 IRR 0.98, 95% CI 0.77–1.25, p = 0.892) (Table 1). Among patients <5 years of age, incidence during 2015/2016 remained significantly lower than that during 2006/2007 (IRR 0.44, 95% CI 0.23–0.80, p = 0.004), similar to that during 2011–2014 (IRR 0.99, 95% CI 0.48–2.07, p = 0.985), and did not significantly increase during 2014/2015 (IRR 1.55, 95% CI 0.68–3.65, p = 0.265) (Table 1).

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Table 1. Number and incidence of invasive pneumococcal disease in North East England, April 2006–March 2016

| Epidemiologic year*	Total no. cases	No. (%) cases, by age group†			Incidence rate (95% CI)‡			
		<5 y	5–64 y	≥65 y	All cases	<5 y	5–64 y	≥65 y
2006/2007	304	35 (12)	137 (45)	132 (43)	11.91 (10.71–13.22)	25.56 (18.99–33.46)	6.89 (5.86–8.09)	30.82 (26.63–35.34)
2007/2008	268	29 (11)	129 (48)	110 (41)	10.46 (9.33–11.71)	20.67 (14.79–28.1)	6.48 (5.48–7.64)	25.57 (21.68–29.9)
2008/2009	263	32 (12)	119 (45)	112 (43)	10.24 (9.12–11.47)	22.21 (16.19–29.67)	5.97 (5.02–7.1)	25.85 (21.95–30.17)
2009/2010	251	27 (11)	114 (45)	110 (44)	9.75 (8.66–10.95)	18.48 (13.03–25.56)	5.73 (4.79–6.83)	25.1 (21.26–29.36)
2010/2011	263	29 (11)	117 (44)	117 (44)	10.17 (9.06–11.39)	19.53 (13.95–26.64)	5.87 (4.92–6.99)	26.27 (22.39–30.54)
2011/2012	237	21 (9)	98 (41)	118 (50)	9.13 (8.08–10.30)	14 (9.34–20.46)	4.92 (4.05–5.95)	26.07 (22.24–30.31)
2012/2013	226	18 (8)	107 (47)	101 (45)	8.68 (7.66–9.83)	11.86 (7.63–17.96)	5.4 (4.49–6.48)	21.55 (18.07–25.5)
2013/2014	169	12 (7)	74 (44)	83 (49)	6.47 (5.59–7.48)	7.91 (4.58–13.31)	3.74 (2.99–4.67)	17.28 (14.17–20.92)
2014/2015	230	11 (5)	100 (43)	119 (52)	8.78 (7.76–9.93)	7.26 (4.1–12.52)	5.06 (4.18–6.12)	24.21 (20.63–28.19)
2015/2016	298	17 (6)	132 (44)	149 (50)	11.38 (10.22–12.65)	11.21 (7.12–17.22)	6.68 (5.66–7.87)	30.32 (26.42–34.52)

*April 1–March 31.
†Percentage of all cases.
‡No. cases/100,000 population. Mid-year population estimates were obtained from the Office for National Statistics (10); for all epidemiologic years other than 2015/2016, incidence rates were calculated by using mid-year population estimates for the first calendar year; for 2015/2016, mid-year estimates for 2014 were used.

The recent rise in IPD is largely attributable to increased cases caused by PPV23-exclusive serotypes (2015/2016 vs. 2011–2014 IRR 2.42, 95% CI 1.80–3.29, p<0.001; 2015/2016 vs. 2006/2007 IRR 3.04, 95% CI 2.20–4.27, p<0.001), notable from 2014/2015 on (Figure 1). Of the 11 serotypes exclusive to PPV23, significant increasing trends were demonstrated by serotypes 8, 9N, and 12F from 2013/2014 on (Table 2; Figure 2). This trend was observed among patients 5–64 and ≥65 years of age; cases among patients <5 years of age were considerably fewer, and temporal changes by serotype were difficult to interpret (data not shown).

Over the longer term, the number of cases caused by NVT serotypes increased between 2006/2007 and 2015/2016 (IRR 2.58, 95% CI 1.52–4.56, p<0.001), particularly from 2008/2009 on (Figure 1). The increased incidence of IPD caused by NVT was not statistically significant between 2015/2016 and 2011–2014 (IRR 1.23, 95% CI 0.80–1.88, p = 0.236). Among NVTs with an observed increase, serotypes 15A, 23A, and 35F increased significantly from 2013/2014 on (Table 2; Figure 2). For 23A, this increase was particularly notable among persons ≥65 years of age; for serotypes 15A and 35F, the increase was among persons 5–64 and ≥65 years of age (data not shown).
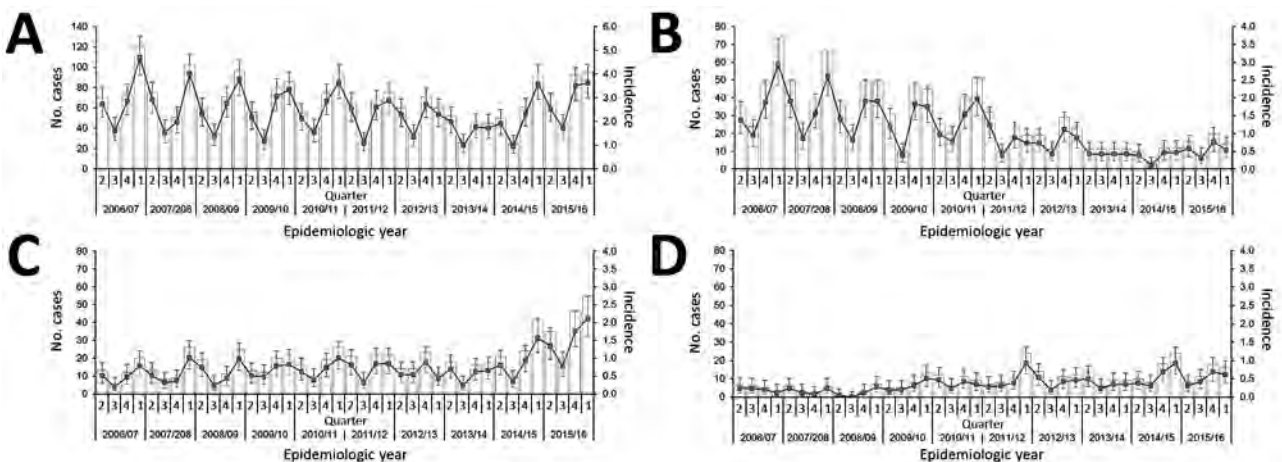
Figure 1. Number and incidence (no. cases/100,000 population) of invasive pneumococcal disease cases by vaccine type serotype subgroups in North East England, by quarter, April 2006–March 2016. A) All cases. B) Cases caused by 13-valent pneumococcal conjugate vaccine serotypes. C) Cases caused by 23-valent pneumococcal polysaccharide vaccine serotypes, excluding those also contained in PCV13. D) Cases caused by nonvaccine types. Bars show numbers of cases. Lines indicate incidence, error bars indicate 95% CIs.

Table 2. Trends in incidence of serotypes causing invasive pneumococcal disease in North East England, April 2013–March 2016*

Serotype group, serotype	IRR (95% CI)†	p value†
PPV23–13		
8	**1.18 (1.08–1.29)**	**<0.001**
9N	**1.19 (1.04–1.36)**	**0.009**
10A	1.07 (0.89–1.29)	0.465
11A	1.03 (0.92–1.15)	0.617
12F	**1.28 (1.20–1.36)**	**<0.001**
15B/C‡	1.29 (0.99–1.69)	0.061
17F	0.84 (0.65–1.09)	0.188
20	1.12 (0.96–1.31)	0.148
22F	1.04 (0.95–1.13)	0.421
33F	1.17 (1.00–1.38)	0.051
NVT§		
6C	1.00 (0.91–1.10)	0.958
15A	**1.13 (1.07–1.19)**	**<0.001**
16F	0.99 (0.85–1.15)	0.924
23A	**1.15 (1.06–1.24)**	**<0.001**
23B	1.06 (0.81–1.40)	0.657
24F	1.03 (0.91–1.17)	0.645
31	1.03 (0.92–1.16)	0.591
35B	1.12 (0.92–1.37)	0.256
35F	**1.23 (1.06–1.44)**	**0.008**
38	0.84 (0.61–1.15)	0.279

*Boldface indicates significance. IRR, incidence rate ratio; PPV23–13, 23–valent pneumococcal polysaccharide vaccine serotype cases, excluding those also contained in the 13–valent pneumococcal conjugate vaccine; NVT, nonvaccine type serotype cases.
†Increase in IRR per calendar quarter, estimated by using negative binomial regression, with counts per calendar quarter (from April 1–June 30, 2013 (Quarter 2) to January 1–March 31, 2016 (Quarter 1), robust standard errors and offset with the natural logarithm of the North East England population (*10*).
‡Includes 15B and 15B/C but excludes 15C serotypes as determined by the Public Health England Respiratory and Vaccine Preventable Bacteria Reference Unit.
§NVT serotypes reported more than once in the epidemiologic year 2015–2016.

Conclusions

Total IPD incidence increased significantly, starting in 2014/2015, reversing the declines in total IPD incidence that followed the introduction of PCVs (*4–8*). The increases were significant for PPV23-exclusive serotypes 8, 9N, and 12F and for NVT serotypes 15A, 23A, and 35F, most notably among persons 5–64 and ≥65 years of age.

We know of no mechanism for increased host susceptibility that could explain these rapid incidence changes. Although associations between influenza and IPD have been reported (*11,12*) and genetically drifted influenza strains contributed to low vaccine effectiveness in the United Kingdom during 2014/2015 (*13*), our primary analysis compared 2015/2016 with 2011–2014 so that any IPD increase associated with the 2014–2015 influenza season had no influence on these findings.

Mechanisms for changes in serotype prevalence include serotype replacement and capsular switching (genetic serotype switch in individual organisms) (*14*). In NEE, serotype replacement and declining IPD incidence were observed among persons of all age groups soon after introduction of PCV7 childhood vaccination (*4*), highlighting the influence of strains affecting young children in determining prevalent pneumococcal serotypes among persons in nonvaccine age groups. With ongoing ≥95% vaccination coverage in NEE, direct protection extends into an ever-increasing proportion of the population, up to those 10 years of age in 2016, increasing pressure on PCV strains. This pressure may be leading to accelerated serotype replacement throughout the population or to increased capsular switching, resulting in some non-PCV serotypes becoming more prevalent. Natural fluctuations in serotype prevalence may also be occurring. However, explanations for the recent IPD increase need to account for the recent and somewhat sudden rise following a long period of decline. For instance, perhaps natural expansion of non-PCV strains into the ecologic niches created has been delayed and therefore the decline observed was only temporary, or perhaps there have been recent changes in invasiveness of the non-PCV strains either naturally or associated with serotype replacement or capsular switching.

Our findings, together with data from all England (*15*), suggest that IPD epidemiology continues to evolve after 10 years of routine childhood vaccination. Observations from other regions that have introduced PCV are merited to determine whether the increase observed in NEE is, or becomes, a widespread phenomenon and, if so, its relationship to the timing of PCV implementation and PCV coverage. Also needed are further studies of the effects of ongoing vaccination on carriage and molecular studies to identify evidence for capsular switching and changes in invasiveness. Clarification of such factors may help guide changes to public health strategies required to tackle persistent IPD.

Figure 2. Trends in incidence of serotypes causing invasive pneumococcal disease associated with recent significantly increasing incidence in North East England, by quarter, April 2006–March 2016. Panels show trends by individual serotypes: A) serotype 8; B) serotype 9N; C) serotype 12F; D) serotype 15A; E) serotype 23F; F) serotype 35F. Bars show observed numbers of cases; broken lines show the percentage of all serotype group cases (A–C PPV23–13; D–F NVT); solid lines show counts of cases predicted by a negative binomial regression model for April 2013–March 2016. NVT, nonvaccine type serotype cases; PPV23-13, 23–valent pneumococcal polysaccharide vaccine serotype cases excluding those also contained in the 13–valent pneumococcal conjugate vaccine.

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Streptococcal Toxic Shock Syndrome Caused by Group G *Streptococcus*, United Kingdom

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We describe successful management of 3 patients with streptococcal toxic shock syndrome (STSS) attributable to group G *Streptococcus* infection. This small series supports recognition of group G *Streptococcus* in the etiology of STSS. We propose intravenous immunoglobulin be used in treatment as it is for STSS caused by group A *Streptococcus*.

Streptococcal toxic shock syndrome (STSS) was defined in 1993 as the isolation of group A *Streptococcus* (GAS) with parameters indicative of multiorgan dysfunction (*1*). STSS remains associated with high mortality rates, even with adequate antimicrobial treatment (*2*). Recently, 12 invasive infections with β-hemolytic streptococci of Lancefield group G also fulfilling the criteria of STSS have been reported (mortality rate 83%) (*3–12*). The benefit of adjunctive intravenous immunoglobulin (IVIg) in the treatment of STSS is difficult to prove because the limited number of cases precludes results of sufficient statistical power. Observational studies (*2,14*) and 1 small, early terminated, randomized control trial (*15*) have demonstrated improved survival among patients with GAS STSS treated with IVIg. We describe 3 patients treated in an 880-bed teaching hospital during 2009–2014; all 3 patients had illness meeting the case definition of STSS that were caused by invasive group G *Streptococcus* (GGS) infection. Successful treatment included IVIg, providing further support for the use of IVIg in treating STSS.

The Patients

In 2009, a 46-year-old woman (patient 1) with multiple sclerosis and lower limb lymphedema was admitted to the hospital with a 1-week history of fever, vomiting, rigors, sore throat, and erythema of the right leg with a healing cut on the foot. Patchy cellulitis extended from the foot to groin and was associated with elevated body temperature (39.4°C). Her creatine kinase level was high at 3,951 IU/L, and she had an elevated C-reactive protein (CRP) level of 396 mg/L. Her illness was initially managed as severe cellulitis. She received intravenous fluids, clindamycin (1.2 g every 6 h), and flucloxacillin (1 g every 6 h). However, her condition rapidly deteriorated. She had onset of

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hypotension (blood pressure 77/24 mm Hg) refractory to 8 L fluid resuscitation, prompting transfer to the intensive therapy unit (ITU) for cardiovascular support. Computed tomography scan results were compatible with adult respiratory distress syndrome. Shock and spreading cellulitis (with patches appearing on the contralateral leg) suggested streptococcal myositis or multifocal necrotizing fasciitis. Flucloxacillin was changed to intravenous daptomycin (4 mg/kg daily), and meropenem (1 g every 8 h) and 2 g/kg polyclonal intravenous immunoglobulin (IVIg) (Privigen; CSL Behring, West Sussex, UK) were administered. Her condition continued to deteriorate. Erythema spread beyond skin markings, her CRP plateaued at levels >300 mg/L, and her serum IgG level was 4.6 mg/L, prompting administration of a second dose of IVIg 2 g/kg on day 3. Surgical exploration released 30 mL of fluid and revealed macroscopically normal fascia and muscle. Histologic evaluation revealed severely necrotic soft tissue. GGS was isolated from all operative specimens. A transthoracic echocardiogram showed no vegetations. Three weeks after admission, the patient was discharged on prophylactic penicillin and remains well.

In 2011, a 63-year-old previously healthy man (patient 2) was admitted to the hospital with a 5-day history of confusion, headache, and bilateral swollen painful knees. Among purpuric lesions on the left lower leg was a small healing wound. Multiorgan failure and septic shock necessitated transfer to the ITU for inotropic support and hemofiltration. Blood tests revealed a reduced platelet count of 54×10^9/L and elevated levels of creatinine at 210 μmol/L, serum lactate at 10.2 mmol/L, CRP at 353 mg/L, and creatine kinase at 2,080 IU/L. Culture of joint fluid from each knee revealed GGS. The patient was treated empirically with intravenous imipenem (500 mg every 12 h), clindamycin (1.8 g every 6 h), vancomycin (1 g stat), and 2 g/kg polyclonal IVIg (Privigen). Despite dramatic clinical improvement, a static CRP level and worsening pain in other joints necessitated repeated arthroscopic washouts of his knees, wrists, hips, and 1 shoulder. Although gram-positive cocci were observed on microscopic evaluation of the knee and shoulder fluids, all aspirates and blood cultures were sterile. A transesophageal echocardiogram found no evidence of endocarditis; however, a repeat transesophageal echocardiogram 1 month after admission revealed a vegetation, necessitating aortic valve replacement. The patient was discharged after 13 weeks and remains well.

In 2014, a 66-year-old man (patient 3) with a history of diabetes mellitus was admitted to the hospital in septic shock. A total knee replacement 6 months earlier had resulted in chronic leg lymphedema. Noteworthy blood test results included a high serum lactate level of 4 mmol/L, an elevated creatinine level of 318 µmol/L, and a high alanine aminotransferase level of 210 IU/L. The patient had a blood pressure of 40/20 mm Hg, rapidly spreading erythema from the knee to the abdomen, and suffused conjunctivae. His illness was managed clinically as toxic shock syndrome. He received inotropic support, mechanical ventilation, hemofiltration, and intravenous linezolid (600 mg every 12 h), intravenous clindamycin (1.2 g every 6 h), and 2 g/kg polyclonal IVIg (Privigen). Arthroscopic washout of the prosthetic knee revealed copious pus that yielded GGS on culture. Although cardiovascular function stabilized, blood parameters continued to deteriorate. On day 3, following open debridement of the knee, the patient began to improve, and his CRP level remained elevated at 280 mg/L. Antimicrobial drugs were changed to intravenous daptomycin (7 mg/kg once daily) to maximize antibiofilm activity (reference 16 in online Technical Appendix, http://wwwnc.cdc. gov/EID/article/23/1/16-1009-Techapp1.pdf). After a negative echocardiography, the patient was discharged home on day 30, completing a 6-week course of daptomycin as an outpatient, and successfully retained the knee prosthesis. He remains well on life-long penicillin prophylaxis.

Conclusions

We describe 3 patients with group G STSS. Isolates were identified by laboratory serotyping of the group G carbohydrate surface antigen. All 3 patients had a favorable outcome after aggressive therapy with a combination of antiexotoxin antibiotics, IVIg, and surgical intervention.

The emergence of GGS causing skin and soft tissue infections (reference 17 in online Technical Appendix) might reflect improved detection, increased virulence, or a growing population of immunocompromised hosts. Similar to GAS, GGS shares multiple virulence factors, including streptokinase, fibronectin, IgG binding proteins, streptolysin O, C5a peptidase (reference 18 in online Technical Appendix), and antiphagocytic M proteins, of which e*mm* types *stg10* and *stg2078* of GGS are significantly associated with invasive disease (reference 19 in online Technical Appendix). Given clinical presentations of STSS by group G and group C *Streptococcus* are indistinguishable from group A STSS, the underlying mechanisms are probably related. However, unlike group A STSS, with group G STSS, underlying co-morbidities predominate, including cardiopulmonary disease, diabetes mellitus, malignancy, or hepatic failure (*6*).

IVIg has superantigen neutralizing activity (reference 20 in online Technical Appendix) and reduces mortality rates in group A STSS (*2,14,15*) A recent review of IVIg use for severe sepsis and septic shock yielded little evidence of benefit (reference 21 in online Technical Appendix). However, in that review, there was no discrimination between superantigen, exotoxin-driven, gram-positive sepsis and primarily endotoxin-driven, gram-negative sepsis, and very few cases of gram-positive sepsis were included (reference 21 in online Technical Appendix).

Twelve cases of group G STSS have been previously documented (*3–12*); 10 of those patients died (mortality rate 83%). With the addition of the 3 cases we reported, group G STSS has an overall reported mortality rate of 66%. The use of IVIg was described in the cases of 4 patients, 3 of whom died (*5,7,11,12*), including 1 who received low-dose IVIg (400 mg/kg) (*12*); for the other patients, the IVIg dosage was unspecified. Although our case series is small, it describes long-term survival in 3 patients with group G STSS. We propose that the definition of STSS extends beyond that of a condition exclusive to GAS infection and recommend that polyclonal immunoglobulin be considered as a potentially lifesaving adjunctive therapy.

Dr. Baxter and Dr. Morgan are clinical microbiologists working at the Royal Devon and Exeter National Health Service Foundation Trust. Both have a particular interest in the diagnosis and management of necrotizing skin, soft tissue infections, and toxic shock.

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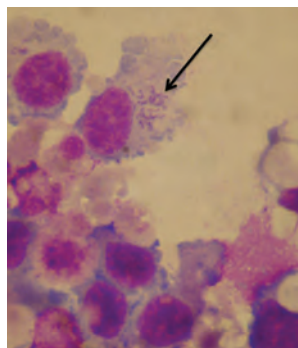
Hepatitis E Virus in Wild Boars and Spillover Infection in Red and Roe Deer, Germany, 2013–2015

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To determine animal hepatitis E virus (HEV) reservoirs, we analyzed serologic and molecular markers of HEV infection among wild animals in Germany. We detected HEV genotype 3 strains in inner organs and muscle tissues of a high percentage of wild boars and a lower percentage of deer, indicating a risk for foodborne infection of humans.

Hepatitis E is an infection of public health concern, leading to an estimated global disease burden of 3.4 million acute cases, 70,000 deaths, and 3,000 stillbirths per year (*1*). Large disease outbreaks in nonindustrialized countries are mainly caused by drinking water contaminated with hepatitis E virus (HEV) (*2*). In industrialized countries, most cases of hepatitis E are sporadic and suspected to be a result of zoonotic HEV transmission from animals to humans (*3*). The numbers of notified hepatitis E cases have sharply increased in several European countries during recent years (*4,5*). Chronic HEV infections among recipients of solid organ transplants pose novel public health concerns (*6*).

HEV belongs to the family *Hepeviridae*, genus *Orthohepevirus,* Its RNA genome comprises 3 open reading frames (ORFs). ORF1 encodes a multifunctional nonstructural polyprotein with methyltransferase and RNA-dependent RNA polymerase genes often used for molecular typing. Human pathogenic HEVs are mainly classified into genotypes 1–4 (*2,3*). The camelid HEV genotype 7 was recently detected in a human (*7*), however. Although genotypes 1 and 2 infect only humans, genotypes 3 and 4 are zoonotic and infect different animal species and humans (*2,3,8*). HEV infection in animals is generally not associated with clinical disease.

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The main animal reservoirs for genotype 3 are domestic pigs and wild boars, although infections among other mammals have been described (*2,3,8*). However, whether these animal species represent true HEV reservoirs or are accidental infections due to spillover events is unclear. In this study, we investigated serologic and molecular evidence of HEV infection in wild boars and different deer species during 2 hunting seasons in a hunting area in Germany.

The Study

We obtained serum samples from wild boars, roe deer, red deer, and fallow deer during 2 hunting seasons (season A, 2013–2014; season B, 2014–2015) and analyzed them by using an ELISA (ID Screen Hepatitis E Indirect; ID Vet, Grabels, France) for HEV-specific IgG (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/23/1/16-1169-Techapp1.pdf). Of 339 serum samples, 81 (23.9%) were positive for HEV IgG; results from 1 sample (0.3%) were questionable. Although all wild deer samples tested negative, the proportion of antibody-positive wild boars increased significantly (p = 0.018) from 13 (27.1%; 95% CI 16.55–37.65) of 48 in season A to 68 (51.5%; 95% CI 44.34–58.66) of 132 samples in season B, with a mean antibody prevalence of 45.0% (Table 1). The capability of the ELISA for detection of HEV-specific antibodies in field serum samples from deer was demonstrated by testing of 153 deer serum samples from another hunting area, which led to 3 positive results (data not shown).

We also tested liver and serum samples from 415 animals for the HEV genome by using real-time reverse-transcription PCR (RT-PCR) (online Technical Appendix). HEV RNA was detected in 46 (11.1%) animals: 39 (16.8%) of 232 wild boars (6/95 [6.3%], from season A and 33/137 [24.1%] from season B); 5 (6.4%) of 78 roe deer; and 2 (2.4%) of 83 red deer (Table 1). Testing of all available organs from the HEV-positive wild boars revealed HEV RNA in >89% of the samples. HEV RNA was detected in all tested muscle samples and in most of the other organ samples of HEV-positive deer (Table 2). Comparison of viral loads in the organs revealed significantly higher genome copy numbers in wild boar liver (median 2.26×10^7 genome equivalents [GE]/g) compared with those for wild boar musculature (median 4.37×10^3 GE/g) or for deer liver (median 2.22×10^3 GE/g) and deer musculature

Table 1. Detection of HEV-RNA and HEV-specific antibodies in wild boars and 3 deer species during 2 hunting seasons in a hunting area in Germany, 2013–2015*

Animal species	HEV-RNA determined by real time RT-PCR, no. positive/no. tested (%)	HEV-specific antibodies determined by ELISA, no. positive/no. tested (%)
Season A, 2013–2014,		
Wild boars	6/95 (6.31)	13/48 (27.08)
Roe deer	2/17 (11.76)	0/8
Red deer	0/25	0/21
Fallow deer	0/2	0/2
Total	8/139 (5.76)	13/79 (16.46)
Season B, 2014–2015		
Wild boars	33/137 (24.09)	68/132 (51.52)
Roe deer	3/61 (4.92)	0/51
Red deer	2/58 (3.45)	0/57
Fallow deer	0/20	0/20
Total	38/276 (13.77)	68/260 (26.15)
Total, 2013–2015	46/415 (11.08)	81/339 (23.89)

*HEV, hepatitis E virus; RT-PCR, reverse transcription PCR.

Table 2. Organ distribution of HEV RNA in positive-screened wild boars and deer during 2 hunting seasons in a hunting area in Germany, 2013–2015*

Animal species	Sample type, no. positive/no. tested (%)					
	Liver	Musculature	Spleen	Kidney	Serum	All
Wild boars	26/26 (100.0)	29/35 (82.9)	23/27 (85.2)	16/19 (84.2)	32/34 (94.1)	126/141 (89.4)
Deer	4/5 (80.0)	6/6 (100.0)	2/4 (50.0)	2/4 (50.0)	3/5 (60.0)	17/24 (70.8)
Total	30/31 (96.8)	35/41 (85.4)	25/31 (80.7)	18/23 (78.3)	35/39 (89.7)	143/165 (86.8)

*HEV, hepatitis E virus.

(median 5.25×10^2 GE/g) (Figure 1; online Technical Appendix Figure 2). However, the low number of positive deer samples limits the interpretation of the statistical results.

A total of 39 of 46 samples were positive in a nested RT-PCR assay targeting the RNA-dependent RNA polymerase gene in the ORF1 (online Technical Appendix)
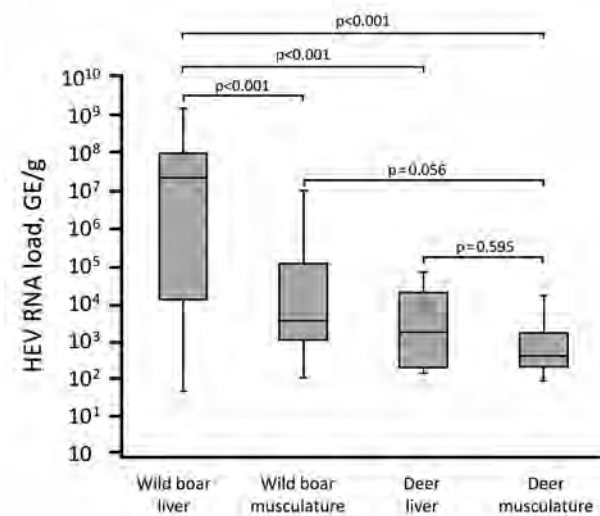
Figure 1. Box plot comparison of HEV RNA load in wild boar and deer specimens from Germany, 2013–2015. Boxes indicate first (bottom) and third quartile; horizontal line within boxes indicate median; error bars indicate minimum and maximum. p values for pairwise comparison of groups are shown. GE, genome equivalents; HEV, hepatitis E virus.

that were suitable for sequencing. The amplicons showed nucleotide sequence identities to each other ranging from 73.6% to 100.0%. A phylogenetic tree set up for the samples together with HEV subtype reference strains indicated that most sequences cluster in a clade containing subtypes 3c and 3i (Figure 2). Within this clade, HEV sequences from wild boar and deer from both hunting seasons clustered very closely together. Four sequences from wild boars of season B clustered in genotype 3f. HEV isolates from human hepatitis E cases from Germany clustered near the wild boar and deer HEV sequences (nucleotide sequence identities up to 86.1% to a German 3f strain and 88.2% to a German 3c strain).

Using a nested RT-PCR assay targeting the methyltransferase gene in the ORF1 (online Technical Appendix), we sequenced a PCR product in 18/46 samples. The nucleotide identities of the sequences ranged from 72.7% to 99.6%. A phylogenetic tree again showed grouping into HEV subtype clade 3ci and subtype 3f (online Technical Appendix Figure 3). All sequences were deposited in GenBank (accession nos. KX455427–KX455478).

Conclusions

We detected HEV-RNA and HEV-specific antibodies in a high percentage of wild boars, with a significant difference between the 2 hunting seasons. The detection rates are consistent with previous reports of infection of wild boars in Germany (9–11). The data underline the high importance of this animal species in the epidemiology of HEV and indicate that wild boars likely represent a persistent reservoir
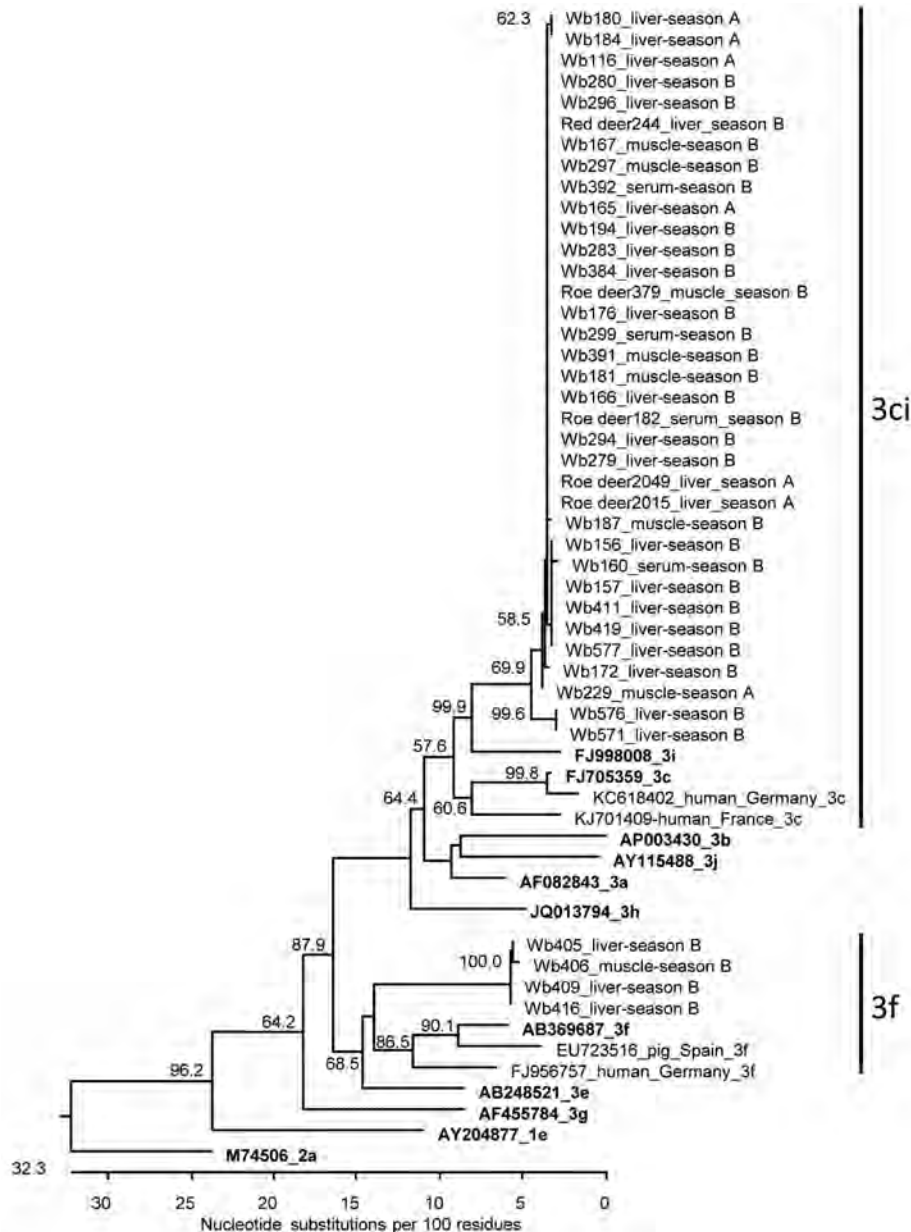
Figure 2. Phylogenetic relationship of HEV sequences derived from wild boars and deer from Germany, 2013–2015. The tree is based on a 280-bp fragment of the ORF1 (RNA-dependent RNA polymerase gene) region. The strain designations, animal species (Wb, wild boar), sample type, and sampling year (season A, 2013–2014; season B: 2014–2015) are indicated for the novel strains. The GenBank accession numbers, the corresponding hosts, the geographic origins and genotypes are indicated for selected additional strains. Reference strains are given in bold; the genotype of the novel strains is indicated at right. Bootstrap values >50% are shown. The tree is scaled in nucleotide substitution units and was constructed using MEGALIGN software (ClustalW [http://www.ebi.ac.uk/Tools/msa/clustalw2/], IUB residue weight table, 1,000 trials and 111 random seeds in bootstrap analysis). HEV, hepatitis E virus; ORF, open reading frame.

for this virus. The detection of high amounts of HEV RNA in wild boar liver, other organs, and especially in muscle tissue highlights the high risk that HEV can be transmitted to humans through the consumption of meat from these animals that has not been cooked properly.

In contrast, only low percentages of samples from roe deer and red deer tested positive for HEV in our study. Data about HEV infection in wild ruminants in Europe are rare, but some reports have demonstrated HEV infection in several deer species (*12,13*). Neumann et al. (*14*) reported serologic and molecular evidence for HEV infection of the indigenous deer species in Germany. We detected HEV RNA in liver, in several organs, and in muscle tissue of the infected deer species. Sequence analysis showed a relationship of HEV from deer with human hepatitis E cases from Germany. In Japan, consumption of deer meat could be linked to acute hepatitis E cases in humans (*15*). Taken together, deer are likely to represent a source of HEV for humans, and consumption of undercooked deer meat should be considered a risk for acquiring HEV infection.

Analysis of the detected HEV sequences indicated that the same strains of genotype clade 3ci circulated in wild boar and deer species. This finding argues against specific HEV strains exclusively circulating in deer species; however, longer sequence parts or whole virus genomes should be analyzed in future studies to support this finding

further. The consistently lower HEV RNA and antibody prevalence in deer than in wild boars indicates a primary circulation in wild boars and only accident transmission to deer. The hypothesis of spillover infections of deer is further supported by the consistent lower viral loads in tissues of infected deer. However, other authors classified deer as a true reservoir for HEV (*8*). Further studies investigating more geographic areas over longer time, including the parallel analysis of different animal species, are necessary to unravel the epidemiology and transmission dynamics of HEV in wildlife.

Dr. Anheyer-Behmenburg is a veterinary specialist for microbiology at the University of Veterinary Medicine, Hannover, Germany. She is interested in the epidemiology of emerging zoonotic diseases in wildlife. Her recent scientific work focused on HEV in wild animals.

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Guillain-Barré Syndrome and Healthcare Needs during Zika Virus Transmission, Puerto Rico, 2016

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To assist with public health preparedness activities, we estimated the number of expected cases of Zika virus in Puerto Rico and associated healthcare needs. Estimated annual incidence is 3.2–5.1 times the baseline, and long-term care needs are predicted to be 3–5 times greater than in years with no Zika virus.

Guillain-Barré syndrome (GBS) is an autoimmune disorder characterized by varying degrees of weakness, sensory abnormalities, and autonomic dysfunction due to peripheral nerve or nerve root damage (1). Annual GBS incidence worldwide is ≈1.1–1.8 cases/100,000 population and varies by geography and age group (2,3). Death is rare and is usually caused by respiratory failure, autonomic dysfunction, or deep vein thrombosis (4).

GBS has been associated with various infectious agents, including Zika virus (5). Zika virus is a flavivirus transmitted primarily by *Aedes* species mosquitoes; symptoms of infection include rash, arthralgia, and fever (6). During a 2013–2014 outbreak in French Polynesia, 42 cases of GBS were reported during a 7-month period, compared with 3–10 cases annually in previous years; all GBS patients during the outbreak had Zika virus antibodies (7).

In December 2015, the Puerto Rico Department of Health reported local transmission of Zika virus (8). In February 2016, the Department of Health reported the first

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case of Zika virus–associated GBS and established the GBS Passive Surveillance System, with support from the Centers for Disease Control and Prevention (9). During January 1–July 31, 2016, a total of 56 cases of GBS were reported; evidence of Zika virus or flavivirus infection was found in 34 (61%) of these (9). As in other locations (5), GBS cases in Puerto Rico are anticipated to increase with ongoing Zika virus transmission. To assist with public health preparedness activities, we estimated the annual number of expected cases of GBS and associated healthcare needs in Puerto Rico (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/23/1/16-1290-Techapp1.pdf).

The Study

We estimated the weekly number of cases of GBS and associated healthcare needs for 3 scenarios: 1) in the absence of Zika virus transmission; 2) in an average week during Zika virus transmission; and 3) during the peak week of Zika virus transmission (Table). Estimates were derived from baseline and Zika virus–associated GBS cases. The population of Puerto Rico in 2015 was estimated at 3,474,182 persons (10).

We calculated baseline GBS incidence in Puerto Rico by using data collected through medical chart review of patients suspected to have GBS at 9 reference hospitals in Puerto Rico during 2012–2015 and for whom neurologic diagnosis was confirmed by the Brighton Collaboration criteria (11). The 2013 incidence of GBS was 1.7 cases (95% CI 1.3–2.1 cases) per 100,000 population (J.L. Salinas, unpub. data). Using this incidence range, in the absence of Zika virus transmission, we estimated that 1 (interquartile range [IQR] 0–2) case occurs each week, and 59 (IQR 52–66) cases occur each year.

We assumed that, during Zika virus transmission, ≈25% of the population could have been infected during 2016, similar to recent chikungunya and dengue virus epidemics in Puerto Rico (12). We used a triangular distribution to characterize uncertainty, with a minimum estimate of 10% infected and a maximum estimate of 70% infected (12,13). Estimated GBS risk associated with Zika virus infection was assumed to be 1.1–2.3 cases/10,000 infections on the basis of a separate analysis of data aggregated from French Polynesia (7), Yap (13), Brazil (14), Colombia, El Salvador, Honduras, the Dominican Republic, and Puerto Rico (L. Mier-y-Teran, unpub. data). We used Monte Carlo sampling to draw 1 million

Table. Estimates of weekly Guillain-Barré syndrome cases and healthcare resource needs, Puerto Rico, 2016

Variable	Scenario*	Estimate		
		Median	Interquartile range	95% Uncertainty interval
New cases and long-term care patients				
Case-patients	No Zika virus	1	0–2	0–4
	Average week during Zika virus	5	3–6	1–11
	Peak week during Zika virus	11	6–17	1–34
Long-term care patients	No Zika virus	0	0–1	0–2
	Average week during Zika virus	2	1–3	0–6
	Peak week during Zika virus	5	2–8	0–16
New patient healthcare resource needs				
Intravenous immunoglobulin	No Zika virus	1	0–2	0–3
	Average week during Zika virus	4	3–6	0–10
	Peak week during Zika virus	10	5–15	0–30
Mechanical ventilation	No Zika virus	0	0–0	0–1
	Average week during Zika virus	1	0–2	0–3
	Peak week during Zika virus	2	1–4	0–8
Regular ward beds	No Zika virus	0	0–1	0–3
	Average week during Zika virus	3	1–4	0–7
	Peak week during Zika virus	6	3–10	0–21
Intensive care unit beds	No Zika virus	0	0–1	0–2
	Average week during Zika virus	2	1–3	0–6
	Peak week during Zika virus	4	2–7	0–15

*The weekly number of Guillain-Barré syndrome cases and associated healthcare needs were estimated for 3 scenarios: 1) in the absence of Zika virus transmission; 2) in an average week during Zika virus transmission; and 3) during the peak week of Zika virus transmission.

values from each of these distributions to estimate each outcome of interest and its associated uncertainty.

We estimated that in an average week of Zika virus transmission, ≈5 (IQR 3–6) GBS cases would occur, comprising cases associated with baseline risk and Zika virus infection. As in previous outbreaks of Zika virus and other arboviral diseases, peak weekly incidence could be 2–4 times higher than average incidence. A maximum of 11 (IQR 6–17) cases could occur during the peak week, 2–4 times more than the average number of cases. We predicted that ≈241 (IQR 191–305) cases would occur in 2016.

Assumptions regarding treatment needs were also based on data collected from the 2012–2015 Puerto Rico hospitalized patient chart review (J.L. Salinas, unpub. data). We made the following assumptions: 90% of patients will require treatment with intravenous immunoglobulin (IVIg), 20% will require mechanical ventilation, all patients will be hospitalized, 40% will require intensive care, and 45% will require long-term care.

In the absence of Zika virus transmission, the estimated weekly number of new patients treated with IVIg was 1 (IQR 0–2) patient and of those requiring mechanical ventilation was low (IQR 0–0 patients). Estimates for the weekly number of new patients requiring a regular or intensive care unit (ICU) bed were also low (IQR 0–1 patient).

During an average week of Zika virus transmission, ≈4 (IQR 3–6) new patients would need treatment with IVIg, and 1 (IQR 0–2) new patient would require mechanical ventilation. An estimated 3 (IQR 1–4) new patients would require a regular ward bed, whereas 2 (IQR 1–3) new patients would require an ICU bed. During the peak week, ≈10 (IQR 5–15) new patients would need treatment with

IVIg, and 2 (IQR 1–4) new patients would require mechanical ventilation. An estimated 6 (IQR 3–10) new patients would require a regular ward bed, whereas 4 (IQR 2–7) new patients would require an ICU bed.

An estimated 0 (IQR 0–1) patients would require long-term care during a week without Zika virus transmission, 2 (IQR 1–3) patients during an average week of Zika virus transmission, and 5 (IQR 2–8) patients during the peak week. During 2016, ≈108 (IQR 85–138) GBS patients would require long-term care.

Conclusions

We estimated that there would be 191–305 new cases of GBS in Puerto Rico in 2016, comprising baseline and Zika virus–associated cases. This estimate represents an annual incidence of 5.5–8.7 cases/100,000 population, which is 3.2–5.1 times the baseline incidence. Associated healthcare resource needs will increase accordingly. Estimated long-term care needs in 2016 were predicted to be 3–5 times greater than in years with no Zika virus transmission.

These estimates have limitations. First, there is considerable uncertainty around key assumptions, including that increases in GBS incidence will mirror those experienced in other Zika virus–affected countries (5,7,13,14). Second, the estimates of associated healthcare needs did not address all possible needs, such as alternative treatments (i.e., plasmapheresis) or additional treatments, such as those for neuropathic pain, cardiac arrhythmias, and deep vein thrombosis. Third, estimates assumed 1 peak week, although GBS cases tend to cluster, and multiple peaks could occur. Finally, a causal association between Zika virus infection and GBS has not been definitively established.

Continued GBS surveillance will monitor for increased incidence and enable adapted public health response. Healthcare workers, including internists, family physicians, and nurses, might need training to ensure adequate patient clinical management if GBS cases increase as predicted. The Puerto Rico Department of Health and the Centers for Disease Control and Prevention have developed training material toward this end. The availability and accessibility of GBS treatment, especially IVIg, and long-term care services should be evaluated, especially given the high costs of GBS patient care (*15*). The Puerto Rico Department of Health is also creating an inventory of available and expandable resources, working with manufacturers and distributors to understand supply chains, and facilitating prompt treatment delivery at points of care.

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Persistent Zika Virus Detection in Semen in a Traveler Returning to the United Kingdom from Brazil, 2016

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Zika virus is normally transmitted by mosquitos, but cases of sexual transmission have been reported. We describe a patient with symptomatic Zika virus infection in whom the virus was detected in semen for 92 days. Our findings support recommendations for 6 months of barrier contraceptive use after symptomatic Zika virus infection.

Zika virus is a mosquito-transmitted flavivirus that was first isolated from mosquitos in the Zika Forest in Uganda. Since its introduction into Brazil in May 2015, the virus has spread rapidly through the Americas, and transmission is now widespread in South America, Central America, the Caribbean, the Pacific islands, Singapore, and Thailand (*1*). The virus is transmitted mainly by *Aedes aegypti* mosquitos and causes symptoms in ≈20% of persons infected (*2*), usually manifesting as a mild illness consisting of fever, arthralgia, myalgia, conjunctivitis, and pruritic rash. Infection in pregnancy can lead to congenital Zika syndrome, which consists of multiple developmental abnormalities, including microcephaly and cerebral calcification, and fetal loss. Zika virus infection is also associated with Guillain-Barré syndrome (*3*).

Although by far the most common route of Zika virus transmission is by mosquito, the virus can also be transmitted sexually (from male to female, female to male, and male to male) (*4–8*). Zika virus RNA has been detected in semen and vaginal fluid (*9–12*). We describe a case in which Zika virus persisted in semen for 92 days after symptom onset.

The Study

A previously healthy 45-year-old man became ill 1 day after returning to the United Kingdom from a 1-week holiday in Rio de Janeiro, Brazil, in February 2016. His illness lasted

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for 10 days and consisted of severe retro-orbital headache, arthralgia, myalgia, and high fevers, followed by a pruritic maculopapular rash. He had no symptoms of prostatitis or gross hematospermia. He had no history of immunocompromise, and he was not on any regular medication. On day 3 of his illness, Zika virus RNA was detected in urine but not in serum; Zika virus IgM and IgG were not detected in serum by an ELISA IgM and IgG kit (EUROIMMUN AG, Lübeck, Germany) (*13*). Results of serologic testing for chikungunya, dengue, and yellow fever were negative by commercial assays.

Seventeen days after the initial testing, Zika virus IgM and IgG were detected in a new serum sample from the patient. The patient and his partner were planning to conceive and were reluctant to wait the 6 months recommended by Public Health England (*1*). Therefore, PCR for Zika virus RNA was performed on serial semen samples by using the Rare and Imported Pathogens Laboratory's in-house real-time reverse transcription PCR (rRT-PCR), based on an assay used by Pyke et al. (*14*). Zika virus RNA was detected 22, 55, and 92 days after symptom onset (cycle threshold values 21.3, 30.1, and 37.2, respectively). No microhematospermia was detected, and Zika virus could not be cultured at any point. No Zika virus RNA was detected in semen at day 132 or day 174. The patient and his partner did not have unprotected sex during this period; his partner remained well and was not tested for Zika virus.

Conclusions

In this case, Zika virus RNA was detected in the semen of a previously healthy, immunocompetent adult who contracted his infection during a short visit to Rio de Janeiro, Brazil, in February 2016. RNA was present in semen samples until 92 days after the onset of symptoms and was subsequently undetectable on 2 occasions.

This report follows several others from the current outbreak in which Zika virus RNA has been detected in semen >90 days after symptom onset in an immunocompetent and previously healthy adult. Previous reports include a man who had visited Haiti, in whom Zika virus RNA was persistently identified in semen until day 188 after symptom onset (*10*). A subsequent report from Italy documented Zika virus RNA being persistently identified in semen until day 181 in a symptomatic traveler returning from Haiti (*11*). In these 2 cases, unlike the present case, disappearance from

semen was not demonstrated at a later point in time. In another case, Zika virus RNA was detected in semen 93 days after symptom onset; in that case, the patient had traveled to a nonepidemic area and had just been diagnosed with a sarcoma, and treatment including aggressive chemotherapy was planned (*12*). This subsequent treatment might have altered the kinetics of virus clearance. Further clarification on whether both symptomatic and asymptomatic patients can persistently shed Zika virus RNA in semen for prolonged periods is needed.

This case is important because it reinforces the possibility raised by Nicastri et al. (*10*) that Zika virus might be sexually transmitted at later points in time than have been documented thus far. The longest known period between Zika virus infection and sexual transmission to another person is 31–42 days after onset of symptoms. It is unclear how the presence of Zika virus (or level of RNA) in semen correlates to infectivity, or how long a person might be infectious by this route after infection with Zika virus. Guidelines from the World Health Organization, Public Health England, and the Centers for Disease Control and Prevention all recommend avoiding unprotected sex for 6 months after symptomatic Zika virus infection in a man (*1,15*). Published literature on sexual transmission and detection of Zika virus RNA in semen, including our report, supports these guidelines. As of press time for this article, published recommendations differ on how long to avoid unprotected sex in asymptomatic couples who have been potentially exposed to the virus.

An important caveat, however, is that virus could not be cultured from semen samples from this patient or from the patients reported by Nicastri et al. (*10*) or Barzon et al. (*11*) at any point. This findings raise the possibility that the detection of RNA does not equate to the detection of infectious virus particles.

An inability to detect Zika virus RNA in semen has been assumed to equate to a lack of infectivity by the sexual route (*10,11*). In this case, in which our patient and his partner planned to try to conceive, the results were useful in encouraging them to defer their plans until 2 sequential specimens were negative for Zika virus RNA. This delay, in fact, corresponded to 6 months after the onset of symptoms, as recommended by current guidelines (*1,15*).

Published studies report an enormous variability in how long after infection Zika virus RNA can be detected in semen, which makes advising patients on the risks for sexual transmission difficult. In this case, testing serial semen samples for evidence of Zika virus RNA helped guide patient management, and doing this routinely in returning travelers in non-Zika virus–endemic countries might be warranted. In such a context, a positive result should be interpreted as indicating possible ongoing risk for sexual transmission.

Further studies on the kinetics of virus isolation and Zika virus RNA detection from semen are needed to help inform guidelines on Zika virus sexual transmission and how to manage the risks. These studies would be especially useful in managing asymptomatic patients in whom evidence of Zika virus RNA detection, virus isolation, and sexual transmission is almost totally lacking.

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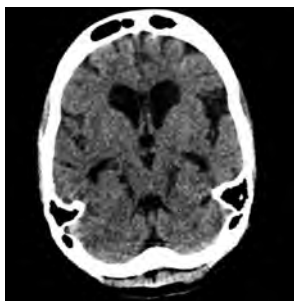
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January 2016: Sexually Transmitted Infections

- Waterborne *Elizabethkingia meningoseptica* in Adult Critical Care
- Human Papillomavirus Vaccination at a Time of Changing Sexual Behavior
- Multiorgan WU Polyomavirus Infection in Bone Marrow Transplant Recipient
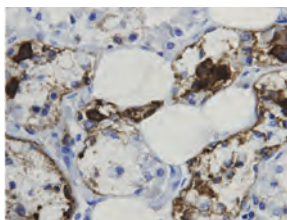- Multifacility Outbreak of Middle East Respiratory Syndrome in Taif, Saudi Arabia
- Falling *Plasmodium knowlesi* Malaria Death Rate among Adults despite Rising Incidence, Sabah, Malaysia, 2010–2014
- Risk Factors for Primary Middle East Respiratory Syndrome Coronavirus Illness in Humans, Saudi Arabia, 2014

- Epidemiology of *Haemophilus ducreyi* Infections
- Human Papillomavirus Prevalence and Herd Immunity after Introduction of Vaccination Program, Scotland, 2009–2013
- Decline in Decreased Cephalosporin Susceptibility and Increase in Azithromycin Resistance in *Neisseria gonorrhoeae*, Canada
- Rapid Emergence and Clonal Dissemination of CTX-M-15–Producing *Salmonella enterica* Serotype Virchow, South Korea
- Avian Influenza A(H7N9) Virus Infection in 2 Travelers Returning from China to Canada, January 2015
- Increase in Sexually Transmitted Infections among Men Who Have Sex with Men, England, 2014

- Surveillance of Bacterial Meningitis, Ethiopia, 2012–2013
- Identification of Source of *Brucella suis* Infection in Human by Using Whole-Genome Sequencing, United States and Tonga
- Porcine Epidemic Diarrhea Virus and Discovery of a Recombinant Swine Enteric Coronavirus, Italy
- Seroepidemiology of Human Enterovirus 71 Infection among Children, Cambodia
- Outbreak of Panton-Valentine Leukocidin–Associated Methicillin-Susceptible *Staphylococcus aureus* Infection in a Rugby Team, France, 2010–2011
- Variations in Spike Glycoprotein Gene of MERS-CoV, South Korea, 2015

- Effectiveness of Ring Vaccination as Control Strategy for Ebola Virus Disease
- Autochthonous *Nocardia cerradoensis* Infection in Humans, Spain, 2011 and 2014
- Asymptomatic Lymphogranuloma Venereum in Men who Have Sex with Men, United Kingdom
- Increased Risk for ESBL-Producing Bacteria from Co-administration of Loperamide and Antimicrobial Drugs for Travelers' Diarrhea
- Hemagglutinin Gene Clade 3C.2a Influenza A(H3N2) Viruses, Alachua County, Florida, USA, 2014–15

EMERGING INFECTIOUS DISEASES
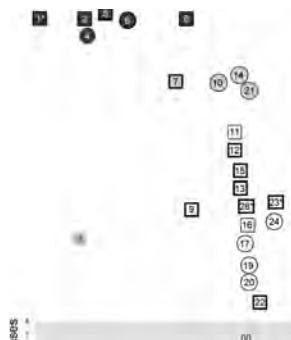
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Upsurge of Enterovirus D68, the Netherlands, 2016

Marjolein Knoester, Elisabeth H. Schölvinck, Randy Poelman, Sylvia Smit, Clementien L. Vermont, Hubert G.M. Niesters, Coretta C. Van Leer-Buter

In June and July 2016, we identified 8 adults and 17 children with respiratory enterovirus D68 infections. Thirteen children required intensive care unit admission because of respiratory insufficiency, and 1 had concomitant acute flaccid myelitis. Phylogenetic analysis showed that all of 20 sequences obtained belong to the recently described clade B3.

The largest enterovirus D68 (EV-D68) outbreak occurred in the United States during the summer and fall of 2014. Approximately 1,100 respiratory EV-D68 infections were recorded, mostly in children, many of whom required intensive care unit (ICU) admission. Most diseases were of respiratory nature, but concurrent with the upsurge of EV-D68, the Centers for Disease Control and Prevention (Atlanta, GA, USA) recorded 120 cases of acute flaccid myelitis (AFM) (1). A Europewide study identified circulation of EV-D68 during the same period. Although numbers were lower, ICU admissions and 3 cases of AFM were reported (2). Before 2014, only a few small outbreaks of EV-D68 had been described worldwide, all with respiratory infections.

Our clinical virology laboratory (University Medical Center Groningen, Groningen, the Netherlands) reported an increase of EV-D68 infections in 2010 and again in 2014, in parallel with the US outbreak (3,4). Very limited activity of EV-D68 was observed in 2015. Since June 2016, we have again identified a substantial increase in respiratory EV-D68 infections in our hospital, just 2 years after the previous upsurge. To raise awareness of this upsurge and the severity of EV-D68 infections, we report on 25 cases. In addition, we show the phylogenetic relationship between the 2016 EV-D68 strains and those that circulated in 2014.

The Study

The University Medical Center Groningen is a tertiary referral center in the northern part of the Netherlands. We perform routine diagnostic real-time PCR for all patients evaluated for respiratory disease by laboratory developed tests for 17 targets, including adenovirus, bocavirus, coronavirus, enterovirus, metapneumovirus, influenza, parainfluenzavirus, rhinovirus, and respiratory syncytial virus (5). From all enterovirus detections with a cycle threshold (C_t) value <31, we sequence part of the viral protein 1 gene on a weekly basis (6). To obtain rapid typing results in the current outbreak, a specific EV-D68 real-time PCR was also used (3), when enterovirus PCR was found positive.

We identified 3 EV-D68 infections in June and 22 additional cases in July. We found a stable seasonal variance for enterovirus and a sudden rise of enterovirus infections in July, mainly caused by EV-D68 (Figure 1).

The EV-D68–positive specimens were nasopharyngeal swabs (n = 8), sputum (n = 2), and nasopharyngeal brushes (n = 15). Two viral respiratory co-infections were detected: rhinovirus A15 in patient 12 (enterovirus C_t 17, rhinovirus C_t 21) and parainfluenzavirus type 4 in patient 23 (enterovirus C_t 14, parainfluenza virus 4 C_t 31). For 20 patients, we obtained sequence results identifying EV-D68; the remaining 5 could not be typed by sequencing but were positive in our specific EV-D68 PCR.

Of the 25 patients, 8 were adults and 17 were children (Table). In the adults, 6 of whom were transplant recipients, symptoms were mild and influenza-like. In children, however, we observed life-threatening respiratory distress, for which ICU admission was necessary for 13 (Table). Twenty-one patients had an underlying or concurrent condition, in the children mostly pulmonary.

One child (patient 16) had the clinical characteristics of AFM. This nearly 4-year-old boy had a history of headaches for 1 week and an influenza-like illness for 3 days. He had rapidly progressing asymmetric weakness of arms and legs, bulbar paralysis, asymmetric facial paralysis, and respiratory distress for which he needed ventilatory support. Cerebrospinal fluid (CSF) analysis showed no abnormalities. Axial fluid-attenuated inversion recovery magnetic resonance imaging (MRI) showed hyperintense nonenhancing gray matter lesions in brainstem and spinal cord. Electromyography findings supported injury on the level of the motor axon or the anterior horn of the spinal cord. No varicella zoster virus, herpes simplex virus, enterovirus, or parechovirus were detected in the CSF. In the nasopharyngeal swab, only EV-D68 was detected.

All patients in this report were treated in our tertiary referral hospital, but they originated from different regions in the north of the Netherlands. Spatial epidemiology data

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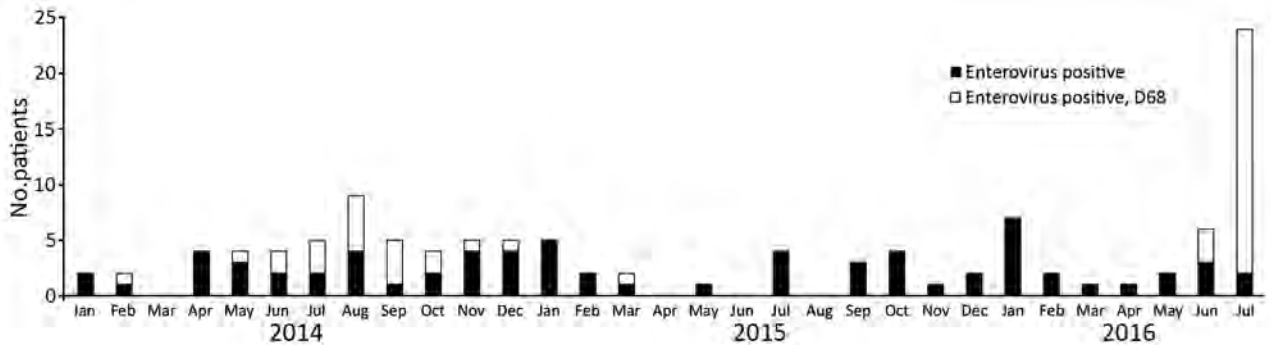
Figure 1. Respiratory enterovirus infections, University Medical Center Groningen, the Netherlands, 2014, 2015, and 2016. The total numbers of respiratory specimens tested for enterovirus in these years during April–July were 784, 828, and 871, respectively. Seventeen (2.2%) of 784 were positive for enterovirus in 2014, 5 (0.6%) of /828 in 2015, and 33 (3.8%) of /871 in 2016. Six (35%) of 17 were typed as enterovirus D68 in 2014, versus 0 of 5 in 2015, and 25 (76%) of 33 in 2016.

did not suggest an epidemiologic link or outbreak (data not shown). In 2 children, EV-D68 symptoms developed >48 hours after admission; the source of possible nosocomial infection was not identified.

We obtained 20 sequences of part of the EV-D68 viral protein gene and aligned them with the sequences from the 2014 epidemic. Clades were assigned as described previously (*7*) by the neighbor-joining method by using BioNumerics software 6.6 (Applied Maths/bioMérieux, Sint-Martens-Latem, Belgium). Sequence analysis (Figure 2) showed that the 2016 strains were closely related to sequences of the recently described subclade B3 (*8*),

Table. Characteristics of patients with EV-D68 infection, University Medical Center Groningen, Groningen, the Netherlands, June 11–August 1, 2016*

| Patient	Date†	Age/sex	Concurrent condition(s)	Clinical characteristics	ICU admission, days in ICU
1	Jun 11	1 y /M	None	Bronchial obstruction, respiratory failure	Yes/4
2	Jun 20	3 y/M	None	Status asthmaticus	Yes/3
3	Jun 30	61 y/F	Lung and liver transplantation	Cough, runny nose	No
4	Jul 4	5 y/M	Asthma	Status asthmaticus	Yes/2
5	Jul 5	3 y/M	Asthma	Status asthmaticus	Yes/3
6	Jul 7	67 y/F	Autologous stem cell transplantation	Influenza-like syndrome, dyspnea	No
7	Jul 7	2 y/M	Pulmonary hypertension, underdeveloped lung vessels	Respiratory failure	Yes/4
8	Jul 11	70 y/F	Allogenic stem cell transplantation	Influenza-like syndrome, dyspnea	No
9	Jul 14	66 y/F	Allogenic stem cell transplantation	Cough, fever	No
10	Jul 16	3 y/M	Asthma	Status asthmaticus	Yes/4
11	Jul 16	3 mo/M	Bronchopulmonary dysplasia of prematurity	Respiratory failure	Yes‡
12	Jul 16	1 y/F	Bronchial hyperreactivity, failure to thrive	Bronchial obstruction, respiratory failure	Yes/8
13	Jul 22	5 y/F	Asthma	Status asthmaticus	Yes/5
14	Jul 22	1 y/M	Tracheal stoma for bilateral vocal cord paralysis	Cough, wheezing	No
15	Jul 23	3 y/M	None	Status asthmaticus	Yes/5
16	Jul 24	3 y/M	Atypical seizures 3 y prior	Acute flaccid myelitis	Yes/ongoing§
17	Jul 25	19 y/F	None	Influenza-like syndrome	No
18	Jul 25	3 y/M	Bronchial hyperreactivity	Asthma exacerbation	No
19	Jul 25	5 mo/M	22q11 deletion, tetralogy of Fallot, tracheal stoma	Acute mechanical airway obstruction, resuscitation	No¶
20	Jul 28	50 y/M	Lung transplantation	Cough, runny nose	No
21	Jul 28	3 y/M	Spinal muscular atrophy	Respiratory failure	Yes/5
22	Jul 29	9 mo/M	Corrected esophagal atresia with related tracheomalacy for which noninvasive ventilation at home	Respiratory distress with bronchorrhea	No
23	Jul 30	3 y/F	Bronchial hyperreactivity	Status asthmaticus	Yes/2
24	Jul 30	51 y/F	Autologous stem cell transplantation	Influenza-like syndrome	No
25	Jul 31	23 y/M	Childhood asthma	Cough, dyspnea, nausea, vomiting	No

*EV, enterovirus; ICU, intensive care unit.
†Date of first EV-D68–positive specimen.
‡Patient 11 contracted EV-D68 in the neonatal ICU.
§Patient still in ICU as of December 6, 2016.
¶Patient 19 acquired EV-D68 while admitted to the special care ward since birth.
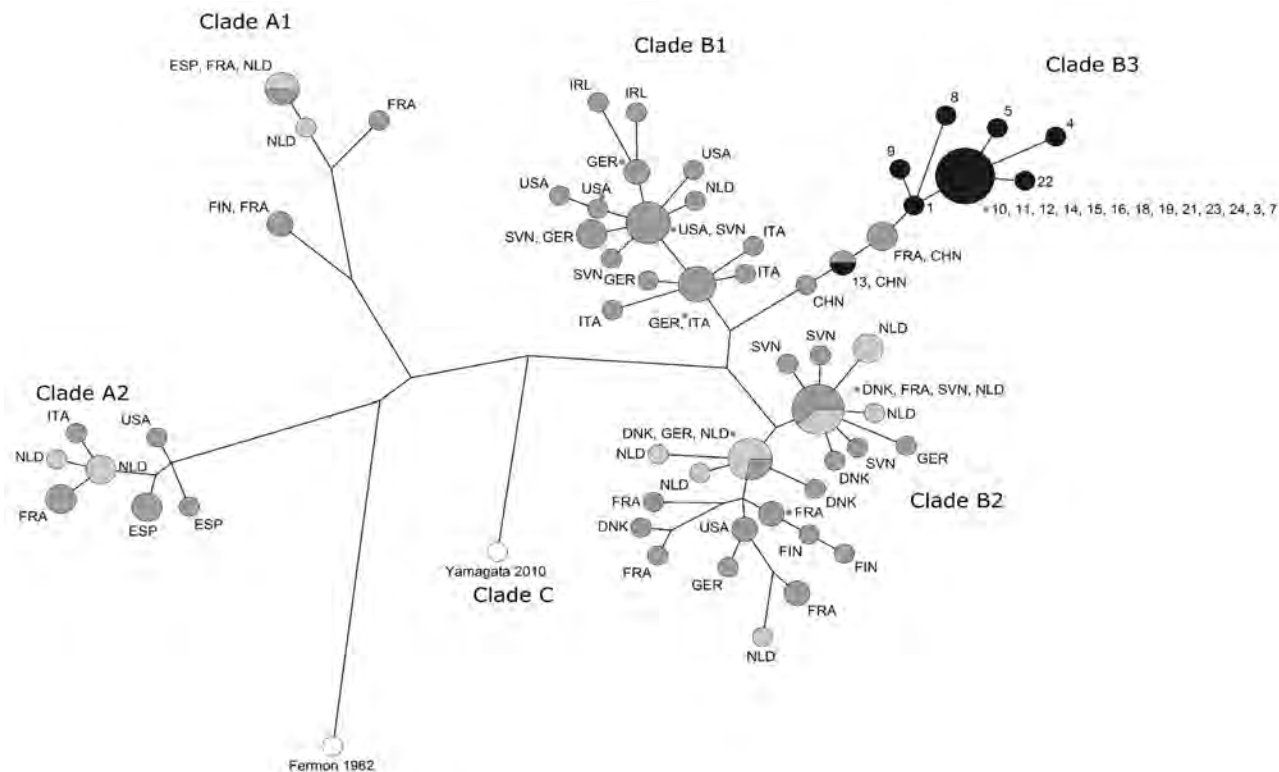
Figure 2. Maximum-parsimony tree of partial viral protein 1 sequences of enterovirus D68 (EV-D68). Included are the strains obtained in the laboratory of the University Medical Center Groningen (Groningen, the Netherlands) in 2014 (light gray, n = 23) and 2016 (black, n = 20) and worldwide isolates from 2014 (dark gray, n = 73). Recent strains cluster in the recently described clade B3, with a nucleotide divergence of 2.1% within clade B3, 5.5% to clade B1, and 7.3% to clade B2. Clades are according to Tokarz et al. and Gong et al (7,8). We submitted the 20 sequences we obtained during 2016 to GenBank under accession nos.: KX685066–KX685084 and KX710328. CHN, China; DNK, Denmark; ESP, Spain; FIN, Finland; FRA, France; GER, Germany; IRL, Ireland; ITA, Italy; NLD, the Netherlands; SVN, Slovenia; USA, United States. White circles indicate reference strains (n = 2).

represented in Figure 2 by 4 sequences from China. The nucleotide divergence was 2.1% within B3, 5.5% between B1 and B3, and 7.3% between B2 and B3. We submitted our 20 sequences from 2016 to GenBank (accession nos. KX685066–KX685084 and KX710328).

Conclusions

After the upsurge of EV-D68 in our region during 2009 and 2010, we set up routine genotypic characterization for several viral pathogens, including enterovirus and rhinovirus, which is also offered to regional referring hospitals and healthcare institutions. Sequencing results are available within 1 week, which provides clinically relevant and epidemiologic information. This so-called REGIOTYPE strategy contributed to timely detection of EV-D68 in our hospital in 2014, as well as in 2016. We recognized a sudden reappearance of EV-D68 with a sharp increase of cases that could not be explained by a change in surveillance strategy.

In line with previous outbreaks, mostly children were affected, and those with underlying pulmonary conditions seemed at higher risk for ICU admission (9). The number

of children who needed ICU admission was higher in 2016 than in our 2010 and 2014 reports (3,4). Although dynamics of viral disease and shedding are not known for EV-D68, we assume a role for EV-D68 in the symptoms of patients 12 and 23, as, in line with rhinovirus infections, higher viral load might indicate symptomatic disease (10).

Evidence that EV-D68 might cause AFM is increasing after recent epidemiologic investigations (1,11,12). In patient 16, atypical Guillain-Barré syndrome initially was diagnosed; however, this diagnosis was later discarded because the electromyography results indicated motoric axon or anterior horn cell disease, and the clinical picture and MRI results were in favor of AFM (13). MRI findings were subtle, and radiologic diagnosis was made only after further review and discussion of the case with the neurologists. The absence of EV-D68 in CSF is consistent with previous reports (1,12).

Sequencing results showed that the strains in our study cluster in the recently described clade B3 (8). During the 2014 outbreak, most EV-D68 sequences belonged to clades B1 and B2, although A1 and A2 were also represented

(*2,14*). Larger epidemiologic and genotyping studies are needed to evaluate whether the distinction within clade B is tenable and whether our clinical findings are typical for subclade B3.

This upsurge could indicate an active EV-D68 season, as highlighted by the epidemiologic curve, with a potential increase in AFM cases. Clinicians should be alert for EV-D68, its clinical implications, and the need for appropriate diagnostics, particularly in children who are admitted with respiratory failure to the ICU or with possible symptoms of AFM.

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Dolphin Morbillivirus Associated with a Mass Stranding of Sperm Whales, Italy

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In September 2014, seven sperm whales were stranded along Italy's Adriatic coastline. Postmortem investigations on 3 female adult whales and 1 male fetus carried by the largest female revealed molecular and immunohistochemical evidence of dolphin morbillivirus infection. A possible role of the virus in the stranding event was considered.

The mass strandings of sperm whales (*Physeter macrocephalus*) are still largely unexplained events. Solar cycles, weather conditions, coastal geographic features, and human activities have been proposed as possible causes (*1*). Although a multifactorial etiology was hypothesized to be responsible for a mass stranding that occurred in December 2009 involving 7 male sperm whales along the southern Adriatic coast of Italy (*1*), well-defined causes of similar events are rarely identified.

In September 2014, seven sperm whales were found stranded along the central Adriatic Italian coastline; 4 of these whales were refloated. Three females (sperm whale 1 [SW1], pregnant, total length 8.95 m; SW2, total length 8.38 m; and SW3, total length 7.33 m) died on the beach, and postmortem analyses revealed that the largest animal,

a pregnant female (SW1), whose male fetus (SW1b) also died, was affected by a prominent hydronephrosis caused by a large stone occupying >50% of the right kidney pelvis. This condition might have resulted in renal function impairment. We observed microscopically evidence of lymphoid cell depletion in several secondary lymphoid tissues from all 3 adult whales, and we found definitive biomolecular evidence of dolphin morbillivirus (DMV) infection in the 3 adult whales and in the fetus.

Our use of 1-step PCR protocols (*2*) failed to sequence any viral fragment, probably because of postmortem changes and subsequent viral RNA degradation by ribonucleases, which progress rapidly in large whales because of their body features and size and thus affect the integrity of the DMV genome. Therefore, we used a more sensitive, nested reverse transcription PCR technique that targeted a highly preserved fragment of the viral hemagglutinin (H) gene (*3*) to investigate the lung (SW1), brain (SW3), and lymphoid tissues (SW2 and SW3) of the 3 adult whales and the lung, kidney, and liver of the fetus (SW1b). We found all of these tissues to be positive for DMV, and we analyzed the gene sequences obtained from the 4 whales' tissues by using an ad hoc computer program (Lasergene package version SeqMan Pro; DNASTAR Inc., Madison, WI, USA). The hemagglutinin consensus fragment obtained from all the positive samples showed 100% sequence homology with the corresponding DMV genome sequence (GenBank accession no. AJ608288). In addition, we observed simultaneous immunohistochemical (IHC) evidence of DMV nucleoprotein antigen in macrophages and follicular dendritic-like cells from the white pulp of the spleen from the youngest female whale (SW3) and in monocytes circulating within splenic blood vessels from the same animal (Figure). Other target tissues from this whale and the 3 others were either negative or unsuitable for IHC and biomolecular analyses.

Although we did not observe any classic DMV-related pathologic changes during postmortem examinations (*2,4*), we strongly suspected a viremic condition in all 4 whales on the basis of IHC (SW3) and biomolecular (SW1, SW1b, SW2, and SW3) findings. In this respect, the consistent immunolabeling of morbillivirus antigens in circulating monocytes and in splenic follicular-like dendritic cells support the hypothesis that DMV infection was in an early developmental stage (*5*). Experimental studies conducted on similar morbillivirus infection models show that during this period, even if no severe clinical signs are observed, a general discomfort condition, secondary to viremic circulation, could be reasonably expected (*6,7*).

DMV infection has been frequently associated with mass deaths during epidemic outbreaks (*2*); however, it has been seldom reported in single mass stranding events among cetaceans in general, much less among sperm
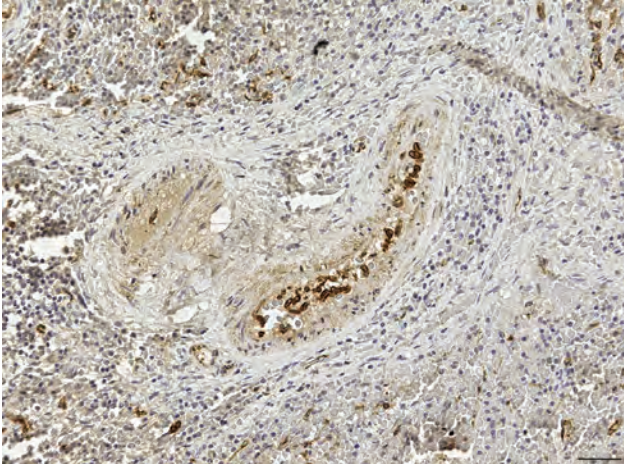
Figure. Spleen of the youngest female sperm whale (SW3) in study of 7 sperm whales stranded along Italy's Adriatic coastline in September 2014. Positive immunostaining (Mayer's hematoxylin counterstain) for morbilliviral antigen is shown in monocytes within vascular lumina and in follicular dendritic-like cells in the splenic white pulp. Morbillivirus immunohistochemistry was conducted with a murine monoclonal antibody against canine distemper virus nucleoprotein (VMRD Inc., Pullman, WA, USA). Original magnification ×20; scale bar indicates 50 μm.

whales. Furthermore, given the documented susceptibility of sperm whales to cetacean morbillivirus and the likelihood of maternal–fetal transfer of the virus (8), the biomolecular evidence of DMV infection obtained in the fetal sperm whale in our investigation strongly supports the assumption of a transplacentally acquired infection in this animal.

Although no clear-cut evidence exists that DMV was the primary cause of the mass stranding of sperm whales we report, a vast body of scientific literature is available to support the primary pathogenicity of morbillivirus genus members for their mammalian hosts, including aquatic mammals (3). In this respect, previous studies would suggest that chemical pollutants, especially methylmercury, were a relevant causative factor in the stranding and subsequent deaths of the 3 sperm whales in our study (9). However, our findings suggest the possible additional role of the DMV infection in this event because it is one of the factors that could explain the whales' northward trajectory into the Adriatic Sea, which is known to be a cul-de-sac for this species (1).

In conclusion, although a vast body of scientific data supports the direct involvement of cetacean morbillivirus clade members such as DMV in the numerous epidemics that have occurred in the last 25–30 years among free-ranging cetaceans worldwide (2), their causative role in single mass stranding events cannot be precisely defined. Several additional biologic, ecologic, and environmental factors should be investigated by using a multidisciplinary approach (10).

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Hepatitis E Virus Infection after Platelet Transfusion in an Immunocompetent Trauma Patient

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Hepatitis E virus (HEV) infection causes acute liver disease, but severe infections are rare in immunocompetent patients. We describe a case of HEV infection in a previously healthy male trauma patient in France who received massive transfusions. Genotyping confirmed HEV in a transfused platelet pool and the donor.

In developed countries, hepatitis E virus (HEV) infection usually results from consumption of contaminated meat or water and causes acute liver disease. Hepatitis E illness is usually self-limiting, and severe, prolonged infections are unusual except in immunocompromised patients. We describe HEV infection in a previously healthy man in France who received massive transfusions of blood, plasma, and platelets after a traumatic skiing accident.

At hospital admission, the patient was in hemorrhagic shock caused by severe blunt splenic injury. He underwent an immediate splenectomy with massive transfusion: 9 packed red blood cells units, 7 fresh frozen plasma units, and 1 whole blood platelet pool. Because of hemopneumothorax, multiple rib fractures, and pulmonary contusions, severe acute respiratory distress syndrome developed, and the patient was treated with venovenous extracorporeal membrane oxygenation for 3 days. The patient received another whole blood platelet pool transfusion at day 5 posttrauma and was treated with renal replacement therapy for 6 weeks.

At day 15 posttrauma, the patient had icterus, and liver blood tests revealed cholestasis; ultrasound findings showed acalculous cholecystitis. Because bile drainage via percutaneous cholecystectomy was insufficient and led to septic shock (day 18), the patient underwent open cholecystectomy. The diagnosis of ulcerated cholecystitis was confirmed by histologic examination; *Enterococcus faecium* was isolated from blood and bile samples. Cholestasis, icterus, and cytolysis gradually resolved over the next week postoperatively; however, liver blood test results did not return to normal (Figure). Histological examination of the liver tissue from the biopsy performed during cholecystectomy gave normal results, and there was no evidence of drug-induced toxicity.

At day 40, liver blood tests indicated a renewed increase in cholestasis and cytolysis (Figure). Meanwhile, the patient's clinical condition had improved, renal function had recovered, and the tracheal tube was removed. Ultrasound examination of the abdomen showed normal findings. Viral serologic tests were negative for Epstein-Barr virus, herpes simplex virus, HIV, and hepatitis A, B, and C viruses. However, reverse transcription PCR testing revealed HEV positivity, with HEV viremia reaching 1.8 $\times 10^5$ copies/mL (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/23/1/16-0923-Techapp1.pdf). Serologic tests for HEV IgM (ASSURE HEV IgM Rapid Test; MP Biomedicals, Singapore) and IgG (HEV IgG ELISA; Wantai, Coutaboeuf, France) were negative at that time, but a blood sample taken on day 75 posttrauma showed HEV IgM.
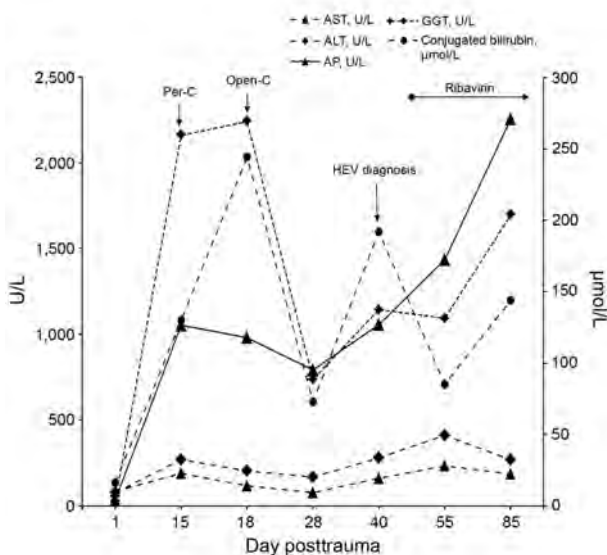
Figure. Time course of liver blood test results from a trauma patient in France who was transfused with an HEV-contaminated blood platelet pool on day 5 posttrauma. ALT, alanine aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; HEV, hepatitis E virus; open-C, open cholecystectomy; per-C, percutaneous cholecystectomy.

The patient was not considered to be immunocompromised. Serologic tests for HIV were negative, leukocyte counts were within reference ranges, and no steroids were given to treat trauma. Neither the patient nor his family reported recent travel to HEV endemic areas or intake of uncooked or poorly cooked pork or game meat in the 3 months before the accident. All transfused blood products were retrospectively tested for HEV, and the blood platelet pool transfused at day 5 was identified as coming from an HEV-infected donor, who had viremia reaching 290 copies/mL. Evidence of direct blood contamination was provided by genotyping, which showed the virus in the donor and blood platelet pool were identical. HEV from the patient and the contaminated platelet pool were both HEV subtype 3f, and a phylogenetic study of the open reading frame 2 coding region by neighbor-joining cluster analysis confirmed the homology.

Because the patient was at high risk for severe acute HEV infection, treatment with 800 mg/day of ribavirin was initiated on day 45; the patient experienced severe nausea and vomiting but had no anemia. The course of the HEV infection reflected a slow response to the treatment. At 80 days posttrauma, the patient was still icteric with unchanged liver blood test results; HEV viremia was higher than before (2.34×10^7 copies/mL; online Technical Appendix Table). After 2 months on ribavirin treatment (day 110), HEV viremia started to decrease, but 3 months of treatment were needed for viremia levels to reach <100 copies/mL (day 135).

The potential for HEV transmission by contaminated blood product transfusion, attributable to the high prevalence of HEV infection in asymptomatic blood donors, has been reported (1). Prevalence of HEV infection is ≈1/1,500 blood donations in Europe, and an HEV transmission rate as high as 42% was observed in immunocompromised patients given HEV-positive blood products (2). Chronic liver infections have developed in immunosuppressed patients (e.g., solid organ transplant recipients, patients with HIV infection, and patients with hematological disease) given HEV genotype 3–contaminated products (3,4). A few cases of prolonged HEV viremia in immunocompetent patients have been described (5,6), but none were transfusion-induced HEV infections in patients after trauma.

Successful treatments with ribavirin have been reported for transplant patients and patients with leukemia or HIV, including those at high risk for severe HEV infection (7). Treatment with ribavirin is usually characterized by rapid viral clearance (8) and ribavirin-induced anemia. Ribavirin could act by direct inhibition of viral replication or an immunomodulatory effect (9).

This case describes HEV infection acquired by an immunocompetent patient through transfusion of a contaminated blood product. Clinicians should consider the risk for HEV infection in trauma patients who receive large transfusions.

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Scrub Typhus Leading to Acute Encephalitis Syndrome, Assam, India

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To determine the contribution of *Orientia tsutsugamushi,* the agent of scrub typhus, as a cause of acute encephalitis syndrome (AES) in Assam, India, we conducted a retrospective study of hospital patients with symptoms of AES during 2013–2015. Our findings suggest that *O. tsutsugamushi* infection leads to AES and the resulting illness and death.

Scrub typhus is a miteborne bacterial disease caused by *Orientia tsutsugamushi*. Clinical features generally include fever, headache, and myalgia, with or without eschar/rash (*1*). In India context, scrub typhus was first reported in Assam during World War II (1944–1945) across the India–Myanmar border (*2*). The northeastern region of India then experienced decades without the disease until it reemerged in 2010 (*3*). Assam, a northeastern state in India, is recognized as an endemic zone for acute encephalitis syndrome (AES), especially that caused by Japanese encephalitis virus (JEV). However, the etiology of >50% of the AES cases in Assam remains unrecognized (*4*). In this study, we aimed to determine the contribution of scrub typhus to AES in this region.

We conducted a retrospective study of patients exhibiting symptoms of AES who were admitted during 2013–2015 to Assam Medical College and Hospital, Dibrugarh (a government-funded tertiary care hospital that provides health care for 8 adjoining districts). Serum samples underwent serologic testing with InBios ST Detect IgM ELISA kit (InBios International, Seattle, WA, USA). An optical density of ≥0.5 was considered positive (*5*). We extracted DNA from the blood samples by using the QIAamp DNA blood mini kit (QIAGEN, Hilden, Germany) and performed nested PCR for amplification of a 56-kDa gene of *Orientia,* targeting a 483-bp fragment (*6*). We compared the sequences obtained with reference strains of *Orientia*.

We enrolled 511 AES case-patients after disease onset (mean 6.23 days; range 1–14 days); 104 (20.3%) had IgM against *O. tsutsugamushi* (suggestive of recent infection) (*7*). Of these 104 patients, 58 (55.7%) were male and 46 (44.2%) were female. Ages ranged from 3 to 80 years (median 25 years). The main clinical features of the 104 IgM-positive case-patients were fever (100%), altered sensorium (100%), headache (67.3%), unconsciousness (55.7%), nausea (40.3%), and neck rigidity (0.9%). None had any record of eschar. Leptospirosis, dengue, and malaria were ruled out for all 104 case-patients. Only 13 (12.5%) of the IgM-positive case-patients were positive for JEV IgM. *Orientia* DNA was detected in 9 (8.6%) of the samples that had IgM against *O. tsutsugamushi*. Percentage similarity of the nucleotide sequences (GenBank accession nos. KU163359, KU163361, KU163362, KU163366, KU163369, KU163370, KU163372, KU163373, and KP067915) demonstrated resemblances with the Karp strain of *Orientia* ranging from 91.9% to 93.7% (Figure). We were able to follow-up 53 of the 104 IgM-positive case-patients and discovered that 26 (49%) died after discharge. These included 4 of the 9 PCR positive case-patients.

The high prevalence of illness and death resulting from scrub typhus in this study can be attributed to various reasons. First, because JEV is the predominant etiologic agent of AES in the northeastern region, especially in Assam, health providers generally do not suspect other etiologic factors, including scrub typhus. Moreover, the nonspecific clinical features of scrub typhus in these patients (i.e., absence of eschar/rash) led to diagnostic dilemmas. In addition, JEV infection, West Nile virus infection, and leptospirosis have been identified as key contributors to AES in the northeastern region (*8,9*). Treatable bacterial diseases such as leptospirosis and scrub typhus are grossly underestimated because of the low index of suspicion and limited diagnostic facilities, especially in developing countries such as India. Proportion of deaths due to untreated scrub typhus varies across different regions from 0% to 70% (*10*). Our study found a high case-fatality rate of 49%. The absence of distinguishable clinical features among AES-identifying etiologic factors makes differential diagnosis difficult, and thus the condition remains untreated. That clinicians are unaware of the presence of the disease remains a major hindrance to its recognition and successful treatment.

In our study, the highest numbers of scrub typhus cases were recorded during July–September, the peak season for JEV transmission. The northeastern region has a subtropical climatic pattern, and the season (May–August) is also an appropriate period for sowing and harvesting crops. Agricultural lands cover 22% of the northeastern region, with ≈70% of the total population dependent on agriculture for their livelihood. Because our study was retrospective, the occupations of the patients with positive cases were unknown. However, the areas where the followed-up case-patients lived were primarily agrarian.

Our findings suggest that the bacterium that causes scrub typhus, *O. tsutsugamushi*, is a notable etiologic
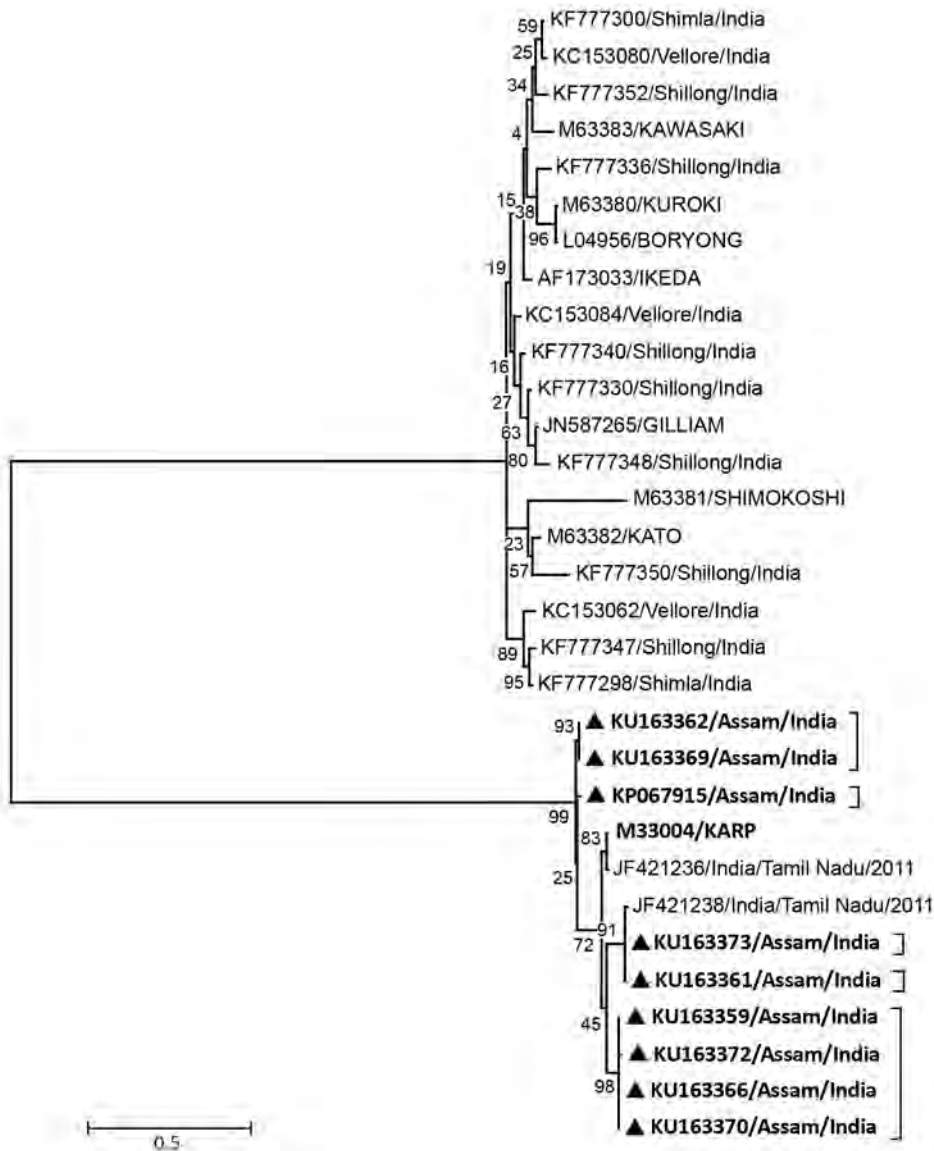
Figure. Dendrogram representing *Orientia tsutsugamushi* sequences (black triangles) from patients with acute encephalitis syndrome, Assam, India, 2013–2015. The phylogenetic tree was constructed on a 56-kDa outer membrane protein gene of *O. tsutsugamushi*. The evolutionary history was inferred by using the maximum-likelihood method based on the Tamura 3-parameter model. Our sequences are found within the brackets. Bold indicates the KARP genotype. Scale bars indicate nucleotide substitutions per site.

agent that contributes to the occurrence of AES and resulting illness and death. We believe that it is urgent that this neglected, treatable disease be considered when diagnosing those with AES, especially during July–September in AES-endemic regions. Recording of occupation details and ecologic background of the attending patients, especially from those in rural areas, could be a vital source for identifying those with suspected scrub typhus. Increasing awareness among clinicians could lead to prompt diagnosis and effective treatment of this almost-forgotten potential source of AES.

Acknowledgments

Dr. Khan is a scientist at the Regional Medical Research Centre, Northeast Region, Dibrugarh, Assam. His research interests include surveillance and molecular epidemiological studies of insect vector-borne diseases, in particular,. identifying disease hotspots and Georgraphic Information Systems mapping.

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Group B Streptococcal Toxic Shock Syndrome and covR/S Mutations Revisited

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Gene mutations in the virulence regulator CovR/S of group A *Streptococcus* play a substantial role in the pathogenesis of streptococcal toxic shock syndrome. We screened 25 group B *Streptococcus* (GBS) isolates obtained from patients with streptococcal toxic shock syndrome and found only 1 GBS clone harboring this kind of mutation.

Streptococcal toxic shock syndrome (STSS) is typically caused by *Streptococcus pyogenes* (group A *Streptococcus* [GAS]) (*1*). Major investigations on host-pathogen interactions have been performed to determine why some persons experience uncomplicated pharyngitis, but STSS develops in others. On a molecular level, mutations in *covS* (a sensor gene of the major virulence regulator CovR/S) have been frequently associated with invasive GAS disease (*2*). In 2009, we reported a case of STSS caused by *S. agalactiae* (group B *Streptococcus* [GBS]) and *covS* mutation (*3*). Here, we reassess those findings in a larger collection of GBS isolates causing STSS.

We tested 26 GBS isolates from 25 patients (22 adults, 3 children) (Table) that were pooled from 3 countries; the United States (22 strains collected 2004–2005), Germany (1 strain, 2006), and Switzerland (2 strains, 2005). For 1 of the 2 case-patients from Switzerland, 2 isolates (same clone) were available for mutation analyses (patient 23 [*4*]). The isolate from our previously published case report (Sweden, 2005 [*3*]) served as a control strain for molecular analyses; the corresponding case-patient was included in the demographic analyses (i.e., 26 patients: 23 adults, 3 children). The median age of the included adult patients was 59 years (interquartile range 45.5–68 years); mortality rate was 35% (8/23). The ages of the 3 children were 0, 30, and 60 days (1 death).

We used standard molecular biology techniques for nucleic acid preparation and analysis. We performed molecular typing by multilocus sequence typing (MLST) as described (*5*) and capsular typing by using latex agglutination and PCR serotype determination (*6*) (Table). To analyze the *cov* gene locus, we amplified the genes *covS* and *covR* by PCR (online Appendix Table, http://wwwnc.cdc.gov/EID/article/22/12/16-1063-Techapp1.pdf). Resulting PCR products underwent DNA sequencing with internal *cov* primers on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany).

Nucleotide sequence analysis showed that, in 1 of the strains (from patient 18), both genes, the sensor histidine kinase *covS* and the response regulator *covR*, had mutated. In *covR*, at nucleotide position 242, cytosine was replaced by thymine, leading to an amino acid exchange from alanine to valine. In addition, the *covS* gene of this strain showed a 1-bp deletion of adenine at position 895 of the gene, causing a frame shift and leading to a truncated CovS with a stop codon at nucleotide position 926 of the

Table. GBS isolates collected from patients with STSS, showing results of capsular and multilocus sequence typing*

Patient no.	Isolate no.	Patient age	Outcome	Capsular serotype	Sequence type	covR/S mutation
1	BSU286	65 y	Died	II	22	–
2	BSU287	71 y	Survived	Ia	23	–
3	BSU288	46 y	Died	V	1	–
4	BSU289	46 y	Survived	IV	397	–
5	BSU290	43 y	Survived	V	1	–
6	BSU291	35 y	Died	III	19	–
7	BSU292	76 y	Survived	V	1	–
8	BSU293	48 y	Survived	Ia	23	–
9	BSU294	61 y	Died	V	1	–
10	BSU295	45 y	Survived	V	1	–
11	BSU296	63 y	Died	Ib	8	–
12	BSU297	0 d	Survived	Ia	23	–
13	BSU298	70 y	Survived	V	1	–
14	BSU299	60 y	Survived	V	1	–
15	BSU300	73 y	Survived	V	1	–
16	BSU301	66 y	Died	III	17	–
17	BSU302	2 mo	Survived	III	17	–
18	BSU303	94 y	Survived	V	23	Yes
19	BSU304	1 mo	Died	Ia	23	–
20	BSU305	34 y	Survived	V	1	–
21	BSU306	59 y	Survived	IV	2	–
22	BSU307	79 y	Died	Ia	88	–
23 (4)	BSU865, VS	38 y	Survived	II	19	–
	BSU866, BC			–	–	–
24	BSU869	32 y	Died	Ib	8	–
25	BSU870	53 y	Survived	Ia	23	–
Control (3)	BSU871	50 y	Survived	Ib	8	Yes

*BC, blood culture; GBS, group B *Streptococcus*; STSS, streptococcal toxic shock syndrome; VS, vaginal swab; –, not found.

gene. In the control strain that harbored a 3-bp deletion, our previously published finding was confirmed (3). In the remaining strains, *cov* alleles matched the gene sequences of completely sequenced GBS strains in the GenBank database (NEM316, 2603V/R, 909A).

During the past few decades, the overall incidence of invasive GBS infections has increased substantially. This trend is particularly noticeable in the elderly and in persons with co-morbid conditions (7). Our results regarding age distribution of patients, mortality rate, and frequencies of different GBS serotypes are in line with results of previous studies. Twelve (52%) of 23 patients were ≥59 years old. The mortality rate for group B STSS (≥30%) was similar to that reported for group A STSS (1). In a previous case series, which included 13 patients with group B STSS, the mortality rate was 23% (3/13) (8). Three-quarter of our strains (19/26 strains) were attributed to serotype V or Ia/Ib. Large epidemiologic studies have frequently implicated serotype s Ia/Ib, III, and V GBS isolates in the etiology of invasive disease in adults (9). Apart from sequence type (ST) 1 and ST23 (15/26 strains), the distribution of MLSTs among the GBS isolates was heterogeneous. The highly virulent GBS lineage ST17 was found in only 2 patients (1 adult and 1 child).

The role of *covR/S* mutations in the switch from colonization to invasion has been demonstrated for GAS in a mouse model (2). Consistent with these findings, GAS strains isolated from STSS patients frequently carry mutations in this operon (10). Similarly, a 3-bp deletion in the *covR* gene was detected in a GBS strain that caused STSS

and necrotizing fasciitis (3). However, our investigations on a larger collection of GBS isolates did not confirm a high *cov* mutation rate. Only 1 of 25 GBS clones demonstrated a mutation. From 1 patient, a colonizing and invasive isolate (same clone) was available (4); that isolate showed no mutation in *covR/S*.

Our results should be interpreted with caution because the absolute number of included patients is small, and the cases were pooled from various centers. Nonetheless, Ikebe et al. found *covR/S* mutations in 76 (46.3%) of 164 GAS strains causing STSS and in only 1.7% of 59 strains without invasive disease (10). In light of these results, the low frequency of mutations found in our collection is surprising. Yet, the association with *covR/S* mutations and GBS TSS in a case report has been shown previously and confirmed here. However, GBS harbors multiple 2-component systems and stand-alone regulators. Our findings indicate that different virulence regulators may be involved in the pathogenesis of fulminant GBS disease.

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Whole-Genome Characterization of a Novel Human Influenza A(H1N2) Virus Variant, Brazil

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We report the characterization of a novel reassortant influenza A(H1N2) virus not previously reported in humans. Recovered from a a pig farm worker in southeast Brazil who had influenza-like illness, this virus is a triple reassortant containing gene segments from subtypes H1N2 (hemagglutinin), H3N2 (neuraminidase), and pandemic H1N1 (remaining genes).

Influenza A(H1N2) viruses have been described in human, avian, and especially swine populations over many years (*1,2*). In contrast to the widespread circulation of seasonal H1N1 and H3N2 viruses, subtype H1N2 has been observed only sporadically in humans (*1,3–7*). Human H1N2 infections were reported during 1988–89 from sporadic cases over the winter in China (*3*). In 2000, another H1N2 subtype strain emerged in the human population and became widespread in Europe, with sporadic cases reported in the Middle East, Asia, Africa, and the Americas during 2001–2003 (*1,4*). In Brazil, this H1N2 subtype strain was detected in humans in the southeast region during the winter of 2002 and in the northern region at the beginning of 2003 (*5*). This 2000–2003 H1N2 subtype strain had a genetic origin similar to the 1988–1989 H1N2 strain from China, both reassortants between human seasonal H1N1 and H3N2 subtype lineages (*3,4*).

In contrast, sporadic cases of zoonotic human infections with swine-origin H1N2 subtype variants (H1N2v) have also been described (*6,7*). In Brazil, the passive monitoring of influenza A viruses in pigs has taken place since 2009 (*8*). Recently, a phylogenetic study revealed that H1N2 subtype viruses have circulated undetected in swine herds in Brazil for more than a decade, and reassortments may have occurred (*9*). These viruses seem to be reassortants originating from an ancestor virus introduced to

pigs from humans in the late 1990s and early 2000s and remained as a relic from a now-extinct human-host hemagglutinin lineage. However, after the emergence in humans of influenza A(H1N1)pdm09 in 2009, reassortment events lead to H1N2 viruses acquiring internal genes segments from the pandemic strain (*9*).

Even though these H1N2 subtype strains from Brazil circulating in the swine population, they have not been detected in humans. We report detection and characterization of a variant H1N2 subtype strain (H1N2v) with a genomic origin not previously reported in humans.

This virus was identified from a nasopharyngeal aspirate collected on November 26, 2015, from a 16-year-old girl from a rural area in Castro City, Paraná, in the southern region of Brazil. Castro has ≈67,000 inhabitants and is a major livestock hub for dairy cattle, poultry, and pigs. The patient did not present any risk factors for influenza but showed development of an influenza-like illness with an onset of symptoms (fever, cough, sore throat, chest pain, and myalgia) on November 23, 2015. The follow-up local investigation reported that she had been working at a swine farm and confirmed direct patient contact with pigs. She had not received a prior influenza vaccine or antiviral treatment, and her clinical recovery was uneventful.

The virus sample was sent to the Central Laboratory of the State of Parana, where an influenza A virus strain was detected. This strain could not be further subtyped using the influenza real-time reverse transcription PCR protocol recommended by the World Health Organization.

Genomic Sanger sequencing of the strain was conducted at the National Influenza Center, IOC, FIOCRUZ, Rio de Janeiro, Brazil. BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was performed for each gene segment sequenced and revealed strong identity with an H1N2 virus subtype genome detected in swine in Santa Catarina, a state in southern Brazil, in 2011 (Table). The human viruses with closest identity to the H1N2v virus detected in this study were a 2003 H1N2 subtype human lineage (hemagglutinin gene), a 1998 H3N2 subtype human seasonal lineage (neuraminidase gene), and A(H1N1)pdm09 subtype lineage for all other genes. This virus isolate was designated as influenza A/Parana/720/2015(H1N2v); the genome sequence is available in the GISAID database (http://platform.gisaid.org; submission no. EPI_ISL_223342).

We conducted phylogenetic reconstructions of each gene segment by the maximum-likelihood method using a dataset with all H1N2 subtype sequences and some representative sequences for H1N1 and H3N2 subtypes available in influenza genetic databases (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/23/1/16-1122-Techapp1.pdf). Our analysis revealed that each gene segment of A/Parana/720/2015(H1N2v) had the same phylogenetic profile as recent swine H1N2 subtype sequences

Table. Highest degree of gene identity of the influenza A/Parana/720/2015 (H1N2)v subtype strain identified from a patient in Brazil with other swine and human strains*

Gene segment	BLAST hits†	% Identity
PB2	A/swine/Brazil/185-11-7/2011(H1N2)	98
	A/San Diego/INS194/2009(H1N1)	98
PB1	A/swine/Brazil/185-11-7/2011(H1N2)	99
	A/Singapore/GN285/2009(H1N1)	98
PA	A/swine/Brazil/185-11-7/2011(H1N2)	98
	A/Texas/67/2009(H1N1)	98
HA	A/swine/Brazil/185-11-7/2011(H1N2)	97
	A/New York/487/2003(H1N2)	95
NP	A/swine/Brazil/185-11-7/2011(H1N2)	99
	A/Tennessee/F1057c56/2010(H1N1)	98
NA	A/swine/Brazil/185-11-7/2011(H1N2)	97
	A/Malaysia/17392/1998(H3N2)	93
M	A/swine/Brazil/185-11-7/2011(H1N2)	99
	A/Mexico City/INER16/2009(H1N1)	99
NS	A/swine/Brazil/185-11-7/2011(H1N2)	98
	A/Singapore/TT454/2010(H1N1)	98

*Sequence submitted to GISAID (http://platform.gisaid.org; submission no. EPI_ISL_223342). HA, hemagglutinin gene; M, matrix gene; NA, neuraminidase gene, NP, nucleoprotein gene; NS, nonstructural protein gene; PB2, polymerase basic 2 gene; PB1, polymerase basic gene; PA, polymerase acid gene.
†Sequence with major identity on BLAST.

from Brazil. This supports the BLAST findings and suggests a recent swine–human infection by the H1N2v strain. Because similar swine strains have been identified in pigs ≈300 km distant from where the human case occurred (*9*), the virus is likely to have been circulating in pigs in Castro City. The H1N2v subtype we report contained the S31N marker in the matrix 2 protein, which confers resistance to the adamantane antiviral class, similar to A(H1N1)pdm09 viruses (*10*).

To date, no further H1N2 subtype human cases have been detected in Brazil; however, influenza virus strains from this region and period are under investigation to confirm whether more H1N2v subtype cases may have occurred. This report highlights the need for influenza surveillance in humans and animals, as well as in their interface, especially during influenza season when transmission is high. To ensure early detection, surveillance should focus on geographic areas when influenza A viruses subtypes co-circulate and where human–animal contact is frequent.

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Avian Pox in Native Captive Psittacines, Brazil, 2015

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To investigate an outbreak of avian pox in psittacines in a conservation facility, we examined 94 birds of 10 psittacine species, including sick and healthy birds. We found psittacine pox virus in 23 of 27 sick birds and 4 of 67 healthy birds. Further characterization is needed for these isolates.

Avian pox is caused by avipoxvirus. Infections occur worldwide in domestic and wild avian species (1), are suggested to be host family- or order-specific, and are modulated by habitat and ecologic niche (2). Avipoxviruses have been described in Brazilian *Amazona* spp. and *Pionus* spp. parrots with severe diphtheritic upper digestive lesions, experimentally causing the formation of cutaneous lesions in chickens; chicken and parrot strains will not provide cross protection (3). The presumptive diagnosis, based on typical pocklike skin lesions of papular or nodular hyperplasic and hypertrophic skin foci or upper digestive diphtheritic form in severe cases (1), may be confirmed by detection of avipoxvirus DNA by PCR (4).

In June 2015, an outbreak of avian pox occurred among 10 species of native Brazilian psittacines (n = 94) maintained in a conservation facility. In addition to the typical pocklike nodular skin lesions, the psittacines had weight loss and reduced activity; 3 died. The outbreak lasted 3 months; remission of lesions occurred within ≈3 weeks in each bird.

Skin scrapings were collected from the cutaneous lesions of affected birds (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/23/1/16-1133-Techapp1.pdf), and conjunctiva and cloaca swabs were collected from all 94 psittacines showing cutaneous lesions (27 birds) or not (67 birds). Skin samples treated with an antibacterial–antimycotic drug mixture (Gibco; Thermo Fisher Scientific, Waltham, MA, USA) were inoculated onto the chorioallantoic membrane (CAM) of 10-day-old specific pathogen–free chicken embryos and typical pocklike CAM lesions were obtained (online Technical Appendix Figure 2). Cutaneous samples and the CAM of inoculated

embryos were subjected to PCR with specific primers forward 5′-CAGCAGGTGCTAAACAACAA-3′ and reverse 5′-CGGTAGCTTAACGCCGAATA-3′ (4) to amplify a partial sequence (576 bp) of the gene encoding the core protein P4b (fpv167 locus) of avipoxvirus (Table). The avipoxvirus P4b gene partial sequence was obtained from skin lesions, conjunctiva and cloacal swabs, and CAM; sequences then underwent phylogenetic characterization (online Technical Appendix Figure 3).

The P4b gene partial sequences obtained from the avipoxvirus isolate Betim-1 of the red-and-green macaw (Ara chloropterus) (GenBank accession no. KT187552), dusky parrot (Pionus fuscus) (Esteves et al., unpub. data), and golden conure (Guaruba guarouba) (Esteves et al., unpub. data) were 100% identical. The obtained sequences were aligned with local avipoxvirus of different species with clinical disease (Esteves et al., unpub. data) and with previously published avipoxvirus sequences in GenBank (online Technical Appendix Figure 3). Evolutionary analyses were performed in MEGA6 (5), evolutionary history was inferred by neighbor joining (6), replicate trees were clustered by using the bootstrap test (7) with 1,000 oversampling, and evolutionary distances were computed by the Tamura 3-parameter method (8). The phylogenetic relationships revealed grouping with psittacine poxviruses, including isolates from the United Kingdom (macaw, GenBank accession no. AM050382.1, and parrot, GenBank accession no. AM050383.1); United States (yellow-crowned amazon [Amazona ochrocephala], GenBank accession no. KC018069.1); and Germany (lovebird [Agapornis sp.], GenBank accession no. AY530311.1). To enable comparisons, a local strain (GenBank accession no. KX863707) of the Atlantic canary (Serinus canaria) (Esteves et al., unpub. data) and an isolate of the Magellanic penguin (Spheniscus magellanicus) (GenBank accession no. KF516679.1) (9) were grouped in the canarypox clade. A local chicken isolate (GenBank accession no. KX863706) was grouped within the fowlpox clade, including chicken (GenBank

accession nos. KF722860.1 KF722863.1) and turkey (GenBank accession nos. KF017961.1 and DQ873808.1) strains.

Birds with pocklike lesions represented 28.7% (27/94) of psittacines in the sanctuary. Laboratory diagnosis was implemented at the early stages of the outbreak; birds were clinically observed up to the final cases (3 months). Of the 27 psittacines with lesions, 23 (85.2%) were PCR positive for avipoxvirus. No blue-fronted parrots (Amazona aestiva) showed lesions, but all were PCR positive (4/94 [4.2%]), suggesting that they were immune, although they were not tested for an immune response. Among the 25 golden conures, the most abundant species in the premises, 9 showed typical pocklike lesions and were PCR positive, but 1 with lesions was PCR negative and 15 had no lesions and were PCR negative, indicating nonuniform immunity. None of the 14 blue and yellow macaws (Ara ararauna) had lesions; PCR results were negative for all, suggesting previous immunity or a lower susceptibility to infection. Four birds with lesions were not PCR positive, possibly indicating an advanced phase of virus-free scars.

The proximity of aviaries suggests that all birds might have had the opportunity for infection during the outbreak. The death rate (3.2%) for the outbreak was low; the death of young golden conures with diphtheritic lesions and 2 adult dusky parrots that demonstrated conjunctivitis and cachexia were accompanied by complications by Candida albicans and Capillaria sp., suggesting the potential aggravation risk. Most psittacines (63/94; 67.0%) were PCR negative and without lesions, as tested in cloacal and conjunctival swabs.

The affected species are declining in wild populations. Our findings emphasize the risk for avipoxvirus among captive psittacines; their relevance for psittacine rehabilitation and conservation may be considerable regarding pathogenicity. As shown, 2 adult dusky parrots and 1 nestling golden conure died; further characterization is needed for the isolates, including their eventual importance for commercial poultry.

Table. Avipoxvirus detection by PCR according to clinical status and psittacine species, Brazil, 2015*

Psittacine species	Clinical status			
	PCR positive		PCR negative	
	Normal	Cutaneous lesions	Normal	Cutaneous lesions
Amazona aestiva (blue-fronted parrot)	4	–	–	–
Amazona brasiliensis (red-tailed Amazon)	–	1	4	1
Anodorhynchus hyacinthinus (hyacinthine macaw)	–	2	4	–
Ara chloropterus (green-winged macaw)	–	–	2	–
Ara macao (scarlet macaw)	–	2	1	–
Ara ararauna (blue and yellow macaw)	–	–	14	–
Deroptyus accipitrinus (red-fan parrot)	–	1	9	2
Guaruba guarouba (golden conure)	–	9	15	1
Pionites leucogaster (white-bellied caique)	–	4	12	–
Pionus fuscus (dusky parrot)	–	3	2	–
Total	4	23	63	4
Ratio, no. birds/no. tested (%)	4/94 (4.2)	23/94 (24.4)	63/94 (67.0)	4/94 (4.2)

*Values are no. birds except as indicated. –, not found.

RESEARCH LETTERS

Dr. Esteves is a veterinarian dedicated to native wildlife conservation. He is the leader of a nongovernmental agency dedicated to the rescue and rehabilitation of wild animals. On his farm, he maintains and cares for about 170 animals that are not fit to reintroduce into nature.

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Chikungunya Fever in Traveler from Angola to Japan, 2016

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Simultaneous circulation of multiple arboviruses presents diagnostic challenges. In May 2016, chikungunya fever was diagnosed in a traveler from Angola to Japan. Travel history, incubation period, and phylogenetic analysis indicated probable infection acquisition in Angola, where a yellow fever outbreak is ongoing. Thus, local transmission of chikungunya virus probably also occurs in Angola.

Simultaneous circulation of multiple arboviruses has been observed several times in many parts of the world. In 1970, Angola reported an outbreak of a dengue-like syndrome, which turned out to be a concurrent outbreak of yellow fever and chikungunya fever (*1*). On April 13, 2016, the World Health Organization declared a yellow fever outbreak in Angola. In response to the outbreak, a nationwide yellow fever vaccination campaign was initiated. As of July 29, 2016, a total of 3,818 confirmed and suspected cases were reported (*2*). In addition, on July 23, 2016, the World Health Organization was notified of a Rift Valley fever case in a man from China working in Luanda, the capital city of Angola, and started an investigation in Angola (*3*). We describe a case of chikungunya fever in a traveler from Angola to Japan.

In May 2016, a 21-year-old woman traveled to Tokyo, Japan, from her home in Luanda. She began to exhibit a high fever on the first day of her visit. On the second day, she sought care at the National Center for Global Health and Medicine (Tokyo). She had been previously healthy and had not traveled out of Luanda in the past 6 months. She claimed to have been vaccinated according to the national immunization plan, which included vaccination against yellow fever. At the first visit, she had high-grade fever (40.7°C) without other signs. Her vital signs were otherwise stable, and physical examination revealed no

abnormality. Complete blood count and biochemistry tests revealed only a slightly elevated C-reactive protein level (1.55 mg/dL). Results of rapid diagnostic testing for malaria and dengue, 3 consecutive thin blood smears, HIV screening, and blood culture for bacteria were all negative.

After hospitalization, her fever gradually subsided but remained above 38°C. On the fifth day, bilateral axillary lymphadenopathy appeared. The lymph nodes were ≈2 cm, painful, and nonfluctuant. Despite the high-grade fever and lymphadenopathy, her general condition improved, and she was discharged on the fifth day. Thereafter, she recovered quickly and returned safely to Luanda.

Although the patient was supposedly vaccinated against yellow fever virus, we performed real-time reverse transcription PCR for yellow fever virus, and the result was confirmed to be negative. Testing for other arboviruses was performed, and real-time reverse transcription PCR for chikungunya virus (CHIKV) showed a positive result. Therefore, the final diagnosis was chikungunya fever. We used phylogenetic analysis based on the nucleotide sequence of the E1 gene from the serum sample, the maximum-likelihood method with 1,000 bootstrap replicates, and MEGA 6.0 software (http://www.megasoftware.net). The sequence was 98% identical to that of a CHIKV strain isolated in the Central African Republic in 1987 (Figure). Considering travel history, incubation period, and phylogenetic analysis, the patient was probably infected with CHIKV while in Luanda.

CHIKV was first isolated in Tanzania in 1953 (4). After a few decades of absence in Africa, the virus caused a large outbreak in the Democratic Republic of the Congo

in 2000 (5) and has subsequently been causing infection across the continent. Although the epidemiology of chikungunya fever is scarcely understood in Africa, an effort has been made to grasp the current burden of CHIKV in Africa. A study in Kenya found the rate of CHIKV IgG positivity among HIV-negative specimens to be 0.96% (6). A serologic study in southern Mozambique found that the rate of seroconversion or a ≥4-fold titer rise of CHIKV IgG among patients with acute febrile illness was 4.3% (7). These studies suggest that the incidence of CHIKV infection in Africa may be higher than previously assumed. This discrepancy may be explained by lack of awareness, diagnostic tools, and surveillance systems. As of April 22, 2016, Angola was not recognized as a country with local CHIKV transmission (8). However, considering that Angola harbors *Aedes aegypti* mosquitoes, which are efficient CHIKV vectors, and that neighboring countries have documented local transmission of the virus, it is reasonable to speculate that local transmission also occurs in Angola.

Co-infection and co-distribution of multiple arboviruses (including dengue viruses, CHIKV, and yellow fever virus) are widely reported (1,9,10). Although these viruses share a common vector, *Aedes* spp. mosquitoes, their interactions within mosquitoes and their effects on vector competence are unknown (9). Arboviruses cause similar clinical presentations, which makes diagnosis challenging without labor-intensive diagnostics, especially in outbreak settings. Because a yellow fever outbreak is ongoing in Angola, the diagnosis of other arboviral infections is needed for conducting appropriate clinical and public health interventions and precise surveillance.
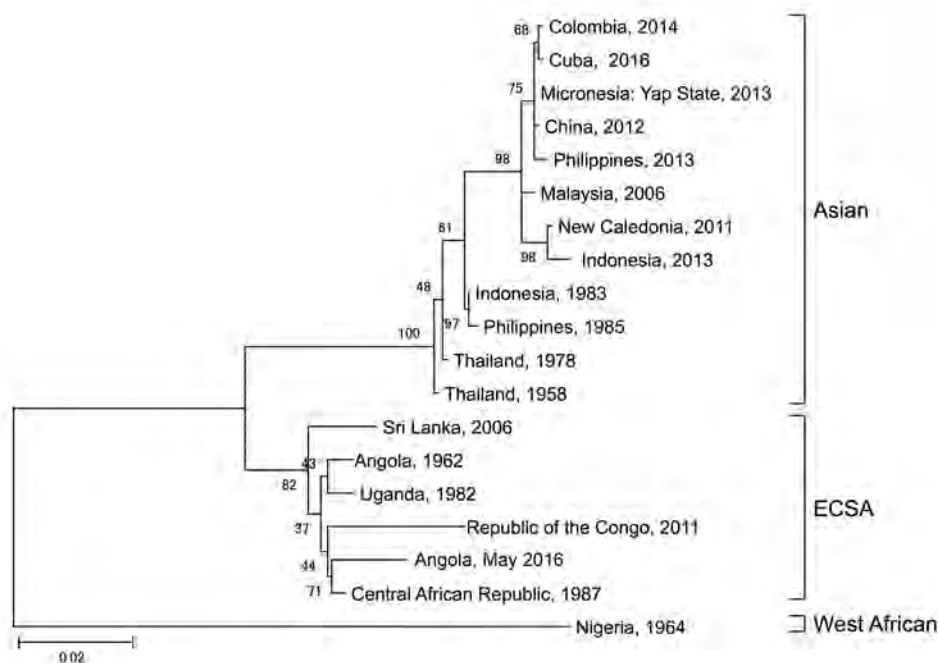
Figure. Phylogenetic comparison of the chikungunya virus sequence obtained from a patient traveling from Angola to Japan in May 2016 and reference sequences. Virus lineages are shown on the right. Scale bar represents substitutions per nucleotide position. ECSA, East/Central/South African lineage.

This case highlights 2 issues: the unknown epidemiology of CHIKV in Africa and the difficulty of diagnosing one arboviral infection during an outbreak of another arboviral infection. Further research is necessary to elucidate the true extent of CHIKV in African countries and to understand the public health implications of co-infection and co-distribution of multiple arboviruses.

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Puumala Virus in Bank Voles, Lithuania

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Little is known about the presence of human pathogenic Puumala virus (PUUV) in Lithuania. We detected this virus in bank voles (*Myodes glareolus*) in a region of this country in which previously PUUV-seropositive humans were identified. Our results are consistent with heterogeneous distributions of PUUV in other countries in Europe.

Puumala virus (PUUV) (family *Bunyaviridae*) is an enveloped hantavirus that contains a single-stranded trisegmented RNA genome of negative polarity (*1*). PUUV harbored by the bank vole (*Myodes glareolus*) is the most prevalent human pathogenic hantavirus in Europe (*2*). A high population density of bank voles can lead to disease clusters and possible outbreaks of nephropathia epidemica, a mild-to-moderate form of hantavirus disease (*3*).

In contrast to the Fennoscandian Peninsula and parts of central Europe (*4,5*), little is known about the epidemiology of PUUV in Poland and the Baltic States. Recent investigations confirmed the presence of PUUV in certain parts of Poland (*5,6*). A molecular study of bank voles in Latvia identified 2 PUUV lineages (Russian and Latvian) (*7*). In Estonia, serologic and molecular screening provided evidence of the Russian PUUV lineage (*8*). For Lithuania, a previous serosurvey indicated the presence of PUUV-specific antibodies in humans from 3 counties (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/23/1/16-1400-Techapp1.pdf). However, molecular evidence of PUUV in humans or in voles is lacking (*9*).

We report a molecular survey of rodent populations in Lithuania at 5 trapping sites, including 2 sites in counties where PUUV-specific antibodies were previously detected in humans (online Technical Appendix Figure 1). A total of 134 bank voles, 72 striped field mice (*Apodemus agrarius*), and 59 yellow-necked field mice (*A. flavicollis*) were captured during 2015. Three trapping sites (Juodkrantė, Elektrėnai, and Lukštas) were located in forests at or near

a cormorant colony, and 2 trapping sites (Žalgiriai and Rusnė) were located in a wet forest and flooded meadows. All applicable institutional and national guidelines for the care and use of animals were followed.

For PUUV detection, we extracted RNA from bank vole lung tissue samples by using the Qiazol Protocol (QIAGEN, Hilden, Germany) and conducting screening by using a small segment RNA–specific reverse transcription PCR (RT-PCR) and primers Pu342F and Pu1102R (6). We detected PCR products for 5 (LT15/164, LT15/165, LT15/166, LT15/174, and LT15/201) of 45 bank voles from the Lukštas trapping site. All 9 striped field mice and 2 yellow-necked field mice from Lukštas showed negative results for the PUUV RT-PCR.

We amplified the complete nucleocapsid protein–encoding region for 3 of the 5 samples positive by RT-PCR with 3 primer pairs: PuNCRS (5′-TAGTAG-TAGACTCCTTGAA-3′)/Pu255R (5′-TGGACACAG-CATCTGCCA-3′), Pu40F (5′-CTGGAATGAGTGACTTA-AC-3′)/Pu393R (5′-TATGGTAATGTCCTTGATGT-3′), and Pu1027F (5′-ATGGCAGAGTTAGGTGCA-3′)/Pu1779R (5′-TCAGCATGTTGAGGTAGT-3′). RT-PCR products were directly sequenced by using the BigDye Terminator Version 1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany). We deposited the sequences of the 5 samples in GenBank under accession nos. KX757839, KX757840, KX 757841, KX751706, and KX751707 (Figure; online Technical Appendix Figure 2).

The 3 nucleocapsid protein–encoding nucleotide sequences showed identities of 98.2%–99.8%, and the 3 deduced nucleocapsid protein amino acid sequences showed identities of 99.8%–100% (online Technical Appendix Table). We found the highest similarity of the 3 nucleotide and corresponding amino acid sequences for the PUUV strain from Latvia (Jelgava1/Mg149/2008; JN657228): nucleotide sequence 89.8%–90.4% and amino acid sequence 99.8%–100% (online Technical Appendix Table).

We generated phylogenetic trees by using MrBayes 3.2.6 software (http://mrbayes.sourceforge.net/download. php) and MEGA6 software (http://www.megasoftware. net/) for complete (1,302 nt; Figure) and partial (465 nt; online Technical Appendix Figure 2) nucleocapsid protein–encoding sequences. Phylogenetic analysis confirmed results of pairwise nucleotide sequence divergence analysis, which indicated clustering of PUUV sequences from Lithuania with sequences from northern Poland (online Technical Appendix Figure 2) and the Jelgava 1 strain from Latvia (Figure). These sequences of the Latvian clade are well separated from the Russian and all other European PUUV clades.

To evaluate a potential association of PUUV with evolutionary lineages of the bank vole, we determined vole cytochrome b gene sequences, deposited them in GenBank
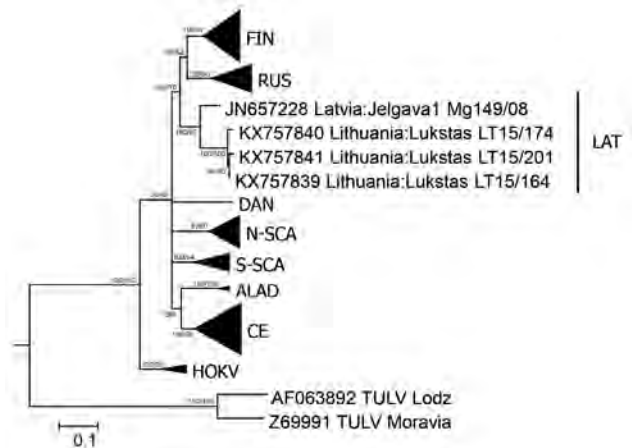
Figure. Phylogenetic tree based on complete nucleocapsid gene sequences of Puumala virus (PUUV) strains from Lithuania (LT), Latvia (Jelgava1), and other PUUV clades. Tula virus (TULV) was used as the outgroup. The tree was generated by Bayesian and maximum-likelihood analysis using MrBayes 3.2.6 (http://mrbayes.sourceforge.net/download.php) and MEGA6 software (http://www.megasoftware.net/). The optimal substitution model was calculated by using jModelTest 2.1.4 (https://code.google.com/p/jmodeltest2). The Bayesian tree was based on transition model 2 with invariant sites and gamma distribution and 4 million generations. For maximum-likelihood analysis, the Kimura 2-parameter model and 1,000 bootstrap replicates were used. Posterior probabilities are indicated before slashes, and bootstrap values are indicated after slashes. Scale bar indicates nucleotide substitutions per site. ALAD, Alpe-Adrian lineage; CE, Central European lineage; DAN, Danish lineage; FIN, Finnish lineage; HOKV, Hokkaido virus; LAT, Latvian lineage; N-SCA, North-Scandinavian lineage; RUS, Russian linage; S-SCA, South-Scandinavian lineage.

under accession nos. KX769843 (LT15/164), KX769844 (LT15/165), KX769845 (LT15/166), KX769846 (LT15/174), and KX769847 (LT15/201), and compared them with cytochrome b prototype sequences of evolutionary lineages. Consistent with results for northern Poland (6), we identified 2 bank vole lineages at Lukštas, and the PUUV sequences were detected in 4 bank voles of the Carpathian phylogroup and in 1 vole of the Eastern lineage.

In conclusion, we detected PUUV in bank voles at 1 site (Lukštas) in Lithuania (prevalence of 11.1%). This site is located in a region where PUUV-seropositive persons were identified (9) and near the border with Latvia (online Technical Appendix Figure 1). The absence of PUUV in bank voles at 4 other sites might have been caused by the small number of voles tested. However, our results are consistent with heterogeneous distributions of PUUV in other countries (10).

Detection of this novel PUUV strain by using a specific RT-PCR confirms the reliability of this assay for molecular diagnostic and epidemiologic studies of this virus in Lithuania. Future large-scale monitoring studies are needed

to evaluate the geographic distribution and temporal fluctuation of PUUV in bank vole populations in Lithuania.

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Loiasis in US Traveler Returning from Bioko Island, Equatorial Guinea, 2016

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The filarial parasite *Loa loa* overlaps geographically with *Onchocera volvulus* and *Wuchereria bancrofti* filariae in central Africa. Accurate information regarding this overlap is critical to elimination programs targeting *O. volvulus* and *W. bancrofti*. We describe a case of loiasis in a traveler returning from Bioko Island, Equatorial Guinea, a location heretofore unknown for *L. loa* transmission.

Loiasis (African eye worm disease) is caused by infection with *Loa loa*, a parasitic vector-borne filarial worm endemic to 10 countries in central and western Africa, including Equatorial Guinea (*1*). The worm, spread by the bite of *Chrysops dimidiata* and *C. silacea* flies, is of public health concern because of its geographic overlap with *Onchocerca volvulus* and *Wuchereria bancrofti* worms, which cause onchocerciasis and lymphatic filariasis, respectively (*2*). Mass drug administration programs for onchocerciasis and lymphatic filariasis often include ivermectin, which can cause serious and occasionally fatal adverse neurologic reactions in persons with high levels of circulating *L. loa* microfilariae (*3*). To avoid such reactions, an accurate picture of the geographic distribution of *L. loa* infection is needed. Given the importance of epidemiologic data in the management of filarial infections, we report a case of loiasis in a US woman who had traveled to Equatorial Guinea.

In May 2016, a 25-year-old woman sought care in Winston-Salem, North Carolina, USA, for fatigue, swelling of her left ankle, right knee pain, and intensely pruritic skin lesions on her lower extremities. She had lived on Bioko Island, Equatorial Guinea, during October 2015–March 2016 while studying local wildlife. On Bioko Island, she frequented local water sources to bathe and wash clothes and consistently took atovaquone/proguanil for malaria prophylaxis. She did not spend time on Equatorial Guinea's mainland or travel to other nations in central or western Africa. Her flight from the United States to Bioko Island connected in Ethiopia; she did not leave the airport.

Symptoms developed soon after her return to North Carolina in late March 2016. Laboratory evaluations

performed at that time showed a leukocyte count of 8.5×10^3 cells/μL (reference range 3.4–10.8×10^3 cells/μL), hemoglobin level of 13.9 (reference range 11.1–15.9 g/dL), platelet count of 219 (reference range 150–379 × 10³ cells /μL), and absolute eosinophil count of 2,300/μL (reference range 40–400/μL).

In May, her physical examination was notable only for edema of the left lower extremity adjacent to her ankle. Three separate midday blood smears for microfilariae were negative. Laboratory tests showed a leukocyte count of 11.5×10^3 cells/μL, absolute eosinophil count of 4,200/μL, and IgE level of 175 IU/mL (reference range 0–100 IU/mL). Results of antifilarial IgG4 and *Strongyloides* IgG tests (performed by LabCorp, Burlington, NC, USA) were negative.

Over the subsequent 4 weeks, new pruritic, erythematous plaques appeared on her right flank and left thigh and behind her left ear (Figure). Blood testing at the Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health (Bethesda, MD, USA), showed negative results for a 1-mL Nuclepore (Whatman GE Lifesciences, Pittsburgh, PA, USA) filtration for microfilariae; *L. loa*–specific PCR (*4*); and rapid diagnostic testing, using the SD BIOLINE Oncho/LF IgG₄ biplex test (Standard Diagnostics, Inc., Seoul, South Korea) for detection of specific antibodies against *O. volvulus* and *W. bancrofti*. Testing also showed a BmA IgG (*5*) level of 100.6 μg/mL (reference value <14.0 μg/mL); a normal BmA IgG4 antibody level; and a luciferase immunoprecipitation systems assay result of 456,969 light units (LU)/mL for LL-SXP1 IgG (negative value <3,000 LU/mL) and 19,193 LU/mL for LL-SXP1 IgG4 (negative value <1,700 LU/mL) (*6*).

The patient was treated with diethylcarbamazine for 21 days. After completion of treatment, her symptoms improved, and her leukocyte and eosinophil counts returned to within reference ranges.

Recent years have seen renewed interest in the epidemiology and geographic distribution of *L. loa* in central and western Africa because of the risk of encephalopathy in patients given ivermectin as part of large programs to control filarial infections. Although the intermediate hosts of *L. loa* are present on Bioko Island, previous loiasis cases were reported only in persons who had been exposed to *Chrysops* flies on mainland Africa (*7*). Given the presence of these vectors on Bioko Island and the patient's lack of exposure to any other *L. loa*–endemic region, transmission of *L. loa* on Bioko Island seems probable. Of note, a previous study found 1 of 541 skin snips tested on Bioko Island to be PCR-positive for *L. loa*, a finding thought to have been caused by skin snip sample contamination with capillary blood (*8*).

The signs and symptoms of *L. loa* infection exhibited by the US patient reinforce the perception that loiasis in returned travelers is often quite distinct from that in persons with lifelong exposure in a region where the disease is endemic (*9,10*). The course of infection also points to differences in IgG- and IgG4-based antifilarial serologic testing early in infection (*5*) and provide evidence that the use of species-specific recombinant antigens can more accurately help with specific parasite diagnosis (*6*).

Knowledge of the geographic distribution of *L. loa* infection is critical because loiasis overlaps with other filarial diseases, such as onchocerciasis and lymphatic filariasis. The intermediate vectors responsible for *L. loa* transmission, *Chrysops* flies, are known to live on Bioko Island; the
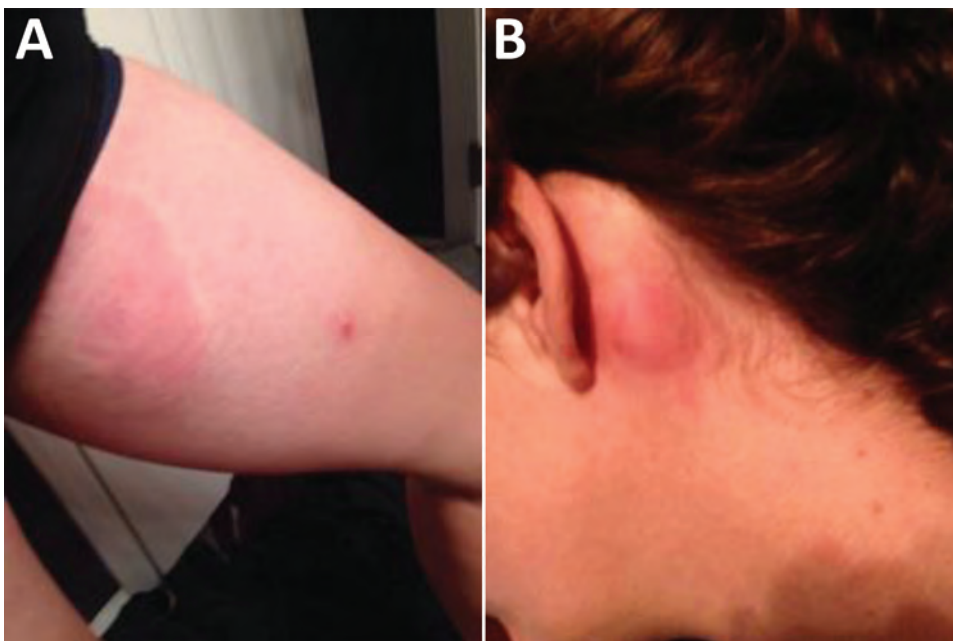
Figure. Cutaneous manifestations of *Loa loa* (African eye worm) infection in a US traveler who returned from a 6-month stay on Bioko Island, Equatorial Guinea, 2016. Urticarial lesions on the left thigh showing a coincident papular eruption (A) and behind the left ear (B).

case we present suggests that local transmission of *L. loa* and prevalence of loiasis on the island may be higher than previously thought.

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Invasive Infections with Multidrug-Resistant Yeast *Candida auris*, Colombia

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Candida auris is an emerging multidrug-resistant fungus that causes a wide range of symptoms. We report finding 17 cases of *C. auris* infection that were originally misclassified but correctly identified 27.5 days later on average. Patients with a delayed diagnosis of *C. auris* had a 30-day mortality rate of 35.2%.

*C*andida auris is an emerging multidrug-resistant fungus that causes a wide range of infections that are sometimes associated with high mortality rates (*1–4*). *C. auris* was first isolated in Japan and described as a new species in 2009 (*5*). In 2011, it was described as a cause of fungemia in South Korea (*4*) and was later isolated from patients in India (*2*), South Africa (*6*), Kuwait (*3*), and Venezuela (*1*).

We report 17 clinical isolates of *C. auris* recovered from 17 patients hospitalized in 6 institutions in the northern region of Colombia from February through July 2016. We reviewed patient medical records; analyzed microbiological, demographic, and clinical variables; and evaluated the mortality rate 30 days after yeast isolation. The initial

pathogen identification was made with the method available at each institution: VITEK 2 system (bioMérieux, Marcy l'Étoile, France); Phoenix (Becton Dickinson, Franklin Lakes, New Jersey, USA); MicroScan AutoSCAN 4 and MicroScan Walkaway (Beckman Coulter, Brea, California, USA); and API Candida (bioMérieux) (Table). Given the unusual prevalence of *C. haemulonii* and discordance in the micromorphologic characteristics of some isolates, we cultured strains in CHROMagar Candida medium (CHROMagar Candida, Paris, France) and identified them by using matrix-assisted laser desorption/ionization time-of-fight (MALDI-TOF) mass spectrometry (Bruker Daltonik, Bremen, Germany). All the isolates showed pink colonies on CHROMagar Candida medium and were identified as *C. auris* by MALDI-TOF mass spectrometry (scores >2.0).

We tested yeast isolates for in vitro susceptibility by VITEK cards (caspofungin, micafungin, fluconazole, voriconazole, and amphotericin B). Additionally, we performed the agar diffusion method using Etest strips (bioMérieux, France) to amphotericin B according to the manufacturer's instructions. *C. parapsilosis* ATCC 22019 was used as the control. The range, mode, MIC_{50}, and MIC_{90} were calculated.

Of the 17 patients, 9 were men (52.9%); age range was 0–77 years (median 36 years). Fifteen (88.2%) were hospitalized in intensive care units and 2 in medical wards; no patients were transferred between hospitals.

Blood samples from 13 (76.4%) patients showed fungemia; for the remaining 4, *C. auris* was isolated from peritoneal fluid, cerebrospinal fluid, bone, or urine (Table). Most patients had a central venous catheter (n = 16, 94.1%), a urinary catheter (n = 15, 88.2%), and mechanical ventilation (n = 10, 58.8%). Additionally, some had risk factors described previously for candidemia: erythrocyte transfusion (n = 12, 70.5%), parenteral nutrition (n = 8, 47%), abdominal surgery (n = 7, 41.1%), hemodialysis (n = 5, 29.4%), diabetes (n = 3, 17.6%), pancreatitis (n = 2, 11.7%), cancer (n = 2, 11.7%), and HIV infection (n = 1, 5.8%).

The average number of days from hospitalization to isolation of *C. auris* was 27.5 days (SD ± 19.9 days). Before the isolation of *C. auris*, 15 (88.2%) and 12 (70.6%) patients received broad-spectrum antimicrobial therapy and antifungal therapy, respectively. Of the latter, 8 received fluconazole, 2 caspofungin, 1 fluconazole and caspofungin, and 1 fluconazole, caspofungin, and anidulafungin (given at a different time periods) (Table). The time from isolation of *C. auris* to the initial application of effective antifungal therapy averaged 3.7 days. The 30-day mortality rate in all patients and in those who had fungemia was 35.2% and 38.4%, respectively.

C. auris is phylogenetically related to *C. haemulonii*, for which it is often mistaken with the methods currently used for identification (*2–6*). Our isolates were originally

Table. Identification and antifungal susceptibilities of *Candida auris* clinical isolates of six hospitals, northern region of Colombia, 2016*

Isolate ID	Hospital no.	Specimen origin	Biochemical identification (system)	Pre-AFT	VITEK cards					Etest/AMB	
					FLC	MCF	CAS	VRC	AMB	24 h	48 h
001	1	Blood	*C. haemulonii* (VITEK)	None	16	0.12	<0.25	0.25	8	0.75	1
002	1	CSF	*C. tropicalis* (MicroScan Walkaway)	CAS	16	0.12	<0.25	<0.12	8	0.75	1
003	1	Blood	*C. famata* (API Candida)	FLC	16	<0.06	<0.25	<0.12	8	1	1
004	5	Blood	*C. haemulonii* (Phoenix)	FLC	16	0.12	<0.25	0.25	>16	1	1.5
005	2	Blood	*C. haemulonii* (VITEK)	FLC	>64	0.25	0.5	2	>16	1	1.5
006	4	Blood	*C. haemulonii* (VITEK)	None	>64	0.12	<0.25	0.5	8	1	1
007	3	Peritoneal fluid	*C. albicans* (MicroScan autoSCAN)	FLC	16	0.12	<0.25	0.25	8	1	1.5
008	2	Blood	*C. haemulonii* (VITEK)	FLC	16	0.12	<0.25	0.25	8	0.38	0.75
009	1	Blood	*C. tropicalis* (MicroScan Walkaway)/ *C. famata* (API Candida)	FLC, CAS	32	0.12	<0.25	0.25	8	2	3
010	5	Bone	*C. haemulonii* (VITEK)	FLC, AFG, CAS	16	0.12	<0.25	0.25	8	0.75	1
011	6	Urine	*C. haemulonii* (Phoenix)	None	32	0.12	<0.25	0.25	8	1.5	1.5
012	3	Blood	*C. albicans* (MicroScan AutoSCAN)	FLC	32	0.12	<0.25	1	8	1	2
013	3	Blood	*C. haemulonii* (VITEK)	None	32	0.12	<0.25	1	8	0.75	2
014	3	Blood	*C. haemulonii* (VITEK)	FLC	>64	0.12	<0.25	2	8	1.5	1.5
015	2	Blood	*C. haemulonii* (VITEK)	None	32	0.12	<0.25	0.25	8	0.75	2
016	2	Blood	*C. haemulonii* (VITEK)	FLC	>64	0.12	<0.25	2	>16	1	2
017	4	Blood	*C. haemulonii* (VITEK)	CAS	>64	0.12	<0.25	2	>16	2	4
MIC (mg/L) range					16 to >64	<0.06 to 0.25	<0.25 to 0.5	<0.12 to 2	8 to >16	0.38 to 2	0.75 to 4
MIC_{50}					32	0.12	<0.25	0.25	8	1	1.5
MIC_{90}					>64	0.12	<0.25	2	>16	2	2

*AFG, anidulafungin; AMB, amphotericin B; CAS, caspofungin; CSF, cerebrospinal fluid; FLC, fluconazole; ID, identification; MCF, micafungin; MIC_{50}, minimal inhibitory concentration for 50% of yeast; MIC_{90}, minimal inhibitory concentration for 90% of yeast; Pre-AFT, use of antifungal therapy before the isolation of *C. auris*; VRC, voriconazole.

misidentified as *C. haemulonii*, *C. famata*, *C. albicans*, or *C. tropicalis*, depending on the method used in the hospital. The identification of isolates by MALDI-TOF mass spectrometry has also been described in the literature as an adequate and fast method for identifying *C. auris* (*7*).

Because the Clinical and Laboratory Standards Institute does not currently provide breakpoints for *C. auris*, no categorical interpretation of results is available; thus, only the MICs obtained for antifungal drugs tested in our study were indicated (Table). Although misleading, elevated MICs of amphotericin B by VITEK card have been previously described (*7*); this study also found discrepancies with Etest strips, which could lead to the selection of inappropriate therapy if only 1 method is used.

The presence of *C. auris* in these patients has clinical and epidemiologic implications, considering the associated mortality rate confirmed in this report and the absence of sufficient technology in clinical laboratories both to confirm their identification and to carry out testing for antifungal susceptibility. The lack of suitable diagnostics complicates patient treatment and changes on the empiric treatment of invasive *Candida* spp. infections are needed.

Our data contributes to the knowledge of the epidemiology of this species at a regional level. Although we had already reported *Candida* spp. in Colombia (*8*), no information regarding these species on the Caribbean coast is available. Given the association of *Candida* spp. with outbreaks in hospitals, according to the Centers for Disease Control and Prevention, it is necessary to further strengthen measures for fungal infection control to prevent possible spread.

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Zika Virus Knowledge among Pregnant Women Who Were in Areas with Active Transmission

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We surveyed women in New York, New York, USA, who were in areas with active Zika virus transmission while pregnant. Of 99 women who were US residents, 30 were

unaware of the government travel advisory to areas with active Zika virus transmission while pregnant, and 37 were unaware of their pregnancies during travel.

Zika virus is primarily transmitted by the bite of infected *Aedes* mosquitoes; the virus can also cross the placenta of infected pregnant women, potentially leading to congenital infection and serious birth defects (*1–3*). As of October 7, 2016, a total of 617 cases of Zika virus infection had been identified among New York City (NYC) residents, including 72 cases among pregnant women (*4*).

Despite government advisories in place since early 2016 recommending that pregnant women avoid travel to areas with active Zika virus transmission (*4,5*), the NYC Department of Health and Mental Hygiene (DOHMH) saw an increase in weekly Zika virus test requests through the summer for women who had been in such areas while pregnant. This increase alerted the DOHMH to the need for additional messaging. To guide this communication, we conducted telephone surveys to evaluate Zika virus knowledge and practices among women in NYC who had been in such areas while pregnant.

In brief, during June 1–July 15, 2016, the DOHMH Zika Testing Call Center facilitated testing for 1,086 women ≥18 years of age because they were pregnant while in an area with active Zika virus transmission (*6*) (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/23/1/16-1614-Techapp1.pdf). At the time of receiving the Zika virus test request, DOHMH collected demographic data, contact information and other pertinent clinical history on the patients; these 1,086 women were potentially eligible for the survey if their telephone number had been provided. The women were called in random order until ≈100 provided consent and completed the survey. Descriptive statistics were calculated for responses to each survey question.

After 642 eligible women had been called, the target number of respondents had provided consent and completed the survey (n = 121; 18.8%); 67 (55.4%) respondents were interviewed in Spanish. We found no statistically significant differences in demographic characteristics between respondents and nonrespondents (online Technical Appendix Table).

Of the 121 respondents, 99 (81.8%) were US residents (considered the United States their home). Approximately one third of the US residents (n = 30; 30.6%) were unaware of the government advisory (recommending that pregnant women avoid travel to areas with active Zika virus transmission) at the time of travel (Table). Nearly half (n = 43; 44.3%) did not know that there was active Zika virus transmission in areas where they traveled, and more than one third (n = 37; 38.5%) did not know that they were pregnant during travel. Of the 30 US residents who were aware of the government advisory, were aware of active Zika virus transmission in areas where they traveled, and knew that they were pregnant during travel, 7 (23.3%) still traveled because their trips were too expensive to cancel. Of 6 US residents who did not know about the government advisory but did know of active Zika virus transmission in areas where they traveled and did know that they were pregnant during travel, 5 (83.3%) said they would not have traveled had they known about the government advisory. The most frequently reported reason for travel among US residents was to visit friends or relatives (n = 68; 70.1%).

Among the women we surveyed, many were unaware of the government travel advisory, unaware of active Zika virus transmission in areas where they traveled, or unaware of their pregnancy during travel. However, our survey had limitations. The small sample size limited our ability to perform sophisticated analyses, and the potential for social desirability and recall bias are inherent to the study design. The survey questionnaire was not a validated instrument. Also, the women described here completed the survey after Zika virus testing; therefore, it is possible that they had a better understanding than the general public.

Most participants in our survey were interviewed in Spanish. This finding underscores the need for providing educational materials in multiple languages.

Although our findings cannot be generalized, they provide insight for increased and improved public health messaging. Public health authorities in the United States should continue to raise awareness among women of reproductive age about the risk for Zika virus infection from travel,

Table. Knowledge about Zika virus infection among US residents who were pregnant at time of travel to areas with active Zika virus transmission, New York, NY, USA, June 1–July 15, 2016*

Characteristic	Total responses	Yes (%)*	No (%)*
Aware of government travel advisory at time of travel to areas with active Zika virus transmission	98	68 (69.4)	30 (30.6)
Aware that areas of travel had active Zika virus transmission	97	54 (55.7)	43 (44.3)
Aware of pregnancy status at time of travel to areas with active Zika virus transmission	96	59 (61.5)	37 (38.5)
Reason for travel			
Visiting friends or relatives	97	68 (70.1)	29 (29.9)
Tourism	97	52 (53.6)	45 (46.4)
Other	87	24 (27.6)	63 (72.4)
Business	97	5 (5.1)	92 (94.9)
Education	97	5 (5.1)	92 (94.9)
Service-related	97	4 (4.1)	93 (95.9)

*Column percentages do not total 100% because categories are not mutually exclusive. Denominator includes only those respondents who answered the question.

enabling them to better make informed decisions. Women who are trying to become pregnant or who are pregnant should avoid travel to areas with active Zika virus transmission and, if they must travel, should talk to their healthcare provider first and take steps to minimize exposure to Zika virus. Furthermore, women who are trying to become pregnant should follow Centers for Disease Control and Prevention (Atlanta, GA, USA) guidelines on how long to wait to get pregnant after a potential Zika virus exposure (7). Women who want to avoid pregnancy and their male partners should use effective birth control correctly and consistently (8). Healthcare providers in the United States caring for pregnant women and women who are trying to become pregnant should routinely discuss travel history and travel plans with their patients.

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Multidrug-Resistant Pathogens in Hospitalized Syrian Children

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Since 2013, wounded and ill children from Syria have received treatment in Israel. Screening cultures indicated that multidrug-resistant (MDR) pathogens colonized 89 (83%) of 107 children. For 58% of MDR infections, the pathogen was similar to that identified during screening. MDR screening of these children is valuable for purposes of isolation and treatment.

As the civil war in Syria enters its sixth year, the United Nations estimates that ≈250,000 persons have been killed, ≈10,000 of them children (1). Preliminary reports indicate a high rate of multidrug-resistant (MDR) pathogen carriage among refugees from Syria, mostly adults (2–5). Preliminary data for 29 wounded Syrian children indicate that 66% carried extended-spectrum β-lactamase–producing Enterobacteriaceae (ESBL) (2).

For ≈3 years, Syrian children who were ill or severely wounded from the civil war have been secretly transported across the border for treatment in Israel, mainly at Galilee Medical Center (GMC; Nahariya, Israel). We characterized carriage of and infections with MDR pathogens among these children.

We prospectively collected demographic and clinical microbiology data for all Syrian children 0–17 years of age who were admitted to GMC during March 2013–February 2016. At admission, contact isolation and screening cultures for MDR were conducted. MDR pathogens belonged to 1 of 5 groups: ESBL, carbapenem-resistant Enterobacteriaceae (CRE), methicillin-resistant Staphylococcus aureus, MDR Acinetobacter baumannii (MDR-AB), and vancomycin-resistant Enterococcus. Culture sites included nares, axilla, groin, rectum, and open wounds. Bacterial identification and susceptibility testing were performed according to Clinical and Laboratory Standards Institute guidelines (http://clsi.org/standards/micro/). For CRE screening, we used CHROMagar plates (hylabs, Rehovot, Israel).

The mechanism of CRE resistance was determined by the GeneXpert Carba-R system (Cepheid, Sunnyvale, CA, USA) with PCR. For MDR-AB screening, we used Mac-Conkey plates with ceftriaxone disks. The study was approved by the GMC institutional review board.

During the study period, 128 Syrian children were admitted to GMC; 92 (72%) were male, median age was 11 (0–17) years, and 87 (68%) were treated for multiple trauma and 41 (32%) for acute or chronic illness. Average hospitalization length was 1 month. Because of trauma severity, 4 (3%) died at arrival.

Of the 128 children, screening cultures for all 5 pathogen groups were performed for 107, and these children were included in the study (Figure). MDR pathogen carriage was found for 89 (83%). Among MDR pathogens carried, all 5 groups were represented. Among CRE, the most common resistance mechanism was New Delhi metallo-β-lactamase. Rates of MDR carriage were similar among wounded and ill children, mean MDR carriage rate among children increased from 1.19 in 2013–2014 to 1.45 in 2015 (p = 0.02). No amikacin resistance to ESBL was detected. Colistin was the only drug to which CRE and MDR-AB were susceptible.

For 24 (19%) children, MDR infections were evident, mostly urinary tract, surgical site, and osteomyelitis.
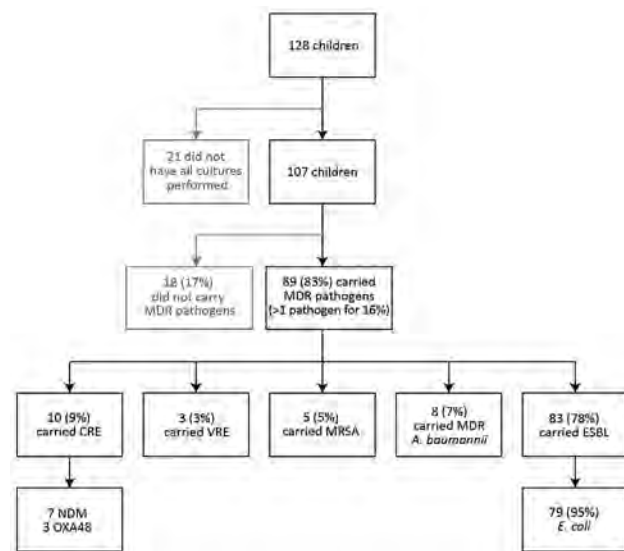
Figure. Carriage of MDR pathogens from 5 pathogen groups in 107 Syrian children treated at Galilee Medical Center, Nahariya, Israel, March 2013–February 2016. Of 128 children included in the study, 29 had been previously reported (2); all MDR carriage rate calculations are based on the 107 children for whom screening cultures for all 5 pathogen groups were performed. *A. baumannii, Acinetobacter baumannii*; CRE, carbapenem-resistant *Enterobacteriaceae*; *E. coli, Escherichia coli*; ESBL, extended-spectrum β-lactamase–producing *Enterobacteriaceae*; MDR, multidrug resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; NDM, New Delhi metallo-β-lactamase; OXA, oxacillinase; VRE, vancomycin-resistant *Enterococcus*.

Infections were more frequent among wounded (90%) than among sick (10%) children (p = 0.035). Two thirds of infections were caused by ESBL, 20% by MDR-AB, and 15% by CRE. In 58% of children with infections, the infecting isolate was similar (by species and antimicrobial drug susceptibility) to the MDR pathogen identified by screening. The empirically prescribed therapy for sepsis was meropenem and amikacin. Colistin was empirically added for severe sepsis or colonization with CRE or MDR-AB.

The rate of MDR carriage among wounded and ill Syrian children who were treated in Israel is extremely high. This finding probably implies a high carriage rate of MDR pathogens (especially ESBL) among healthy children in Syria and acquisition of MDR pathogens (especially CRE and MDR-AB) in the healthcare system in Syria. Indeed, before the Syrian war, prevalence of ESBL urinary tract infections associated with wide use of antimicrobial drugs was high (6). In addition, sale of antimicrobial drugs without prescriptions was common in Damascus and other areas (7). The war added inadequate sanitation, hospital and infrastructure destruction, and suboptimal infection control measures. Increased rates of MDR pathogen carriage in 2015, compared with 2013–2014, along with the recent polio and measles outbreaks, suggest further deterioration of the healthcare system in Syria (8).

Neighboring countries and European centers (for healthcare and asylum seekers) report finding MDR pathogens among wounded adult patients and refugees from Syria; in Germany, among refugees from Syria in 2016, the rate of colonization with gram-negative MDR pathogens was 60% (3–5,9). Earlier in the Syrian conflict, we described an MDR pathogen carriage rate of 66% (almost all ESBL) among 29 children in Israel (2). Since then, many more Syrian children have been treated at GMC. Approximately 80% of these children are colonized by MDR pathogens; ESBL predominate, followed by CRE and MDR-AB.

Our data, relevant to other centers caring for patients from Syria, show that contact isolation at the time of admission of patients from Syria is crucial for preventing transmission of MDR pathogens. We did not observe cross-infections, but another center in Israel that cares for patients from Syria has documented nosocomial spread of oxacillinase-48 CRE (10). Thoughtful decisions about empiric antimicrobial drug therapy for patients from Syria suspected of having infection are complex, given the multitude of MDR pathogens carried and the severity of trauma. Our study demonstrates that in almost 60% of MDR infections, the pathogen was similar to that found on screening cultures. Therefore, screening cultures at admission can provide valuable information for infection control, isolation decision-making, and tailoring appropriate empiric antimicrobial drug therapy.
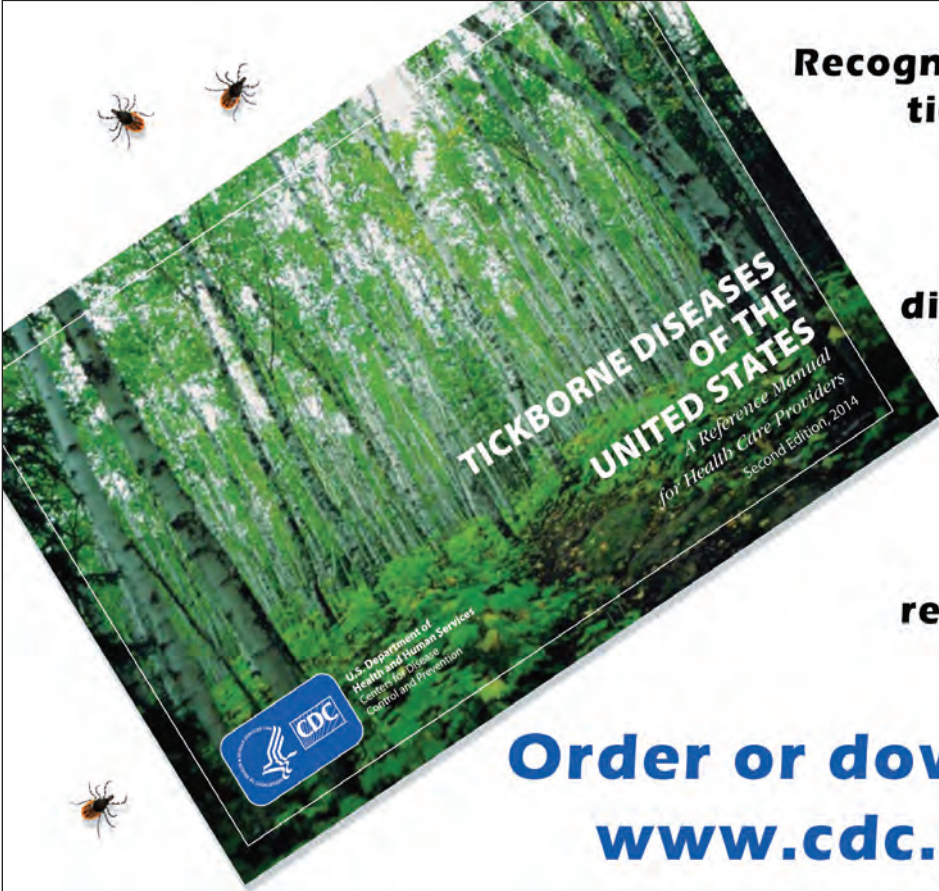
Acknowledgment

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Human Tick-Borne Encephalitis, the Netherlands

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To the Editor: We read with interest the report by Henningsson et al. (*1*) on a recent human case of tick-borne encephalitis (TBE) in Sweden. This case has multiple similarities to a case in the Netherlands. Until recently, TBE virus (TBEV) was not thought to be present in the Netherlands. However, detection of TBEV in ticks from a forested area in the Netherlands (S. Jahfari et al., Centre for Infectious Disease Control (CIb), RIVM, the Netherlands, pers. comm., 2016, Jun 30) led to identification of an autochthonous case of TBE (*2*). Of note, the presumed incubation period in both cases was very short, only 2 days. In both cases, quantitative reverse transcription PCR of a tick removed from the patient's leg showed high levels of TBEV RNA. Another similarity between the cases was the absence of intrathecally produced TBEV antibodies (no IgM and a negative antibody index for IgG for the case in the Netherlands) at hospital admission. Although 16% of TBE patients have no intrathecally produced TBEV antibodies at hospital admission, these antibodies are usually important for diagnosis (*3*). These cases underscore the point that absence of these antibodies does not rule out TBE.

By the time patients undergo TBE testing, the virus is typically no longer detectable in clinical samples (*4*). Therefore, laboratory diagnosis is done on the basis of the presence of antibodies in blood. Flavivirus serologic testing is, however, challenging due to cross-reactivity between the flaviviruses. Thus, confirmation by virus neutralization test is typically required.

These 2 studies (*1,2*) show that virus detection in ticks from TBE patients can be useful for the definitive diagnosis of TBE. However, in low-prevalence settings, it would be inefficient to test ticks from all patients with tick bites. In these cases, ticks should be tested only when TBE is suspected on the basis of compatible clinical symptoms and laboratory findings, such as positive TBEV serologic test results.

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Disease Selection: The Way Disease Changed the World

Disease Selection: The Way Disease Changed the World by Roger Webber is written in the tradition of Rats, Lice and History by Hans Zinsser; Guns, Germs and Steel: The Fates of Human Societies by Jared M. Diamond; and Viruses, Plagues, and History by Michael B.A. Oldstone. These volumes focus on the role of diseases, particularly infectious diseases, in history. Compared with the others, however, Dr. Webber's book is more focused on the effects of diseases on natural selection and evolution. These factors sometimes work in parallel and sometimes in opposition but always in highly complex and often unpredictable ways. The book is clearly written for a knowledgeable lay audience, a difficult task when covering evolution, microbiology, a wide variety of diseases and modes of their transmission, and immunologic responses to those diseases.

The book begins with a brief review of the origins of life on earth, including a discussion of the earliest life forms, the archaea and bacteria. Dr. Webber then describes the development of the various means of reproduction and their effects on the evolution of species and increasing complexity of life forms. In the first chapter, he covers the evolution of sexual reproduction and its effect on survival of species. Later, he discusses the role of diseases and the likely influence of endogenous viruses in the evolution of higher life forms, including humans.

Human ancestors originated in Africa and, to this day, this continent contains a unique variety of infectious agents that have likely effected human evolution. Malaria, for example, has probably led to the ability of populations to sustain sickle cell anemia because of the relative resistance of sickle red cells to *Plasmodium* spp., the cause of malaria. When the role of mosquitoes in transmission of malaria and other diseases was recognized in the 19th century, the complexity of biological relationships at all levels was revealed. For instance, because mosquitoes are damaged by blood infected with filarial parasites, those mosquitoes are less likely to transmit other disease agents such as those that cause malaria.

Smallpox, plague, influenza, and other diseases have killed large percentages of the world's populations at various times in the past. When smallpox and measles were brought to the New World, by Europeans who were carrying variola and measles viruses, indigenous populations in South America suffered great loss of life. After these epidemics, mortality rates for survivors and their progeny were lower than for newly exposed persons who became ill. This lends support to the concept of selection of populations resistant to diseases after substantial proportions of the populations have been killed by these diseases. Later chapters deal with the effects of war, breast feeding, diet, climate change, migration, and domesticated animals on the evolution of human beings.

Dr. Webber has succeeded in the ambitious task of describing the evolution of a broad range of organisms and their interrelationships during that process, with special emphasis on how diseases have influenced the evolution of humans. The book is clearly written, and many will find the index and extensive references useful. It will be an enjoyable read for audiences with a broad range of expertise in the biological sciences.

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Crockett Johnson (1906–1975), Mystic Hexagon (Pascal) (1965) (detail). House paint on Masonite, 49 in × 25 in / 24.5 cm × 63.5 cm. National Museum of American History, Kenneth E. Behring Center, Constitution Avenue, NW, between 12th and 14th Streets Washington, DC, USA.

Extrapolation Yields Painting, Probability, and Predictions

Byron Breedlove, Martin I. Meltzer

". . . in a matter which so closely concerns the wellbeing of the human race, no decision shall be made without all the knowledge which a little analysis and calculation can provide."

—Daniel Bernoulli, 1760

According to the National Museum of American History, "Inspired by the allure of the space age, many Americans of the 1960s took great interest in mathematics and science." Included among these was Crockett Johnson, a well-known cartoonist, book illustrator, and children's author best remembered for his *Harold and the Purple Crayon* series.

From 1965 until his death in 1975, Johnson painted what he described as "a series of romantic tributes to the great geometric mathematicians from Pythagoras on up." Initially, Johnson drew inspiration from figures he found in James R. Newman's book *The World of Mathematics* (1956) and other mathematics books but later began to develop his own geometric constructions. He completed more than 100 of these distinctive paintings of layered, precise geometrical shapes during the last decade of his life.

Critics and art historians have noted that Johnson showed little interest in the technical details of painting. Eschewing convention, Johnson instead preferred to use house paints from a local hardware store and to paint on the rough side of small pieces of Masonite instead of canvas—though he did on occasion both use the smoother side and complete some larger works. Although other contemporary

painters such as such as Piet Mondrian, Josef Albers, Alexander Calder, Richard Anuszkiewicz, and Ad Reinhardt (who was a close friend) also used mathematical ideas and geometric shapes, Johnson differed from them in that he linked his geometric paintings with specific mathematicians and he delved into researching and understanding the mathematical ideas that he found inspiring.

Among the earliest of these paintings is this month's cover art, *Mystic Hexagon* (*Pascal*), which Johnson based on a theorem devised by 16-year-old Blaise Pascal in 1640. In essence, Pascal had postulated that if the opposite sides of an irregular hexagon inscribed in a circle are extended, they meet in 3 points that lie on a straight line. In his depiction of Pascal's work, Johnson positioned the circle and cream-colored hexagon near the center of the painting. Overlapping wedges of green, blue, and gray form the different pairs of lines. He did not paint the line that would serve to join the 3 intersections (now dubbed the Pascal Line), but the right edge of the painting fulfills that function.

Pascal, like Johnson, was intrigued by numbers, and he made notable contributions to mathematics and science. He is credited with laying the foundation for probability theory through a series of letters he exchanged with Pierre de Fermat. The pair pondered a problem related to expected outcomes in a dice game that vexed an acquaintance who gambled professionally. That correspondence is credited with developing a fundamental theory of probability—the branch of mathematics concerned with analyzing random, or seemingly random, phenomena—with its roots in Pascal's "Treatise on the Arithmetical Triangle."

Similar to Pascal's geometrical extrapolations as depicted in Johnson's painting, mathematical extrapolations of data have long provided essential information to aid public health officials with decision making. An early example is that of Daniel Bernoulli, who in 1766 used the then relatively new method of calculus to estimate that smallpox elimination via routine vaccination would reduce the risk of death by age 25 years from ≈57% to 50%. Ronald Ross's model on malaria transmission, first introduced in 2 reports published in 1908 and 1911, is a particularly important example of such modeling for public health decision making. Versions of that model are still used today to inform critical public health decision making regarding malaria control.

Today, mathematical models have become essential tools for public health officials, providing estimates of disease burden, potential impact of interventions, and duration of disease outbreaks. They are particularly useful in situations for which little or no data exist, such as estimates of number of cases of disease in the future, or potential impact (benefit) of a yet-to-be-licensed vaccine. In such situations, mathematical modelers typically use data from different sources, along with assumptions about the underlying transmission, to build (or extrapolate) models to provide estimates for the current problem. Such mathematical models have, with the advent of more powerful and cheap computing capabilities, become ever more diverse in methods and degrees of complexity. Mathematical models of infectious disease can now range from the simple, such as the two-dimensional representation found in Johnson's painting, to large multidimensional models that simulate the daily contacts between individuals within a community and the resultant risk for onward transmission of infectious disease.

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- Fatal Infection with Murray Valley Encephalitis Virus Imported from Australia to Canada, 2011

- Low Circulation of Zika Virus, Cambodia, 2007–2016

- Biofilm-Forming Capability of Highly Virulent and Resistant *Candida auris*

- Norovirus GII.17 Natural Infections in Rhesus Monkeys, China

- Characteristics of US Travelers to Zika Virus–Affected Countries in the Americas, March 2015–October 2016

- Oral Transmission of L-Type Bovine Spongiform Encephalopathy Agent among Cattle

- Persistent Infections with Diverse Co-Circulating Astroviruses in Pediatric Oncology Patients, Memphis, Tennessee USA

- Determination of *Elizabethkingia* Diversity by MALDI-TOF Mass Spectrometry and Whole-Genome Sequencing

- Fatal Novel *Emmonsia* sp. Infection with Fungemia after Orthotopic Liver Transplantation

- Cerebrospinal Fluid Findings in an Adult with Human Metapneumovirus-Associated Encephalitis

- Reoccurrence of Avian Influenza A(H5N2) Virus Clade 2.3.4.4 in Wild Birds, Alaska, USA, 2016

- Novel Reassortant Clade 2.3.4.4 Avian Influenza A(H5N8) Virus in Wild Aquatic Birds, Russia, 2016

- Outbreak of Infection with *Legionella pneumophila* Serogroups 1 and 13, Japan, 2015

- *Diphyllobothrium nihonkaiense* Tapeworm Larvae in Salmon, North America

- Hepatitis E Virus Infection in Solid Organ Transplant Recipients, France

Complete list of articles in the February issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

February 6–8, 2017
American Society for Microbiology
2017 Biothreats
Washington, DC USA
https://www.asm.org/index.php/
biothreats-2017

February 13–16, 2017
CROI
Conference on Retroviruses and
Opportunistic Infections
Seattle, WA, USA
http://www.croiconference.org/

March 29–31, 2017
SHEA
Society for Healthcare
Epidiemiology of America
St Louis, MO, USA
http://www.shea-online.org/

April 22–27, 2017
ECCMID
European Congress of Clinical
Microbiology and Infectious Diseases
Vienna, Austria
http://www.eccmid.org/

June 1–5, 2017
ASM
American Society for Microbiology
New Orleans, LA, USA
http://www.showsbee.com/fairs/
25161-ASM-Microbe-2017.html

June 19–21 2017
Transmission of Respiratory Viruses
Harbour Grand Hong Kong
https://transmission2017.med.hku.hk/
mass_email.html

March 1–4, 2018
18th International Congress
on Infectious Diseases (ICID)
Buenos Aires, Argentina
http://www.isid.org/icid/

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 75% passing score) and earn continuing medical education (CME) credit, please go to http://www.medscape.org/journal/eid. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the "Register" link on the right hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/about-ama/awards/ama-physicians-recognition-award.page. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for AMA PRA Category 1 Credits™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Epidemiology of Hospitalizations Associated with Invasive Candidiasis, United States, 2002–2012

CME Questions

1. You are advising a large hospital about anticipated needs regarding cases of invasive candidiasis (IC). According to the analysis of inpatient hospitalization records from the Healthcare Cost and Utilization Project (HCUP) by Strollo and colleagues, which of the following statements about rates of IC-associated hospitalizations in the United States from 2002–2012 is correct?

A. Average annual age-adjusted rate of IC-associated hospitalizations in the United States from 2002–2012 was 5.3 hospitalizations per 100,000 population
B. Age-adjusted annual rates decreased significantly from 2005–2012 among men but not among women
C. Among all 50 states, there was significant, marked variation in state-specific rates of IC
D. Age-adjusted annual rates decreased significantly from 2005–2012 among adults 50 to 65 years old, but not in other age groups

2. According to the analysis of inpatient hospitalization records from HCUP by Strollo and colleagues, which of the following statements about risk factors for rates of IC-associated hospitalizations in the United States from 2002–2012 is correct?

A. Children were at highest risk for IC-associated hospitalization

B. Adults 65 years or older were at highest risk, with risk peaking among adults older than 80 years
C. Age groups with the highest rates of IC-associated hospitalizations in this study differ from those of previous reports from population-based surveillance for candidemia
D. Blacks had a 4-fold higher incidence of IC-associated hospitalization

3. According to the analysis of inpatient hospitalization records from HCUP by Strollo and colleagues, which of the following statements about healthcare utilization, complications, and costs of IC-associated hospitalizations in the United States from 2002–2012 is correct?

A. Median length of hospital stay was 21 days overall, with a significant increase during the study period
B. An estimated 12% of patients died during hospitalization
C. There were 97% of IC-associated hospitalizations that were coded as disseminated candidiasis, 3% as candidal endocarditis, and 1% as candidal meningitis
D. Median costs varied more by patient sex than by survival status

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

Analysis of Anthrax Immune Globulin Intravenous with Antimicrobial Treatment in Injection Drug Users, Scotland, 2009–2010

CME Questions

1. What should you consider regarding the response to severe anthrax disease and the application of anthrax immune globulin intravenous (AIG-IV)?

A. Antitoxin agents are most effective as monotherapy
B. AIG-IV is stored as part of the Strategic National Stockpile
C. AIG-IV is derived from bovine hosts
D. AIG-IV has been tested for efficacy among humans

2. Which of the following variables was most associated with receiving AIG-IV among patients in the current study?

A. Higher risk for mortality
B. Male sex
C. Presenting originally with signs of systemic infection
D. Weak microbiologic evidence of infection

3. Which of the following statements regarding the treatment of patients with systemic anthrax in the current study is most accurate?

A. AIG-IV promoted angioedema in approximately 30% of treated patients
B. AIG-IV was associated with increased rates of hypotension

C. Patients treated with AIG-IV were more likely to undergo surgery
D. Patients treated with AIG-IV received less antibiotics

4. Which of the following statements regarding outcomes of treatment with AIG-IV in the current study is most accurate?
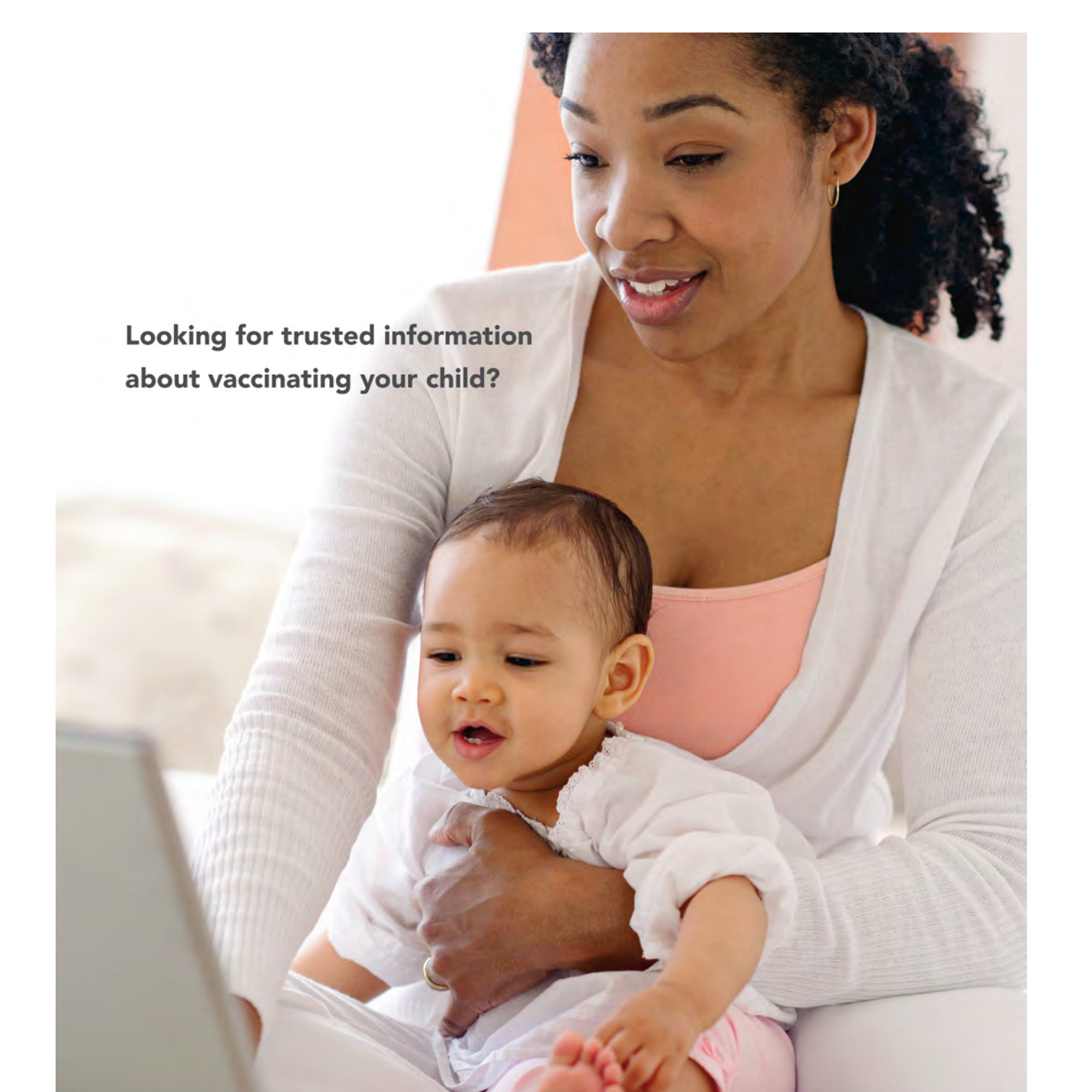
A. AIG-IV was associated with a broad survival benefit
B. AIG-IV reduced the risk for mortality only among severely ill patients
C. AIG-IV reduced the risk for mortality only among individuals with moderate illness
D. Treatment with AIG-IV was associated with longer stays in the intensive care unit and hospital

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

Looking for trusted information about vaccinating your child?

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.

U.S. Department of Health and Human Services
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Summary of Authors' Instructions

Author's Instructions. For a complete list of EID's manuscript guidelines, see the author resource page: http://wwwnc.cdc.gov/eid/page/author-resource-center.

Manuscript Submission. To submit a manuscript, access Manuscript Central from the Emerging Infectious Diseases web page (www.cdc.gov/eid). Include a cover letter indicating the proposed category of the article (e.g., Research, Dispatch), verifying the word and reference counts, and confirming that the final manuscript has been seen and approved by all authors. Complete provided Authors Checklist.

Manuscript Preparation. For word processing, use MS Word. Set the document to show continuous line numbers. List the following information in this order: title page, article summary line, keywords, abstract, text, acknowledgments, biographical sketch, references, tables, and figure legends. Appendix materials and figures should be in separate files.

Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Use terms as listed in the National Library of Medicine Medical Subject Headings index (www.ncbi.nlm.nih.gov/mesh).

Text. Double-space everything, including the title page, abstract, references, tables, and figure legends. Indent paragraphs; leave no extra space between paragraphs. After a period, leave only one space before beginning the next sentence. Use 12-point Times New Roman font and format with ragged right margins (left align). Italicize (rather than underline) scientific names when needed.

Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

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 50 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 in the main body of the text or include more than 50 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (not to exceed 150 words), a 1-line summary of the conclusions, and a brief biographical sketch of first author or of both authors if only 2 authors. This section comprises case series papers and concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome. If detailed methods are included, a separate section on experimental procedures should immediately follow the body of the text.

Research. Articles should not exceed 3,500 words and 50 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 50 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.

Research Letters Reporting Cases, Outbreaks, or Original Research. EID publishes letters that report cases, outbreaks, or original research as Research Letters. Authors should provide a short abstract (50-word maximum), references (not to exceed 10), and a short biographical sketch. These letters should contain no more than 850 words (including the abstract) and may include either 1 figure or 1 table. Do not divide Research Letters into sections.

Letters Commenting on Articles. Letters commenting on articles should contain a maximum of 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication.

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.

In This Issue

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Dispatches