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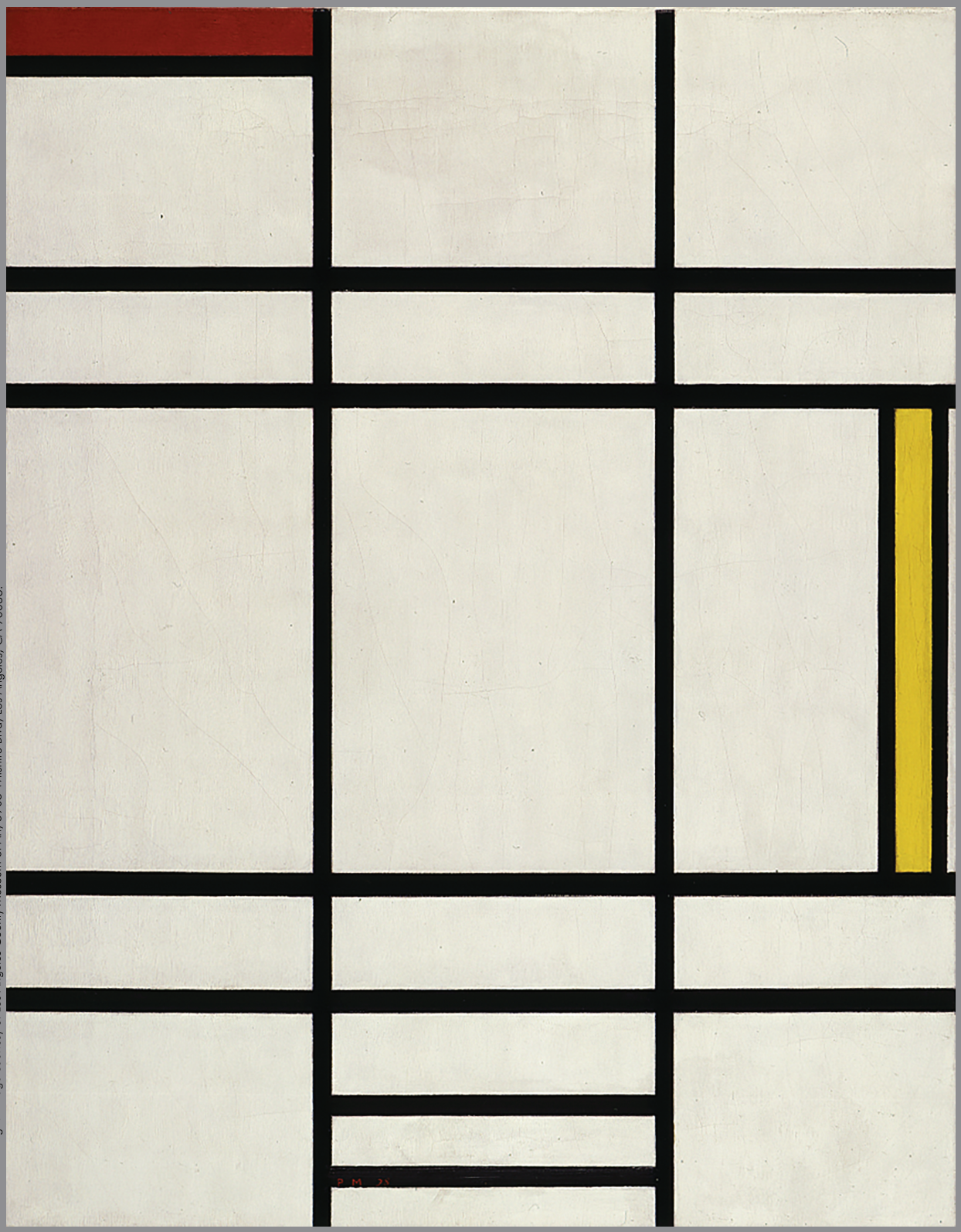

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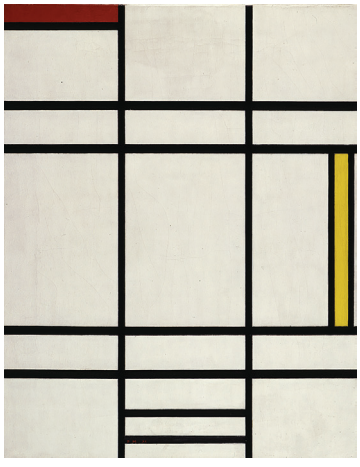

On the Cover

Piet Mondrian (1872–1944), Composition in White, Red, and Yellow (1936).

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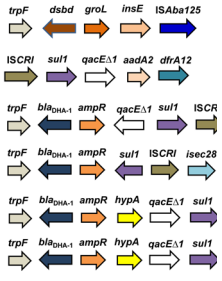
About the Cover p. 1863

Synopses



p. 1758

p. 1772

Research

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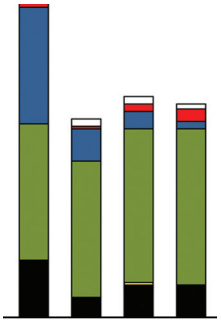


p. 1786

Dispatches

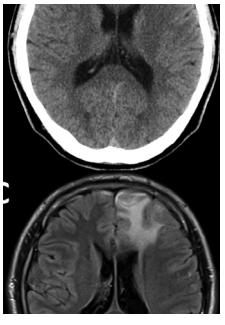
p. 1795

EMERGING INFECTIOUS DISEASES®
October 2016

Letters

p. 1831

p. 1847



Books and Media

About the Cover

Etymologia

Corrections

Outbreaks of Human *Salmonella* Infections Associated with Live Poultry, United States, 1990–2014

Colin Basler, Thai-An Nguyen, Tara C. Anderson, Thane Hancock, Casey Barton Behravesh

Backyard poultry flocks have increased in popularity concurrent with an increase in live poultry–associated salmonellosis (LPAS) outbreaks. Better understanding of practices that contribute to this emerging public health issue is needed. We reviewed outbreak reports to describe the epidemiology of LPAS outbreaks in the United States, examine changes in trends, and inform prevention campaigns. LPAS outbreaks were defined as ≥2 culture-confirmed human *Salmonella* infections linked to live poultry contact. Outbreak data were obtained through multiple databases and a literature review. During 1990–2014, a total of 53 LPAS outbreaks were documented, involving 2,630 illnesses, 387 hospitalizations, and 5 deaths. Median patient age was 9 years (range <1 to 92 years). Chick and duckling exposure were reported by 85% and 38% of case-patients, respectively. High-risk practices included keeping poultry inside households (46% of case-patients) and kissing birds (13%). Comprehensive One Health strategies are needed to prevent illnesses associated with live poultry.

Salmonella species are zoonotic bacteria found in the intestinal tract of many animals, including cattle, pigs, horses, other mammals, reptiles, amphibians, and poultry (e.g., chickens, ducks, geese, and turkeys) (*1*). Nontyphoidal salmonellosis causes an estimated 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths annually in the United States (*2*). *Salmonella* infection typically manifests as acute gastroenteritis that develops 12–72 hours after exposure. Young children, persons ≥65 years of age, and immunocompromised persons are at greater risk for serious complications, including septicemia, joint or brain infections, and death (*3*).

Although *Salmonella* is commonly transmitted through food, recent outbreaks have highlighted direct or indirect contact with animals as a frequent route of transmission (*4*). An estimated 11% of all *Salmonella* infections are attributed to animal exposure annually, with the highest rates of illness and death occurring among children (*1*). Since 2007, numerous outbreaks of human *Salmonella* infections linked to contact with animals and their environments have been investigated, including those involving contact with turtles, bearded dragons, African dwarf frogs, hedgehogs, and backyard poultry (*5*). Poultry can be persistent subclinical shedders and can appear healthy while shedding *Salmonella* bacteria (*6*). Zoonotic salmonellosis outbreak investigations require a One Health approach because they occur at the intersection of human and animal health (*7*).

In the United States, live poultry–associated salmonellosis (LPAS) outbreaks have been documented since 1955 (*8*). Historically, these outbreaks involved young children, occurred in the spring months around Easter, and were associated with birds obtained as pets (*9*). Baby poultry were often dyed bright colors, making them more attractive to young children. Currently, public health officials are identifying LPAS outbreaks linked to backyard poultry flocks that are affecting adults and children. Most of these outbreaks begin in the spring but continue over many months. The first multistate outbreak where the association with backyard flocks was recognized occurred in 2007 (*10*). Since that time, the popularity of backyard flocks has increased substantially (*11*). Most chicks sold for backyard flocks are produced by a network of mail-order hatcheries (*9*). Disease control guidance for hatcheries is provided by the US Department of Agriculture National Poultry Improvement Plan, which is a voluntary state, federal, and industry cooperative program aimed at eliminating certain diseases from poultry breeding flocks and hatcheries (*12*). We reviewed outbreak reports from 1990–2014 to describe the epidemiology of LPAS outbreaks in the United States, to identify changes in trends, and to identify practices of concern among case-patients to better inform future prevention campaigns.

Methods

We defined LPAS outbreaks as ≥2 culture-confirmed human *Salmonella* infections in the United States with a combination of epidemiologic, laboratory, or traceback evidence linking illnesses to live poultry contact. Data sources included PulseNet, the national molecular subtyping network for foodborne disease surveillance in the United States; the Centers for Disease Control and Prevention (CDC) Outbreak Response and Prevention Branch's outbreak management database; and CDC's National

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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Outbreak Reporting System (*13–15*). Through these data sources, we collected outbreak summaries from 50 states and 4 US territories. Additionally, we conducted a literature review to identify any additional LPAS outbreaks that had not been reported to CDC. In January 2015, we searched PubMed without date or language restrictions and used combinations of the terms "salmonella," "salmonellosis," "outbreak," "poultry," and "United States." To avoid including duplicate reports, we further reviewed outbreaks that occurred in the same year and for which identical *Salmonella* serotypes were reported.

A standardized live poultry exposure questionnaire was developed by officials at CDC, state and local health departments, and the National Poultry Improvement Plan. The questionnaire focused on patient demographics, baby and adult poultry contact, poultry purchases, flock management, and *Salmonella* awareness. The questionnaire was created in 2008, and since its creation, it has been administered to case-patients (or their parents/guardians) who were part of 21 multistate outbreak investigations during 2008–2013. To identify common patient characteristics and practices that might have increased the risk for *Salmonella* transmission from poultry to humans, we analyzed the results of these questionnaires by using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

A total of 53 LPAS outbreaks were documented in the United States during 1990–2014 (Table 1); these 53 outbreaks were associated with 2,630 illnesses, 387 hospitalizations, and 5 deaths (Figure 1). Median outbreak size was 26 case-patients (range 4–363 case-patients). Approximately 77% (41/53) of outbreaks were multistate outbreaks.

The number of LPAS outbreaks reported annually has increased substantially in recent years (Figure 1). During 1990–2005, a total of 17 outbreaks were documented (1.06/year), with a median size of 12 case-patients per outbreak (range 4–53 case-patients). In comparison, during 2006–2014, a total of 36 outbreaks were documented (4/year), with a median size of 41 case-patients per outbreak (range 4–363 case-patients). The number of LPAS outbreaks peaked in 2012, with a total of 8 individual outbreaks. The 4 largest outbreaks occurred during 2012–2014.

Reported outbreak onset dates ranged from January to July. Most (80%) outbreaks began in the months of February, March, and April. Outbreak duration ranged from 1 to 12 months, with an average duration of 4.9 months.

Montevideo was the serotype identified in 36% (19/53) of LPAS outbreaks, making it the most common *Salmonella* serotype reported. Among the *Salmonella* strains associated with LPAS outbreaks, 62% (38/61) were serogroup C_1; serogroup B accounted for 16% (10/61), and serogroup C_2 accounted for 13% (8/61). Serogroups C_3, D_1, and R were also reported. Additionally, 4 outbreaks consisted of multiple *Salmonella* serotypes. In the outbreaks with available information, 54% (1,026/1,898) of case-patients were male, and 46% (872/1,898) were female. Median case-patient age was 9 years (range ≤1 to 92 years); 31% (467/1,488) of case-patients were ≤5 years of age, and 42% (628/1488) were ≤10 years of age (Figure 2).

A total of 62% (511/822) of case-patients reported exposure to baby poultry (Table 2). Chick exposure was reported by 85% (434/511) of case-patients and duckling exposure by 38% (195/511). Among case-patients exposed to baby poultry, 62% reported exposure to only chicks (316/511), 15% (77/511) exposure to only ducklings, and 22% (118/511) exposure to both chicks and ducklings. Approximately 23% (117/582) of respondents reported contact with adult poultry. Among all outbreaks, the median time between purchase of poultry and illness onset was 17 days (range 1–672 days). Approximately 66% of case-patients reported <30 days between obtaining poultry and illness onset. However, 7% of case-patients reported ≥60 days between obtaining poultry and illness onset.

Among respondents with baby poultry exposure, 74% (276/373) reported that exposure occurred at the home. Approximately 76% (303/400) of respondents reported touching baby birds, 61% (227/373) reported touching the cage/coop of the baby birds, 49% (196/400) reported snuggling baby birds, and 13% (53/400) reported kissing baby birds.

Nearly 46% (188/413) of respondents reported keeping poultry inside the house. Of these, 22% (41/188) reported keeping live poultry in the living room, 12% (22/188) in the kitchen, 10% (18/188) in a bedroom, and 10% (18/188) in a bathroom. Approximately 52% of respondents reported owning poultry for <1 year. When asked if they were aware of a connection between poultry contact and *Salmonella*, 58% (167/290) of respondents reported that they were aware of the risk.

Discussion

The number of LPAS outbreaks reported annually has increased substantially in recent years. Because only a small proportion of *Salmonella* infections are diagnosed and reported to public health departments, the actual number of illnesses in these outbreaks might be much larger with an estimated 29 additional infections going unreported for every reported case (*2*). These outbreaks are not only happening with increased frequency but are also generally affecting more persons. In addition, 62% of case-patients reported contact with baby chicks or ducklings, and 45% were ≤10 years of age. This finding is possibly attributable to the fact that children's immune systems are not fully developed and that young children typically have poor hand hygiene practices. Most contact occurred at the patients' home, and high-risk behaviors included keeping poultry

Table 1. Details on live poultry–associated salmonellosis outbreaks, by year, United States, 1990–2014

Year	Serotype	No. illnesses	No. hospitalizations	No. deaths	Month of first illness in outbreak	Outbreak duration, mo	Reference(s)
1991	Hadar	22	4	0	April	2	(*16*)
1995	Montevideo	12	3	0	April	2	(*17*)
1996	Montevideo	11	0	0	April	2	(*17*)
1996	Montevideo	16	2	0	March	4	(*17*)
1999	Infantis	21	3	0	April	2	(*18*,*19*)
1999	Typhimurium	40	3	0	April	2	(*18*)
2000	Infantis	5	2	0	May	1	(*19*)
2000	Montevideo	4	0	0	May	2	
2000	Agona	4	0	0	February		
2000	Montevideo	7	0	0			
2002	Montevideo	21	0	0	March	2	
2003	Thompson	31	4	0	May	2	
2003	Unknown	5	0	0			
2004	Montevideo	4	0	0	March	2	
2004	Typhimurium	18	0	0	March		
2005	Montevideo	53	6	0	April		(*20*)
2005	Ohio	12	0	0			
2006	Typhimurium	14	7	0	May		
2006	I 4,[5],12:i:-	64	7	0	April	6	(*21*)
2006	Montevideo	84	8	0			(*20*,*21*)
2006	Ohio	4	1	0			(*21*)
2007	Montevideo	64	8	0	February	11	(*20*)
2007	Montevideo	65	3	0	March	7	(*22*)
2008	Kiambu	32	0	0	March	4	
2008	Montevideo	12	4	0			(*20*)
2008	Montevideo	66					
2009	Montevideo	96	16	1	January	12	(*20*)
2009	Johannesburg	7	2	0	May	1	(*23*)
2009	Thompson	26	1	0	February	6	(*22*)
2009	Typhimurium	36	7	0	May	4	(*23*,*24*)
2009	Pomona	6	0	0	March		
2009	Montevideo	15	1	0			(*23*)
2010	Typhimurium	54	0	0	May	4	
2010	Montevideo	55	7	0	February	4	(*20*)
2010	Braenderup	7		0	February		
2011	Johannesburg, Altona	96	20	0	February		(*25*,*26*)
2011	Berta	9	1	0	April	3	
2011	Hadar	25	2	0	March	5	
2011	Montevideo	28	2	0	March		(*20*)
2012	Infantis	54	4	0	February	8	
2012	Braenderup	48	6	0	July	6	
2012	Infantis	27	7	0	April	5	
2012	Muenchen	21	1	0	March	6	
2012	Hadar	46	13	0	March	5	(*27*)
2012	Montevideo	93	21	1	February	7	(*28*)
2012	Infantis, Newport, Lille, Thompson	195	34	3	March	6	(*29*,*30*)
2012	Thompson	33	4	0	February	8	
2013	Braenderup	53	1	0	March	5	
2013	Infantis, Mbandaka, Lille, Newport	158	29	0	March	7	(*31*–*33*)
2013	Montevideo	12	0	0	April		
2013	Typhimurium	356	62	0	March	7	(*33*)
2014	Infantis, Newport, Hadar	363	76	0	February	9	
2014	Typhimurium	20	5	0	March	7	
Total		2,630	387	5	March (median)	4.9 mo (median)	

inside the house and having close contact, such as holding, snuggling, or kissing poultry. These findings highlight the need for additional consumer education, especially on the risk for illness in children, the necessity for keeping live poultry outside of the home, and the recommendation to wash hands after coming in contact with live poultry.

Instead of being sold as novelty pets around the Easter holiday, chicks, ducklings, goslings, and turkey poults are now additionally being sold for backyard flocks; these birds can be purchased at agricultural feed stores across the United States. The practice of keeping backyard flocks of live poultry has gained popularity during the past decade

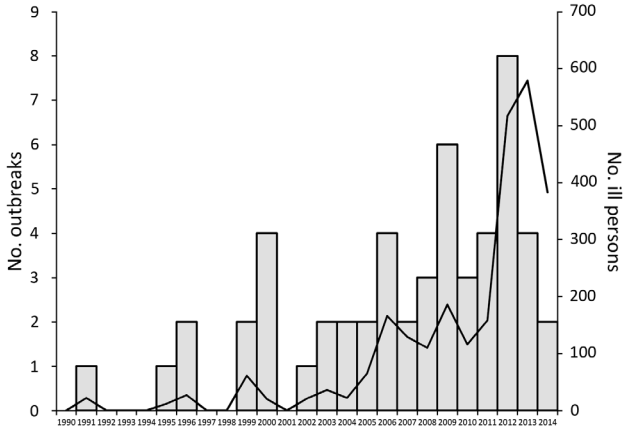

Figure 1. Number of live poultry–associated salmonellosis outbreaks and number of ill persons reported, by year, United States, 1991–2014.

(*11*). This increase is attributable to various reasons, including growing interest in local and organic food production, animal welfare concerns, environmental concerns, the desire to provide a learning experience for children, and the perception that local eggs are healthier and of better quality than store-bought eggs (*34*). In addition, backyard flocks are becoming increasingly common in urban and suburban areas (*11*). The fact that half of respondents to the live poultry questionnaire owned poultry for <1 year could signify that new owners might be unfamiliar with appropriate husbandry practices.

Most poultry sold for backyard flocks are produced by a core group of ≈20 mail-order hatcheries. These hatcheries sell more than 50 million chicks annually, and most distribute chicks nationwide (*20*). Distribution of baby poultry occurs through the US Postal Service, by which a small proportion are mailed directly to owners, whereas most baby poultry are sold to agricultural feed stores. Baby poultry are shipped in cardboard boxes that can contain 120 chicks, 60 ducklings, 32 goslings, or 80 turkey poults. One box may contain multiple species, and shipment can provide ample opportunity for cross-contamination. Increased shedding of *Salmonella* can occur when poultry are subjected to stressful conditions, such as transportation through the mail (*6*). The nationwide distribution as well as the opportunity for cross-contamination might help to explain the multistate distribution of outbreaks.

The serotypes identified in LPAS outbreaks are different from *Salmonella* serotypes, such as Enteritidis and Heidelberg, that are traditionally associated with foodborne poultry outbreaks (*35*,*36*). This finding might be attributable in part to differences in poultry that originate from mail-order hatcheries and commercial poultry hatcheries. Mail-order hatcheries typically operate on a much smaller scale, with more species and breeds of poultry sourced from breeding stock within their own farm or eggs from other mail-order hatcheries, which could explain the diversity of *Salmonella* serotypes identified in these outbreaks. In comparison, commercial poultry operations are typically larger scale, closed operations with 1 species and fewer breeds of bird on site (*37*).

The seasonality of these outbreaks might be attributable to the fact that most agricultural feed stores sell large numbers of chicks during spring or fall promotional events or "chick days." These events provide additional opportunity for cross-contamination in the stores because of the increase in volume of chicks during these events. In addition to the increase in volume of chicks being sold in the spring, some households might keep chicks inside the home because of concerns that the chicks will not do well in cold weather.

Poultry can appear healthy and still shed *Salmonella* bacteria intermittently for extended periods of time (*38*). This intermittent shedding could contribute to the fact that some case-patients reported illness onset >1 year after poultry purchase. In addition, intermittent shedding could partially explain why recent outbreaks have been of a longer duration, some lasting up to 12 months.

The findings of this investigation are subject to several limitations. Smaller, single-state outbreaks might have been missed if they were not reported to National Outbreak Reporting System or not documented elsewhere. Additional outbreaks might have been missed if they were not detected by PulseNet or if, during the course of the investigation, public health practitioners did not ask case-patients about exposure to live poultry. Finally, results of the supplemental poultry questionnaires were only available for multistate outbreaks that occurred in 2008 or later. Case-patients from earlier outbreaks might have had different characteristics and

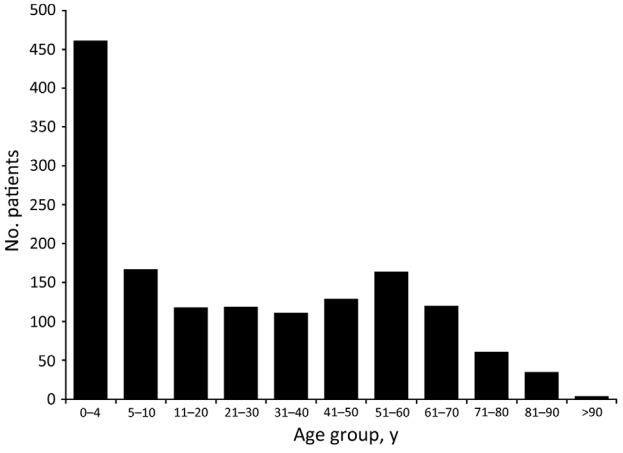

Figure 2. Number of patients in reported live poultry–associated salmonellosis outbreaks, by age group, United States 1991–2014.

had different types of poultry exposure in comparison to case-patients from the more recent outbreaks. Because our study relied on aggregated outbreak data, we could not calculate the relative magnitude of risk for different handling practices; therefore, we are unable to state which practices contribute the most to *Salmonella* transmission from live poultry to humans.

This review highlights the need for an integrated One Health response to LPAS outbreaks. One Health is defined as the collaborative effort of experts in multiple disciplines, including healthcare professionals, veterinarians, epidemiologists, and environmental scientists, working to attain optimal health for humans, animals, and the environment (*7*). Prevention and control efforts for LPAS outbreaks include interventions that target hatcheries, agricultural feed stores, health professionals, and consumers. Detailed recommendations for a comprehensive One Health prevention approach are available (*9*).

To prevent future outbreaks, the general public needs to be educated about the risk for LPAS. Persons need to be aware that healthy poultry can shed *Salmonella* intermittently, that persons need to wash their hands after contact with live poultry, that young children are at an increased risk for salmonellosis, and that poultry should never be allowed inside the house. Mail-order hatcheries, agricultural feed stores, public health officials, local and federal departments of agriculture, pediatricians, and veterinarians can all help to spread awareness about the association between live poultry and *Salmonella* infections. CDC has developed various educational resources that mail-order hatchery Web sites can link to (Figure 3). Posters and additional educational material can be displayed at points of sale (*39*). CDC has participated in a series of online consumer educational webinars with the US Department of Agriculture and other poultry interest groups (*40*). Healthcare providers can talk to parents about the risk for zoonotic *Salmonella* in children, especially if high-risk pets are in the home (*41*,*42*). The Journal of the American Veterinary Medical Association recently reported on the increased need for veterinarians who are willing to treat backyard poultry (*43*). Veterinarians have a unique opportunity to educate poultry owners about *Salmonella* prevention and control strategies (*9*).

Figure 3. A "Tips to Stay Healthy around Backyard Poultry Flocks" web graphic produced by the Centers for Disease Control and Prevention.

Poultry are acquiring a new position in many households. Instead of being treated as production animals, they are increasingly being considered household pets. However, recurring LPAS outbreaks highlight the need for strategies to prevent human illnesses associated with live poultry contact through a comprehensive One Health approach involving human, animal, and environmental health.

Table 2. Selected exposure characteristics of patients in 21 multistate live poultry–associated salmonellosis outbreaks, United States, 2008–2013*

Characteristic	No. (%) patients
Type of poultry, n = 822	
Adult poultry	161 (20)
Baby poultry	511 (62)
Chicks only	316 (62)
Ducklings only	77 (15)
Chicks and ducklings	118 (23)
Contact location, n = 413	
Indoors	188 (46)
Living room	41 (11)
Basement	57 (15)
Kitchen	22 (12)
Bedroom	18 (10)
Bathroom	18 (10)
Utility room/laundry room	17 (9)
Other indoor	44 (23)
Type of contact, n = 400	
Touched	303 (76)
Held/snuggled	196 (49)
Kissed	53 (13)

*n values indicate number of respondents for each question.

Acknowledgments

We would like to thank the mail-order hatchery and feed story industries, many state and local health departments, departments of agriculture, the US Department of Agriculture, and the US Centers for Disease Control and Prevention staff for their contributions to this public health issue.

Dr. Basler completed this analysis while he was an Epidemic Intelligence Service officer through the Centers for Disease Control and Prevention. Currently, he is working as a veterinary epidemiologist with the Outbreak Response and Prevention Branch of the Division of Foodborne, Waterborne, and Environmental Diseases, National Center for Emerging and Zoonotic Infectious Diseases, CDC. His primary research interests include foodborne and enteric zoonotic outbreak investigations.

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Address for correspondence: Colin Basler, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop A38, Atlanta, GA 30329-4027, USA; email: cbasler@cdc.gov

Vaccine-Derived Polioviruses and Children with Primary Immunodeficiency, Iran, 1995–2014

Mohammadreza Shaghaghi,[1] Shohreh Shahmahmoodi,[1] Hassan Abolhassani, Saeed Soleyman-jahi, Leila Parvaneh, Sussan Mahmoudi, Zahra Chavoshzadeh, Reza Yazdani, Seyed Mohsen Zahraei, Mohsen Ebrahimi, Mohammad H. Eslamian, Hamideh Tabatabaie, Maryam Yousefi, Yaghoob M. Kandelousi, Aliasghar Oujaghlou, Nima Rezaei, Asghar Aghamohammadi

Widespread use of oral poliovirus vaccine has led to an ≈99.9% decrease in global incidence of poliomyelitis (from ≈350,000 cases in 1988 to 74 cases in 2015) and eradication of wild-type poliovirus serotypes 2 and 3. However, patients with primary immunodeficiency might shed vaccine-derived polioviruses (VDPVs) for an extended period, which could pose a major threat to polio eradication programs. Since 1995, sixteen VDPV populations have been isolated from 14 patients with immunodeficiency in Iran. For these patients, vaccine-associated paralysis, mostly in >1 extremity, was the first manifestation of primary immunodeficiency. Seven patients with humoral immunodeficiency cleared VDPV infection more frequently than did 6 patients with combined immunodeficiencies. Our results raise questions about manifestations of VDPVs in immunodeficient patients and the role of cellular immunity against enterovirus infections. On the basis of an association between VDPVs and immunodeficiency, we advocate screening of patients with primary immunodeficiency for shedding of polioviruses.

In 1988, the World Health Assembly of the World Health Organization resolved to eradicate poliomyelitis through the introduction of vaccination on a worldwide scale as the main tool against transmission of poliovirus (*1*,*2*). Endemic poliomyelitis has been eliminated from most parts of the world by vaccination with live attenuated oral poliovirus vaccine (OPV) and inactivated poliovirus vaccine (IPV) (*3*). The annual incidence of wild-type poliomyelitis has decreased by ≈99.9% from ≈350,000 cases in 1988 to 74 cases in 2015, and only 2 countries, Afghanistan and Pakistan, still have transmission of wild-type poliovirus (*2*).

As the polio eradication program is proceeding, emergence of vaccine-derived polioviruses (VDPVs) could be a major threat to the success of current strategies. VDPVs are variants of 3 OPV serotypes (PV1, PV2, and PV3), which during prolonged intestinal replication show >1% (PV1 and PV3) or >0.6% (PV2) nucleotide divergence in the viral protein 1 (VP1) coding region (*4*). Only a small number of genetic mutations are responsible for reduced neurovirulence in OPV strains (*5*). Revertant vaccine viruses with increased neuropathogenicity might cause vaccine-associated paralytic poliomyelitis (VAPP) in OPV recipients or unimmunized contacts. VAPP is the most common adverse event after OPV administration (*6*). Because of impaired systemic or mucosal immunity, patients with primary immunodeficiencies are at a markedly increased risk for VAPP (*6*). In addition, patients with primary immunodeficiencies might shed VDPVs (immunodeficiency-associated VDPV [iVDPV]) in stool samples for an extended period after exposure to OPV strains (*7*). Vaccine-derived polioviruses might also circulate in the community (circulating VDPV) through person-to-person transmission and occasionally cause symptomatic infections in immunodeficient or healthy persons (*6*).

In recent years, several studies have focused on environmental detection of VDPVs and their community circulation. Some studies investigated VDPVs in immunodeficient persons, which lead to identification of ≈100 cases

Author affiliations: Tehran University of Medical Sciences, Tehran, Iran (M. Shaghaghi, S. Shahmahmoodi, H. Abolhassani, S. Soleyman-jahi, L. Parvaneh, M. Ebrahimi, M.H. Eslamian, N. Rezaei, A. Aghamohammadi); Universal Scientific Education and Research Network, Tehran (M. Shaghaghi, H. Abolhassani, S. Soleyman-jahi, R. Yazdani, N. Rezaei, A. Aghamohammadi); Research Center for Immunodeficiences, Tehran (M. Shaghaghi, H. Abolhassani, N. Rezaei, A. Aghamohammadi); Tehran University of Medical Sciences School of Public Health, Tehran (S. Shahmahmoodi, H. Tabatabaie, M. Yousefi, Y.M. Kandelousi, A. Oujaghlou); Ministry of Health and Medical Education, Tehran (S. Mahmoudi, S.M. Zahraei); Shaheed Beheshti Medical University, Tehran (Z. Chavoshzadeh); Isfahan University of Medical Sciences School of Medicine, Isfahan, Iran (R. Yazdani); Children's Medical Center, Tehran (M. Ebrahimi, M.H. Eslamian)

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[1]These authors contributed equally to this article.

of infection with iVDPV worldwide to date (*1,8*). We have previously reported several cases of infection with iVDPV in Iran and emphasized the need for surveillance of iVDPV (*9–13*). We report newly identified persons who shed iVDPV and review additional characteristics of cases reported.

Objectives

In this study, we report clinical characteristics of 14 VAPP patients and virologic properties of their iVDPV isolates. All patients were identified in Iran during 1995–2014. We sought to determine relationships between clinical features of underlying immunodeficiency disorders, VDPV manifestations, and patient outcomes.

Study Design

Patients in Iran with acute flaccid paralysis (AFP) were tested for shedding of polioviruses in stool specimens during national AFP surveillance up to the end of 2014. Stool specimens were collected at the earliest convenience within 48 hours after onset of paralysis. Fecal shedding of vaccine virus strains was simultaneously investigated in close contacts of patients.

Specimens were processed at the Iranian National Polio Laboratory (Tehran, Iran). Virus isolation, serotype identification, and intratypic differentiation were performed by using the World Health Organization protocol for poliovirus detection in stool specimens (*14*). Screening for VDPV shedding was performed by using an ELISA, reverse transcription PCR, or real-time reverse transcription PCR. Genome sequencing of isolates was conducted at the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA). The VP1 divergence rate was defined as the value (percentage) of VP1 divergence divided by years of virus replication from the original Sabin strain. To calculate this rate, we considered the interval between first OPV administration and date of the most recent isolation of iVDPVs as the duration of virus replication.

Clinical and laboratory data were obtained from the national primary immunodeficiency registry and AFP surveillance database (*15,16*). Patients with undetermined immune status were analyzed to determine serum levels of immunoglobulins and absolute leukocyte and different lymphocyte subpopulation counts. Immunity disorders were defined according to the Expert Committee of the International Union of Immunological Societies (*17*). Agammaglobulinemia, hypogammaglobulinemia, and mu heavy chain disease were considered humoral immunodeficiencies. Combined immunodeficiency (CID) included severe combined immunodeficiency, major histocompatibility complex class 2 deficiency, and Nijmegen breakage syndrome.

We evaluated patients by monthly collection of stool specimens and analysis until patients cleared their infections or died of any cause. Patients were classified into separate subgroups according to types of immunodeficiency (humoral or combined) and whether virus shedding in stool specimens was cleared before death. Patients were considered as having cleared an iVDPV infection if 2 consecutive stool specimens were negative for iVDPVs.

Statistical Analyses

Continuous variables are reported as means ± SD. We used the Shapiro-Wilk normality test to assess distribution of study parameters. We used the χ^2 test to analyze relationships between categorical parameters, the Mann-Whitney U test to compare duration of shedding and VP1 divergence rate between different subgroups, and an independent *t*-test to compare age of onset of VAPP and absolute VP1 divergence. p values <0.05 were considered statistically significant.

Results

Patients and Manifestations

During 1995–2014, a total of 14 patients (12 boys and 2 girls) infected with iVDPV were identified in Iran. Mean ± SD age at onset of VAPP was 12.4 ± 5.8 months for all patients. Mean ± SD age at onset of VAPP was 14.1 ± 4.1 months for patients with humoral immunodeficiency and 10.2 ± 7.5 months for patients with combined immunodeficiency. However, this difference was not significant (p = 0.26). Eight patients had paresis in >1 extremity, and 4 patients had monoparesis. Data were discordant for 2 patients for defining monoparesis or polyparesis.

We analyzed the patients for a mean duration of 18 months; by the end of follow-up, 9 (64.3%) patients had died. As expected, patients with humoral immunodeficiency disorders survived longer than patients with CID (p<0.05). For 5 patients who were alive at the last follow-up (March 2015), mean ± SD age was 4.2 ± 3.3 years. Detailed clinical characteristics for all patients are shown in Table 1, and virologic properties of iVDPVs are shown in Table 2.

Types of Primary Immunodeficiencies

Except for 1 patient who died before immunologic studies were completed (patient 12), all patients with VDPV shedding had primary immunodeficiency. Seven patients had humoral immunodeficiencies and 6 patients had CIDs (Table 3). Further investigations and genetic analysis were performed for 8 patients to identify the genes responsible for immunodeficiency. Four patients had mutations in the Bruton tyrosine kinase gene, and a final diagnosis of X-linked agammaglobulinemia was established. Low expression of human leukocyte antigen DR on B lymphocytes indicated major histocompatibility complex class 2

Table 1. Clinical characteristics for 14 patients infected with iVDPV, Iran, 1995–2014*

Patient no.	Age, mo, at VAPP onset/sex	No. doses OPV	Duration, mo, from last OPV dose to VAPP onset	Duration from VAPP onset to death†	Site of paralysis
1	17.4/F	0	NA	8 d	NA
2	7.5/M	4	1.1	4 mo	Left leg, right leg, right arm, respiratory muscles
3	10.6/M	4	3.3	1 mo	Left leg, right leg, right arm
4	15.1/M	4	9	11 mo	Left leg, right leg
5	5.3/F	2	3.2	<1 mo	Left leg, right leg
6	20.2/M	4	1.1	Alive	Right leg
7	6.2/M	3	2	28 mo	Right leg
8	15.7/M	4	9.2	Alive	Left leg, right leg, left arm
9	25.2/M	4	6.7	<1 mo	Left leg
10	6.6/M	1	6.6	2 mo	Left leg, right leg
11	11.4/M	3	5.3	Alive	Left leg
12	13.1/M	2	7	18 d	Not available
13	10.0/M	4	3	Alive	Left leg, right leg, left arm
14	9.0/M	4	2.8	Alive	Left leg, right leg

*iVDPV, immunodeficiency-associated vaccine-derived poliovirus; NA, not applicable (patient 1 did not receive any OPV): OPV, oral poliovirus vaccine; VAPP, vaccine-associated paralytic poliomyelitis.
†Five patients were alive at the most recent follow-up. In March 2015, patient 6 was 9.5 years old, patient 8 was 5 years old, patient 11 was 3.5 years old, and patients 13 and 14 were 1.5 years old.

deficiency in patient 2 (*12*). Patient 3 had severe combined immunodeficiency caused by a mutation in recombination activating gene 2 (R229W) (N. Parvaneh, unpub. data). Mutations in Nijmegen breakage syndrome 1 confirmed a diagnosis of Nijmegen breakage syndrome in patient 7, and mu heavy chain genes confirmed mu heavy chain disease in patient 11 (H. Abolhassani, unpub. data).

Temporality of VAPP with Diagnosis of Primary Immunodeficiency

Primary immunodeficiencies in patients were detected mostly after onset of paralysis (11 of 14 patients). Only 3 patients had been given a diagnosis of primary immunodeficiency before onset of VAPP. Patient 1 was screened and given a diagnosis of primary immunodeficiency at birth. Patients 9 and 10 received *Mycobacterium bovis* BCG vaccine and a first dose of OPV at birth. These 2 patients had multiple BCG-adenitis and respiratory infections at 10 months and 4 months of age, respectively, which led to a diagnosis of primary immunodeficiency. Despite initiation of immunoglobulin replacement, they subsequently had VAPP.

VDPV Characteristics in Primary Immunodeficiency Patients

Sixteen iVDPV populations were isolated from the 14 patients; 2 patients were simultaneously shedding iVDPV types 1 and 2. Twelve of 16 viruses isolated were serotype 2, three were serotype 1, and 1 was serotype 3 (Table 2). Overall mean ± SD VP1 divergence was 1.88% ± 0.79%. No differences in values for VP1 divergence were observed between the 2 subgroups of humoral immunodeficiency and combined immunodeficiency patients ($p = 0.58$). This divergence was also comparable in both subgroups of patients who stopped shedding virus or continued to shed virus ($p = 0.75$).

The mean ± SD VP1 divergence rate was 1.8% ± 1.1% (range 0.6%–4.3%) per year. Similar to absolute VP1 divergence, the VP1 divergence rate was comparable between 2 subgroups of primary immunodeficiency patients ($p = 0.61$).

Shedding Duration and Clearing Infection in Primary Immunodeficiency Categories

Duration of iVDPV isolation for the patients ranged from 3 days to 15 months (median 1 month). Seven patients stopped shedding iVDPV. Seven other patients shed iVDPVs in their last stool specimen before death. If we excluded patient 12, who did not have a definite primary immunodeficiency category, 6 (85.7%) of 7 patients with predominantly humoral immunodeficiencies stopped shedding VDPV, but only 1 (16.7%) of 6 CID patients cleared the infection before death. This association observed between type of primary immunodeficiency and rate of clearing VDPV infection was statistically significant ($p = 0.013$). Patients with humoral immunodeficiencies cleared VDPV infection more frequently than CID patients (Table 2).

Duration of shedding after VAPP onset was similar in both subgroups of patients with humoral immunodeficiency and combined immunodeficiency ($p = 0.66$). Analysis also showed a trend of longer shedding duration in patients who stopped shedding iVDPV than in patients who shed the virus until death ($p = 0.07$).

Discussion

We analyzed 14 iVDPV patients identified in Iran during 1995–2014. Detailed characteristics of patients 1–7 have been described (*9–13*); among these patients, only patient 6 is alive, and this patient has residual paresis. Patient 7, who had AFP at 6 months of age, continued shedding iVDPV2

for 2 months after the most recent published data and then stopped shedding virus. He died at 34 months of age from pneumonia. Among the 7 newly identified case-patients, patient 12 died before he underwent specific immunologic investigations. This patient had a history of recurrent infections, and sequencing data showed quasispecies of VDPV2 from multiple lineages, which suggested immunodeficiency for this patient. This patient was identified as an iVDPV patient in CDC annual reports (*18*).

Serotype 2 iVDPV has been the most prevalent serotype detected in immunodeficient patients (64%), followed by serotypes 1 (21%) and 3 (15%) (*18*); our series showed similar findings. We analyzed 2 patients who were simultaneously shedding serotypes 1 and 2 iVDPVs. Concurrent infection with >1 VDPV serotype has been documented in 3 other patients from China and the United States (*4,6,19*). Paralysis in poliomyelitis is usually asymmetric (*6,20*). In contrast, most of our iVAPP patients had bilateral flaccid paresis. Further studies are required to differentiate manifestations of VDPV infection in primary immunodeficiency patients with poliomyelitis caused by wild-type poliovirus.

Patient 1 received only IPV; however, all his contacts had received OPV as routine national vaccination in Iran. Subsequently, he had VAPP and died of an unknown etiology. All other patients in our study had severe complications of live vaccines (BCG and OPV) given at birth. These complications were the earliest manifestations of primary immunodeficiencies in these patients, which further aggravated their course of disease. Use of screening programs to detect immunodeficiencies at birth could prevent administration of live vaccines to primary immunodeficiency patients and their contacts and minimize the burden of adverse complications.

We observed an association between type of primary immunodeficiency and frequency of clearing iVDPV infection; patients with humoral immunodeficiency cleared VDPV infection more frequently than CID patients. Patients with CID have more severe complications and shorter survival times than patients with humoral immunodeficiency (*16,21*). This finding could limit the ability of CID patients to clear infections by use of proper immunoglobulin therapy before death. Although this finding might be an explanation for lower rate of infection clearance in CID patients, comparable durations of virus shedding between 2 subgroups of primary immunodeficiency patients do not support this hypothesis and imply a probable contribution of other mechanisms to this observation.

Another explanation for the relationship observed in our study between type of primary immunodeficiency and rate of clearing iVDPV infection might be differences in inherent capacity of these immunodeficiency disorders to respond to polioviruses or other enterovirus infections. To our knowledge, enteral mucosal immunity against enteroviruses is a prominently antibody-mediated mechanism, and patients with major B-cell dysfunction are at increased risk for poliomyelitis (*6,22*). Nevertheless, our observations suggest other major contributions of cellular immunity to clearance of poliovirus infections. The index case-patient for an outbreak of poliovirus infection in Minnesota, USA, in 2005 had been given a diagnosis of severe combined immunodeficiency. She continued to shed iVDPVs while receiving immunoglobulin therapy for several months and finally stopped shedding virus after a second bone marrow transplant (*23*).

Similarly, regular intravenous immunoglobulin (IVIg) administration could not protect patients 9 and 10 in our study from VAPP, and neither patient cleared the infection.

Table 2. Virologic characteristics for 14 patients infected with iVDPV, Iran, 1995–2014*

Patient no.	Report year	Age, mo, at VAPP onset	Virus shedding duration from VAPP onset	iVDPV serotype	VP1 nt divergence, %†	Cleared infection	No. contacts‡
1	1995	17.4	8 d	2	2.2	No	0
2	2005	7.5	3 mo	2	1.5	No	6 (all negative)
3	2006	10.6	2 wk	2	1.7	No	8 (all negative)
4	2006	15.1	5 mo	3	2	Yes	7 (all negative)
5	2007	5.3	5 d	2	2	No	8 (all negative)
5	2007	5.3	5 d	1	1.7	No	8 (all negative)
6	2007	20.2	3 d	2	1.2	Yes	5 (all negative)
7	2011	6.2	15 mo	2	2	Yes	22 (21 negative; 1: P3 SL)
8	2011	15.7	3.5 mo	2	3.8	Yes	6 (4 negative; 1: P1 SL; 1: P1 SL and P2 SL)
9	2011	25.2	4 d	2	3.3	No	6 (all negative)
9	2011	25.2	4 d	1	1.6	No	6 (all negative)
10	2012	6.6	1.5 mo	2	2.3	No	0
11	2012	11.4	2 wk	2	1.5	Yes	6 (all negative)
12	2013	13.1	2 wk	2	0.9	No	4 (3 negative; 1: P1 SL)
13	2014	10.0	2.1 mo	1	1.8	Yes	3 (all negative)
14	2014	9.0	3.3 mo	2	0.6	Yes	3 (all negative)

*iVDPV, immunodeficiency-associated vaccine-derived poliovirus; P1, poliovirus type 1; P2, poliovirus type 2; P3, poliovirus type 3; SL, Sabin-like; VAPP, vaccine-associated paralytic poliomyelitis; VP1, viral protein 1.
†Nucleotide divergence from the prototype Sabin strain.
‡No. contacts investigated for VDPVs. Results are indicated in parentheses.

Table 3. Immunologic findings for 14 patients infected with iVDPV, Iran, 1995–2014*

Patient no.	Underlying immunodeficiency	Cells/μL†						Concentration, mg/dL‡		
		Leukocytes	ALC	CD3	CD4	CD8	CD19	IgG	IgM	IgA
1	Undefined hypogammaglobulinemia	NA	NA	NA	NA	NA	NA	NA	NA	NA
2	MHC2	6,300	3,642	1,216	608	607	1,460	200	<10	<10
3	SCID	1,700	731	138	96	32	10	45	<10	<10
4	XLA	6,500	3,375	2,700	1,404	1,290	35	556	<10	<10
5	SCID	6,800	2,589	336	184	185	160	<10	<10	<10
6	XLA	8,500	4,000	2,760	1,920	835	40	20	58	25
7	NBS	6,050	1,040	527	206	264	10	30	22	<10
8	XLA	9,400	4,470	4,201	2,547	1,564	10	80	<10	<10
9	SCID	7,500	3,825	1,092	841	279	2,371	<10	45	<10
10	SCID	7,200	2,174	652	543	163	1,413	40	<10	<10
11	Mu heavy chain	14,400	10,080	8,769	4,636	4,132	110	600§	<10	<10
12	NA¶	NA	NA	NA	NA	NA	NA	NA	NA	NA
13	XLA	10,300	4,738	4,264	3,506	663	30	292	<10	<10
14	Agammaglobulinemia#	10,600	6,254	5,628	4,377	1,375	81	297	34	14

*ALC, absolute lymphocyte count; iVDPV, immunodeficiency-associated vaccine-derived poliovirus; MHC2, major histocompatibility class 2 deficiency; NA, not available; NBS, Nijmegen breakage syndrome; SCID, severe combined immunodeficiency; XLA, X-linked agammaglobulinemia.
†Reference range for total leukocytes, 5,000–10,000 cells/μL; reference range for ALC, 3,000–10,800 cells/μL; reference ranges for lymphocyte subpopulations: CD3, 1,900–5,900 cells/μL; CD4, 1,400–4,300 cells/μL; CD8, 500–1,700 cells/μL; CD19, 610–2,600 cells/μL.
‡Reference ranges for serum immunoglobulins: IgG, 246–904 mg/dL; IgM, 40–143 mg/dL; IgA, 27–66 mg/dL.
§Patient received intravenous immunoglobulin at a primary medical center before blood sampling was initiated.
¶Not determined because patient died.
#Patient was candidate for gene analysis for definitive diagnosis.

Shedding of iVDPV by patient 7 is also notable because he is one of the few case-patients with CID who eliminated the infection before death (*23,24*). He had the longest period of iVDPV shedding among our patients before clearing the infection. His duration of virus shedding was several times longer than shedding durations in each of our patients with humoral immunodeficiencies. These observations appear to support the hypothesis of a probable role for cellular immunity in clearing enteroviral infections. Our patients were generally treated with monthly administration of IVIg. However, different IVIg preparations, doses, and settings in immunoglobulin replacement therapy might contribute to efficacy of treatment. Thereafter, to assess the independent role of immune status in clearance of infection, a comprehensive profile of potential confounding factors and therapeutic conditions, which might contribute to capability of virus clearance, is needed. This profile is missing in our study. Additional studies with comprehensive and appropriate adjustments for these factors are required to assess the immunologic response against enteroviral infections in patients with various types of primary immunodeficiencies.

A search for VDPV infection in contacts of our patients yielded negative results, and only Sabin-like polioviruses were isolated from few contacts, which indicates high vaccination coverage in Iran. Spread of iVDPVs to unimmunized contacts in the United States (*6*) and Morocco (*25*) showed potential risk for iVDPV transmission in populations with low immunity, which can lead to an outbreak. Maintaining high vaccination coverage reduces this risk.

The overall rate of accumulative nucleotide substitution in the genome of polioviruses is believed to be ≈1%/year (*6,26*). Some investigators consider a range of 1%–2%/year as the overall rate of nucleotide variation in polioviruses (*27,28*). Most of our VDPV isolates showed a divergence rate of 1%–2%/year. However, we observed unexpectedly high divergence rates beyond this range in some of our isolates. We propose 3 possible explanations for this difference.

First, for isolates with high divergence rates, the patient could have been infected with an already divergent virus, rather than the original vaccine strain. Thus, the virus had already replicated in a previous host before transmission to the patient. However, lack of any report of circulating VDPV from Iran does not support this explanation. High vaccination coverage and herd immunity against polioviruses in Iran would prevent any circulation of VDPVs. Negative results for VDPV screening in contacts of patients also does not support this explanation.

Second, it has been frequently observed that shortly after OPV administration, the initial rate of capsid region gene evolution is high in Sabin strains, which leads to VDPV emergence (*28,29*). A high proportion of these changes are nonsynonymous substitutions in the genomic region, which codes for structural proteins. However, the synonymous mutations, mostly in nonstructural regions, occur at the expected rates. Changes in specific antigenic sites might increase additional nonsynonymous substitutions (*29*). These changes could be caused by effects of direct selection against attenuating mutations in the early stages of VDPV emergence. Selected mutations might carry unselected hitchhiker mutations in these early stages, which would contribute further to a higher initial evolution rate. After these initial selection events, VDPVs appear to evolve at rates closer to the unselected steady-state rate of ≈1%–2%/year. This alternative view is supported by only

limited data from more prolonged infections because dates of initiation of most VDPV infections can only be estimated from clinical records but is consistent with available evidence (*27*).

Third, another explanation for higher rates of divergence in some of our isolates could be presence of mixed lineages or quasispecies of VDPVs in stool specimens from corresponding patients. This explanation is consistent with previous evidence, which indicated shedding of mixed-lineage VDPVs by patients with primary immunodeficiency (*27,28*). Earlier detection of poliovirus shedding in our patients, possibly after exposure to OPV strains, could have provided precise information about timeline of VP1 divergence in our isolates. This detection could be another advantage of early screening for primary immunodeficiencies and VDPV shedding.

Eradication of wild-type poliovirus serotypes 2 and 3 indicates that eradication of all 3 serotypes can be expected in the near future. However, emergence of VDPVs still remains a threat to global eradication of poliomyelitis (*2*). A review of reports published by CDC showed that ≈100 primary immunodeficiency patients with VDPV shedding have been documented (*1*,*4,18,19,30–32*). By the end of 2014, Iran had reported the highest incidence of iVDPV infections (14 case-patients with AFP), followed by the United States (11 case-patients), and the United Kingdom, Egypt, and China (each with 6 case-patients). Similar to the United Kingdom and other industrialized countries, which reported few VAPP cases after switching to vaccination with IPV, only 3 cases occurred in the Unites States after OPV cessation in 2000 (*3,23,33*). In contrast, the number of iVDPV cases reported from developing and middle-income countries has increased in recent years because of increased extended VDPV surveillance in these countries that used OPV (*18,34*).

More than 20 outbreaks of circulating VDPV infection have been documented, mostly in countries with low OPV coverage (*9*). These outbreaks occurred while no cases of infection with iVDPV were reported from some of these countries, such as Nigeria, Democratic Republic of the Congo, Somalia, and Niger, where VDVPs have circulated for a long time (*4,9*). To explain this observation, one should consider that patients with primary immunodeficiency do not have a chance for long survival in low-income communities because of lack of proper supportive therapies (*35,36*). Thus, even if they are infected with OPV strains, they may die of severe consequences of primary immunodeficiency before emergence of VDPV through prolonged enteral replication.

Environmental isolation of highly divergent VDPVs without an obvious source of shedding has been reported in different countries (*37–39*). In Finland, although only IPV vaccine has been administered since 1985, neurovirulent and highly divergent VDPVs have been recurrently detected in sewage since 2008. Epidemiologic data and virologic characteristics suggest that these VDPVs might have originated from chronically infected patients with immunodeficiencies; however, the source of shedding remains to be identified (*38*). Such unidentified chronic shedding of VDPV might reintroduce neurovirulent viruses into the population and initiate outbreaks in the posteradication era if population immunity to poliovirus is not maintained.

High coverage of OPV vaccination disrupted wild-type poliovirus transmission in Iran in the late 20th century, and the last case of wild-type poliomyelitis was detected in 2000 through sensitive AFP surveillance (Figure). Iran has borders with 2 polio-endemic countries (Afghanistan and Pakistan), which necessitates maintaining high levels of serum and mucosal immunity in the population. Although no wild-type poliomyelitis case has been reported from Iran for many years, vaccine-associated paralysis occurs at a rate of a few cases every year and increases illnesses in immunodeficient patients.

Routine administration of trivalent OPV would inevitably lead to emergence of VDPVs and new VAPP cases. Conversely, vaccination with IPV might not stop virus transmission because of inefficient induction of mucosal immunity (*40*). However, vaccination with IPV might be too expensive for some low-income countries. Accordingly, the switch to a combination of bivalent OPV (serotypes 1 and 3) and IPV vaccination was implemented worldwide in April 2016 (*2*). We believe that immediate achievement of maximum immunization coverage is another vital point to be addressed in this strategy. Moreover, attempts to develop safer and more efficient poliovirus vaccines and polio antiviral drugs are warranted.

This study highlights the need for efficient iVDPV surveillance and the crucial role of patient registry.

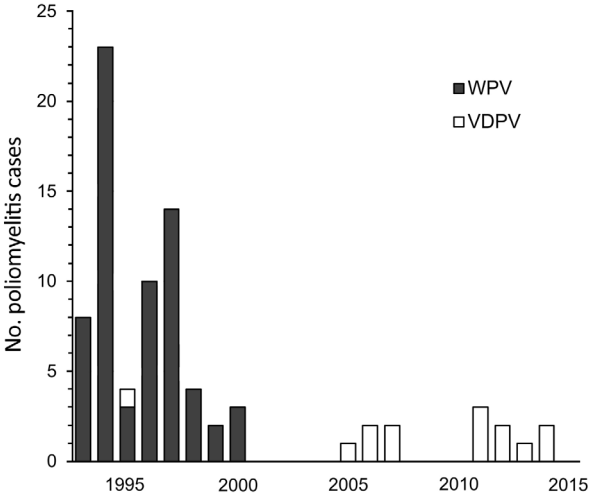

Figure. Poliomyelitis cases in Iran, 1995–2014. WPV, wild-type polioviruses; VDPV, vaccine-derived polioviruses.

Vaccine-derived polioviruses not only endanger countries using OPV but also remain a threat to industrialized countries and the global polio eradication program. Regarding the association of immunodeficiency with this public health issue, we advocate establishment of sensitive clinical and laboratory surveillance to screen primary immunodeficiency patients for shedding of polioviruses.

Eradication might not be accomplished without robust registry and tracking capacities in every country to closely monitor the persistence of polioviruses in the environment and persons at high risk for infection. Achievement of this goal requires providing public health infrastructures, which is missing in some developing countries. In addition, global vigilance of final steps of the eradication program should be increased to attain maximum public contributions. Finally, worldwide IPV immunization should continue with high levels of coverage long after OPV cessation until reliable data indicate worldwide elimination of any poliovirus.

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Dr. Shaghaghi is doctor of medicine and master of public health at the Tehran University of Medical Sciences, Tehran, Iran. His primary research interests are clinical and molecular studies of immunodeficiencies.

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Address for correspondence: Asghar Aghamohammadi, Children's Medical Center Hospital, 62 Qarib St, Keshavarz Blvd, Tehran 14194, Iran; email: aghamohammadi@tums.ac.ir

Infection-Related Death among Persons with Refractory Juvenile Idiopathic Arthritis

Mario Abinun, Jonathan P. Lane, Mark Wood,[1] Mark Friswell, Terence J. Flood, Helen E. Foster

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Release date: September 15, 2016; Expiration date: September 15, 2017

Learning Objectives

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

- Assess the prognosis and management considerations of juvenile idiopathic arthritis (JIA)
- Distinguish characteristics of fatal infections in the current case series
- Evaluate the relationship between biologic disease-modifying antirheumatic drugs (DMARDs) and the risk for serious infections
- Analyze the clinical presentation of macrophage activation syndrome

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CME Author

Charles P. Vega, MD, Clinical Professor of Family Medicine, University of California, Irvine. *Disclosure: Charles P. Vega, MD, has disclosed the following financial relationships: served as an advisor or consultant for Allergan, Inc.; McNeil Consumer Healthcare; served as a speaker or a member of a speakers bureau for Shire Pharmaceuticals.*

Authors

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Severe infections are emerging as major risk factors for death among children with juvenile idiopathic arthritis (JIA). In particular, children with refractory JIA treated with long-term, multiple, and often combined immunosuppressive and antiinflammatory agents, including the new biological disease-modifying antirheumatic drugs (DMARDs), are at increased risk for severe infections and death. We investigated 4 persons with JIA who died during 1994–2013, three of overwhelming central venous catheter–related bacterial sepsis caused by coagulase-negative *Staphylococus* or α-hemolytic *Streptococcus* infection and 1 of disseminated adenovirus and Epstein-Barr virus infection). All 4 had active JIA refractory to long-term therapy with multiple and combined conventional and biological DMARDs. Two died while receiving high-dose systemic corticosteroids, methotrexate,

Author affiliations: Great North Children's Hospital, Newcastle upon Tyne, UK (M. Abinun, J.P. Lane, M. Friswell, T.J. Flood, H.E. Foster); Newcastle University, Newcastle upon Tyne (M. Abinun, H.E. Foster); Leeds General Infirmary, Leeds, UK (M. Wood)

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[1]Current affiliation: Leeds General Infirmary, Leeds, UK.

and after recent exposure to anti–tumor necrosis factor-α biological DMARDs, and 2 during hematopoietic stem cell transplantation procedure. Reporting all cases of severe infections and especially deaths in these children is of paramount importance for accurate surveillance.

Juvenile idiopathic arthritis (JIA), a group of clinically heterogonous conditions with arthritis of unknown origin beginning before 16 years of age and persisting for >6 weeks, is the most common childhood chronic rheumatic disorder (*1*). The most severe forms are those with systemic (So-JIA) or polyarticular (poly-JIA) onset, progressing to polyarticular disease. Despite the success of conventional and new biological disease-modifying antirheumatic drugs (DMARDs), a substantial percentage (>30%) of patients will have ongoing active disease into adulthood that includes sequelae from chronic inflammation and considerable morbidity from joint damage, osteoporosis, growth retardation, psychosocial morbidity, and reduced quality of life and education or employment (*2*).

Rapidly evolving guidelines (*3*) include the window-of-opportunity concept, where biological DMARDs are used as tailored therapy, depending on the disease category, and in naive patients not previously treated with conventional DMARDs (e.g., corticosteroids) (*2*). In the United Kingdom, treatment guidelines are regulated by the National Institute for Clinical Excellence, which specifies indications for tumor necrosis factor-α (TNF-α), interleukin (IL) 6, T-cell activation, and IL-1 blocking agents (https://www.nice.org.uk/guidance/ta373; https://www.nice.org.uk/advice/esnm36). However, no good evidence exists to guide clinicians when they confront failure of the initial biological DMARD (*4*), and switching to a second (or third) (*5*) or combining ≥2 biological DMARDs (*6*) raises concern about risks from severe infections and development of malignancy and new autoimmune disorders (*7*,*8*). For this small group of patients with refractory JIA, hematopoietic stem cell transplantation (HSCT) might be the only treatment option (*9*).

We report details of 4 patients referred for HSCT because of refractory JIA who died of overwhelming infection from central venous catheter (CVC)–related bacterial sepsis (3 patients) or disseminated viral infection (1 patient). Two died while receiving high-dose systemic corticosteroids and methotrexate and after recent exposure to anti–TNF-α agents, before they underwent stem cell collection, and were not started on conditioning chemotherapy at the time of death; 2 died during HSCT (*10*,*11*).

Case Reports

Parents provided informed consent for data and tissue collection. Consent was obtained when the child started treatment with new anti–TNF-α agents (British Society for Paediatric and Adolescent Rheumatology database) or at assessment for HSCT (Newcastle upon Tyne Hospitals National Health Service [NHS] Foundation Trust, Newcastle upon Tyne, UK).

Patient 1

Patient 1, a 13-year-old girl with refractory So-JIA who was previously reported with osteoarticular tuberculosis while treated with etanercept (*12*) (Table 1), never achieved complete disease control (Juvenile Arthritis Disease Activity Score [JADAS]–10 score 25–30) (Table 1) (*13*). She was assessed for HSCT on February 17, 2004 (Table 2). After CVC insertion for treatment with weekly intravenous methylprednisolone pulses (IVMPs) and 1 dose of infliximab, routine CVC cultures grew fully sensitive

Table 1. Disease characteristics and treatment at time of death for 2 children with JIA, Newcastle upon Tyne, United Kingdom*

Characteristic	Patient 1	Patient 2
Year of disease onset/year of death	1996/2004	2000/2004
Age at So-JIA diagnosis/at death, y	5/13	2/6
JIA symptom or sign, ever		
Fever	Yes	Yes
Rash	Yes	Yes
Arthritis	Yes	Yes
Lymphadenopathy	No	No
Hepato/splenomegaly	Yes	Yes
Serositis	No	No
Macrophage activation syndrome	No	Yes
Disease remission, ever	No	No
JADAS-10†	20–35	12–32
Treatment		
Corticosteroids‡	Yes§	Yes§
Methotrexate¶	Yes§	Yes§
Cyclosporin A	No	Yes#
Intravenous immunoglobulins**	No	Yes††
Etanercept	Yes‡‡	No
Infliximab	Yes§,§§	Yes§¶¶
Side effects of treatment		
Cushingoid	Yes	Yes
Cataracts	Yes	No
Osteoporosis	Yes	Yes
Osteoarticular tuberculosis	Yes	No
Stunted growth	Yes	Yes

*JADAS, Juvenile Arthritis Disease Activity Score; JIA, juvenile idiopathic arthritis; So-JIA, systemic JIA.
†Linear sum of the scores of the 4 JADAS components (0–40): physician global assessment of disease activity (measured on a 10-cm visual analog scale; 0 = no activity and 10 = maximum activity); parent/patient global assessment of well-being (measured on a 10-cm visual analog scale; 0 = very well and 10 = very poor); count of joints with active disease (0–10); erythrocyte sedimentation rate (actual value/10; range 0–10) (*13*).
‡Methylprednisolone pulses (intravenous 30 mg/kg/day; maximum. 1 g) given as a 3-day course or a single dose (weekly or otherwise), when indicated based on clinical decision (for treating a disease flare). Prednisolone maintenance dose (orally 0.5–1 mg/kg/d) with adjusting and aiming for alternate day regimen whenever possible depending on the clinical course or disease activity.
§Treatment at time of death.
¶Subcutaneously 15 mg/m^2/wk.
#March–December 2002.
**2 g/kg/mo.
††December 2003–January 2004.
‡‡0.4 mg/kg/wk during March–May 2000 (*12*) and again during May 2003–January 2004.
§§5 mg/kg single dose, February 2004.
¶¶6 mg/kg/mo during December 2002–December 2003.

Table 2. Laboratory parameters of inflammatory activity for 2 children with juvenile idiopathic arthritis, Newcastle upon Tyne, United Kingdom, 2004*

Patient no., date	CRP, mg/dL	Ne, × 10^9 cells/L	PLT, × 10^9/L	ESR, mm/h	Ferr, μg/L	Fib, g/L	TG, mmol/L	Alb, g/L	ALT, U/L	Na, mmol/L
Patient 1										
Feb 17	100	20.2	322	35	485	5.4	0.7	34	24	142
Feb 28	107	28.5	266	–	–	–	–	31	35	136
Feb 29	157	15.8	140	–	–	–	–	22	48	134
Mar 1	63	7.1	79	–	–	–	–	23	120	140
Patient 2										
May 27	122	17.2	475	45	339	6.3	1.9	44	10	135
Jun 1	260	34.9	603	–	2815	8.0	–	38	23	137
Jun 6	365	37.5	710	105	7692	8.1	–	37	50	134
Jun 10	127	70.5	244	–	–	2.9	–	13	125	134

*Laboratory parameters for diagnosing macrophage activation syndrome: Ferr >684 μg/L and any 2 of the following signs: PLT count <181 × 10^9/L, ALT >48 U/L, TG >156 mg/dL, fibrinogen <360 mg/dL (*14*). See section in text on MAS versus Multiorgan Failure. Alb, serum albumin level (reference 38–53 g/L); ALT, serum alanine transaminase level (reference 0–40 U/L); CRP, C-reactive protein (reference 0–5 mg/dL); ESR, erythrocyte sedimentation rate (Westergren; reference 1–10 mm/h); Ferr, serum ferritin level (reference 20–60 μg/L); Fib, serum fibrinogen level (reference 1.5–4.0 g/L); Na, serum sodium level (reference 135–145 mmol/L); Ne, absolute neutrophil count; PLT, absolute platelet count; TG, serum triglyceride level (reference 0.5–1.8).

coagulase-negative *Staphylococcus*. She was treated with systemic teicoplanin and vancomycin locks to all 3 CVC lumens for 7 days (*15*). On February 28, three days after antimicrobial treatment ended, she was readmitted with fever (40°C), generalized macular erythematous rash, abdominal discomfort, nausea, vomiting, and diarrhea. Alongside empirical treatment with systemic antimicrobial drugs (vancomicin and cefotaxime) and intravenous fluids, presumed adrenal insufficiency was treated with hydrocortisone, but she remained febrile (38.6°C); on February 29, she had a sudden episode of hypotension (blood pressure 70 mm Hg) requiring fluid bolus resuscitation and further hydrocortisone. On March 1, as the CVC was accessed for administration of antimicrobial drugs, she became pale and light-headed and collapsed; the CVC was removed, but she died of cardiorespiratory arrest. Multiple peripheral blood and CVC cultures taken during this period remained sterile. Autopsy showed congested lungs and pleural effusions, atrophic adrenal glands, no identifiable thymus, and no erythrophagocytosis in liver or spleen. Cultures from the CVC tip, both lungs, and the pleural fluid samples taken post mortem grew α-hemolytic *Streptococcus*.

Patient 2

Patient 2, a 6-year-old girl, had refractory So-JIA (Table 1) with macrophage activation syndrome (MAS), a form of hemophagocytic lymphohistiocytosis and a potentially fatal complication characterized by unremitting fever, pancytopenia, liver failure with coagulopathy, and central nervous system dysfunction (*14*). Several months before referral, she was brought for care again with possible MAS; symptoms and signs included fever, hepatomegaly, diarrhea, anemia, thrombocytopenia, high serum ferritin (5,670 μg/L [reference 20–60 μg/L]), albeit with leukocytosis and normal clotting (*16*). She was treated with IVMP and high-dose intravenous immunoglobulin. A previously inserted CVC was removed because of *Enterobacter intermedius* infection. Because her disease was never in full remission (JADAS-10 score 20–30; Table 1), she was referred on May 27, 2004, for HSCT (Table 2). On June 1 (one week after new CVC insertion), she was admitted with fever (38°C), macular erythematous rash, vomiting, swelling and pain of several joints, and cough (Table 2). Chest examination and radiographic findings were normal, and she was treated empirically with systemic antimicrobial drugs (teicoplanin and meropenem) for 1 week (*15*) and a 3-day course of IVMP for presumed MAS. After transient improvement during the next few days, on June 6 the child again became unwell, with fever (38.5°C), rash, hepatomegaly, and joint pain (Table 2). Empirical treatment with systemic antimicrobial drugs (teicoplanin and ceftriaxone) was restarted with another 3-day course of IVMP, but she remained febrile (38.5°C) and became restless with abdominal pain and vomiting. She was transferred to the pediatric intensive care unit (PICU), where surgical reasons for acute abdomen pain were excluded. Ciprofloxacin and ambisome were added to the treatment regimen, but the child's condition rapidly progressed into multiorgan failure (Table 2), and she died on June 10. Multiple peripheral blood and CVC cultures taken during this period remained sterile. The family did not agree to autopsy. Culture from the CVC tip removed postmortem grew coagulase-negative *Staphylococcus*.

Patients 3 and 4

Both children received immunosuppressive conditioning with anti–T-cell globulin (rabbit, 10 mg/kg), fludarabine (150 mg/m^2), and cyclophosphamide (120 mg/kg) (*10*,*11*). Both died during T-cell–depleted (by CD34+ positive selection; Miltenyi Biotec, San Diego, CA, USA) autologous HSCT.

Patient 3

Patient 3 was an 18-year-old woman whose So-JIA was diagnosed at 10 years of age. She had active systemic disease,

MAS, and progressive polyarthritis refractory to conventional (corticosteroids, methotrexate, cyclosporine, leflunomide) and biological DMARDs, both TNF-α (infliximab, etanercept) and IL-1 (anakinra) inhibitors (JADAS-10 score over the years 22–35). She underwent HSCT in 2007 with MAS prevention (prednisolone, cyclosporine, and anakinra) during conditioning, but after uneventful engraftment, she died 2.5 months after HSCT of disseminated adenovirus (blood, feces, brain) and Epstein-Barr virus (EBV) (blood, cerebrospinal fluid) infection/reactivation. *Aspergillus fumigatus* was grown from a paranasal sinus washout sample in terminal stage; autopsy was not performed (*10*).

Patient 4

Patient 4 was a 13-year-old girl whose rheumatoid factor + poly-JIA was diagnosed at 4 years of age. She had progressive and debilitating polyarthritis refractory to conventional (corticosteroids, methotrexate) and biological (infliximab, etanercept, anakinra, rituximab) DMRADSs (JADAS-10 score over the years 24–30). She underwent HSCT in 2009. α-hemolytic *Streptococcus* grew from CVC culture taken during a febrile episode after receipt of anti–T-cell globulin, and she was treated empirically with meropenem and teicoplanin; unusually, she rapidly progressed into multiorgan failure requiring ventilatory, inotropic, and renal support in the PICU. Because results of initial liver function tests, including clotting, were normal, and C-reactive protein (CRP) response was adequate, the impression was of bacterial (or fungal) septicemia and renal failure. After transient improvement, she completed conditioning and HSCT and, despite renal failure, maintained stable neutrophil engraftment but remained platelet dependent. Bone marrow biopsy was hypocellular and showed some evidence of macrophage activation. Subsequently, and in parallel with acute pancreatitis, encephalopathy, and progressive enteral and liver failure, the girl manifested prolonged hyperinflammatory response (CRP 100–170 mg/L [reference 0–5 mg/L]; fibrinogen 6–10 g/L [reference 1.5–4.0 g/L]; raised neutrophil count >20 × 10^9 cells/L) despite broad-spectrum antimicrobial and antifungal therapy. Multiple cultures and viral PCRs from different sites (blood, CVC, and other line tips; bone marrow and intestine biopsy; cerebrospinal fluid; maxillary sinus washing) remained negative. She died on day 43 after HSCT; autopsy confirmed multiorgan failure with severe secondary pancreatitis (*11*).

Possible Risk Factors for Severe Infection and Death

Treatment with Combined DMARDs, Including Biologicals

At death, patients 1 and 2 had active disease treated with high-dose systemic corticosteroids and methotrexate; because they previously had been exposed to long-term and multiple DMARDs, including anti–TNF-α biologicals (Table 1). Patients 3 and 4 underwent autologous T-cell–depleted HSCT after a severely immunosuppressive conditioning regimen.

Clinical observation of increased risk from severe infections in children with JIA, often requiring treatment in a hospital (*17*), recently was confirmed in a study of a large JIA cohort (*18*). The increased risk for concurrent immunosuppressive therapy, in addition to the underlying disease-related immune dysfunction (*8*,*17*), was supported by this study, in which high-dose systemic corticosteroids, but not methotrexate and/or anti–TNF-α agents, substantially increased susceptibility to severe infections (*18*). Although simultaneous use of different biological DMARDs is not common (*5,6*), a recently published study highlighted the exposure to multiple and often combined immunosuppressive drugs, such as corticosteroids, methotrexate, cyclosporine, cyclophosphamide, and a variety of biological anti-inflammatory DMARDs, including TNF-α, IL-1, and IL-6 blocking agents, as a major risk factor for severe infections and the unusually high rate of death for a selected group of children with refractory So-JIA, often complicated by MAS, and associated with pulmonary hypertension, interstitial lung disease, and alveolar proteinosis (*19*). It is well recognized that severe immunosuppression targeting B- and/or T-lymphocyte functions (e.g., B-cell–depleting agents, such as rituximab, T-cell activation blocking agent abatacept, anti-CD52 monoclonal antibody alemtuzumab, HSCT procedure) is often complicated with infections caused by a wide spectrum of pathogens, such as pyogenic bacteria, viruses, and fungi (*7*,*9–11*). In contrast, blocking specific inflammatory pathways (e.g., IL-1, IL-6, TNF-α) mimics some of the very rare primary immunodeficiencies of the innate immune system (*20*), with susceptibility to a relatively narrow range of pathogens (*21*,*22*). Infections with encapsulated pyogenic bacteria have been reported in patients with deficiencies of IL-6 function (*23*), whereas the essential role of TNF-α in defense against intracellular pathogens (*24*) was highlighted by the initially observed increased risk for mycobacterial infections associated with anti–TNF-α biologicals (*7*,*12*), prompting the introduction of effective screening and prevention measures (*24*). Although inconsistently reported (*25*), unusually prolonged, severe, and life-threatening infections with common bacterial pathogens are being seen in children with JIA treated with combined DMARDs, including anti–TNF-α agents (*26*,*27*). As observed in these patients (*23*,*28*), because of blocking of the inflammatory response mediated by TNF-α, IL-1, or IL-6 cytokines, these patients might not have high fever and raised CRP, a fact of which clinicians should be aware (*20–24*,*29*). Regardless of active disease and combined immunosuppressive therapy, patients 1 and 2 (i.e.,

those not undergoing HSCT) had preserved inflammatory responses (Table 2) and appropriate routine immunologic testing (data not shown).

CVC-Related Bacterial Infections

Three patients died of CVC-related bacterial sepsis with α-hemolytic *Streptococcus* and coagulase-negative *Staphylococcus*, common and fully sensitive organisms, despite timely administered appropriate antimicrobial therapy (*15*). Long-term CVCs are essential for patients requiring frequent blood tests and intravenous treatments. Unfortunately, CVC-related bloodstream infection is a well-recognized and potentially severe complication. Coagulase-negative *Staphylococcus* species are the most common pathogens causing CVC-related infections. Guidelines recommend treatment with 10–14 days of systemic antimicrobial drugs and antibiotic locks, but routine CVC removal is not recommended because most patients have a benign course and rarely develop sepsis or poor outcome (*15*). *S. aureus*, enteric gram-negative bacilli and *Candida* are less frequent but potentially more severe pathogens. Coagulase-negative *Staphylococcus* species (*S. epidermidis* in particular) were the most common (>50%) pathogens identified from 146 episodes of bacteremia in 64 children with primary immunodeficiencies undergoing HSCT in Great North Children's Hospital, whereas *Enterococcus* species, gram-negative organisms, and *Candida* were isolated only in few cases each (*30*). Most (80%) episodes were successfully treated with appropriate systemic and antibiotic locks; CVC was removed in 12 patients, and the only death resulted from overwhelming *C. albicans* infections despite CVC removal (*30*). Contrary to that study, severe and life-threatening CVC-related sepsis caused by *S. epidermidis* has been reported in a significant percentage of children with systemic vasculitis treated with infliximab and combined immunosuppressive and/or antiinflammatory therapies (*31*). Two deaths from CVC-related sepsis resulting from coagulase-negative *Staphylococcus* and combined *Escherichia coli* and *Candida* infection were reported from a cohort of children with inflammatory bowel disease treated with adalimumab in combination with other immunosuppressive medications (*32*). This high risk for severe CVC-related infections associated with active disease that requires multiple immunomodulatory therapies was also reported from a group of children with pediatric rheumatic disease–related complications admitted to PICU, with substantially high rate of death (50%), of which 44% resulted from multiorgan failure (*33*).

MAS versus Multiorgan Failure

Because the 3 patients we describe who died of CVC-related bacterial sepsis manifested unusually severe and rapidly progressing multiorgan failure, we considered the possibility of MAS in the differential diagnoses (*14*,*16*,*33*,*34*). MAS is a well-recognized major risk factor for death in children with JIA admitted to PICU (*33*,*34*). A recent international study of a large cohort of So-JIA patients identified the most common clinical features as fever, organomegaly, central nervous system involvement, and hemorrhage (*16*). The most useful laboratory parameters were thrombocytopenia; hyperferritinemia; increased liver transaminases, lactate dehydrogenase, triglycerides, and D-dimer levels; and decreased leukocyte count, erythrocyte sedimentation rate, and fibrinogen (*16*).

Some features that manifested near death, such as persisting fever, falling neutrophil and platelet counts, and increased serum transaminase levels (Table 2; patient 1 on March 1) suggest MAS in patient 1 (*16*). Unfortunately, not all of the laboratory parameters highlighted as essential for the clinical diagnosis of MAS were available (*14*) (Table 2). However, the fact that she collapsed after CVC was accessed and that α-hemolytic *Streptococcus* grew from the CVC line tip, lung tissue, and pleural effusion samples after death favors infection as the cause of death. In patient 2, persisting fever, hepatomegaly, and high serum ferritin level suggested MAS, but increasing platelet and neutrophil counts, erythrocyte sedimentation rate, and fibrinogen and normal liver transaminase levels did not support MAS (*14*) (Table 2; patient 2 on June 6). Although rapid deterioration to terminal multiorgan failure was associated with falling platelet count, deranged liver function, and clotting (Table 2; patient 2 on June 10), the high neutrophil count suggested overwhelming fungal infection (which cannot be ruled out because autopsy was not performed), regardless of negative fungal cultures. In patient 4, unusually severe, progressive multiorgan failure developed after CVC-related sepsis, but normal liver function results and clotting and adequate CRP response did not support MAS. Features of macrophage activation in bone marrow biopsy were seen later in the course of progressive multiorgan failure and in parallel with unusual hyperinflammation, suggesting possible fungal infection. However, multiple cultures from different sites remained negative, and autopsy confirmed severe pancreatitis. In patient 3, disseminated adenovirus and EBV infection/reactivation during HSCT led to bone marrow aplasia. In the terminal stage of multiorgan failure with *A. fumigatus* infection, results of liver function and clotting tests were normal, and inflammatory markers were raised (erythrocyte sedimentation rate 80 mm/h [Westergren method; reference 1–10 mm/h]; CRP 200 mg/L [reference 0–5 mg/L]; ferritin 11,000 μg/L [reference 20–60 μg/L]).

Deaths and Reporting Deaths

Although the death rate for JIA has decreased since the 1970s, 1 of 2 recent studies referring to the period before the use of biological DMARDs reported a standardized mortality ratio of 3.4 (95% CI 2.0–5.5) for boys and

5.1 (95% CI 3.2–7.8) for girls (*35*). This nationwide cohort study from Scotland (1,246 children with JIA during 1981–2000) reported 39 JIA-associated deaths, for which the most common causes were the underlying disease (9 cases), circulatory complications (8 cases), and respiratory complications (6 cases) (*35*). In the United States, the standardized mortality ratio for the JIA pediatric rheumatology mortality database (9,604 children with JIA during 1992–2001) was lower at 1.8 (95% CI 0.66–3.92), with 6 of 19 reported deaths occurring among children with So-JIA caused by MAS and heart failure (2 each) and infection and secondary malignancy (1 each) (*36*).

In addition to uncontrolled disease activity and its complications, in particular amyloidosis in the past and MAS today, several recent reviews highlighted the substantial risk for death from severe infections in children with JIA who are receiving biological DMARDs (*7*,*8*). Most of these children were treated with multiple and combined classical DMARDs before or alongside biological DMARDs. Although the range of causing pathogens is broad, pyogenic bacteria and herpes viruses were the most common (*7*,*8*). An unexpectedly high death rate (68% [17/25]) in a group of children with refractory So-JIA associated with pulmonary complications was recently reported from an international group of 25 patients (*19*). Although disease onset ranged from the 1980s onward and most children in the cohort had received multiple immunosuppressive and anti-inflammatory drugs, 19 (76%) diagnoses were made after 2000; contrary to previous reports (*35*,*36*), 68% had been exposed to biological DMARDs (*19*). Strikingly, the calculated death rate for these 19 patients increased by almost 50% over the figure recently reported from the US pediatric rheumatology mortality database for the period up to 2000, before the use of biological DMARDs (*36*).

The regional pediatric rheumatology service at Great North Children's Hospital is closely linked to the national center providing expertise in investigating and treating children with primary immunodeficiencies for northern England and a leading center for HSCT in children with severe rheumatic diseases. This database holds ≈1,250 children in whom JIA has been diagnosed and who were treated and followed during 1994–2013; each year, 50–80 new patients are referred. The calculated death rate for children in this cohort is 0.032% (4 patients died during this period). Three had So-JIA and 1 rheumatoid factor + poly-JIA, all with progressive polyarthritis and poorly controlled systemic disease for many years, including fever, rash, organomegaly, and high acute-phase reactants, with features of MAS in 2 So-JIA patients (*14*,*16*). All died during the early 2000s after long-term treatment with multiple and combined conventional and biological DMARDs: corticosteroids and methotrexate (all 4 children), cyclosporine and leflunomide (1 each); anti–TNF-α agents (3 etanercept, 4 infliximab); IL-1 blocking agent (2 anakinra); and B-cell–depleting (anti-CD20) monoclonal antibody (1 rituximab). Although considered in all 4, only 2 patients underwent autologous T-cell–depleted HSCT (*10*,*11*). With the expertise in pediatric rheumatology, immunology, and infectious diseases available in centers best placed to care for these most severe and often refractory cases, fatalities are rare but do occur.

Monitoring the safety and reporting the side effects, including severe infections and deaths, of new biological DMARDs is a priority of national and international patient registries and multicenter, international collaborative research consortiums, such as the Childhood Arthritis and Rheumatology Research Alliance, Pediatric Rheumatology International Trials Organisation, Pediatric Rheumatology Collaborative Study Group, and Single Hub and Access Point of Care for Pediatric Rheumatic Diseases in Europe (*37*,*38*). However, reporting of deaths is still inconsistent. In children with JIA treated with multiple DMARDs alongside anti–TNF-α biologicals, a systematic review from 2013 reported 4 deaths, 3 of which were associated with severe infections: 2 treated with etanercept (group A *Streptococcus*–related purpura fulminans) and 1 with adalimumab (bacterial sepsis); for 1 treated with infliximab, infection cause was not given (*27*). However, Hashkes et al. (*8*) commented on 6 deaths, all in children treated with combined DMARDs, including anti–TNF-α agents, and associated with serious infections: 3 treated with etanercept (1 each with suspected sepsis, MAS, and tuberculosis [previously treated with infliximab]); 2 with infliximab (sepsis); and 1 with adalimumab (MAS and interstitial pneumonia). Furthermore, neither Woerner and Ritz (*7*) nor Swart et al. (*24*) referred to severe infections and infection-related deaths associated with biological DMARDs from 2 clinical trials reported in 2012 (*39*,*40*). Six deaths were reported in trials of the anti–IL-6 agent tocilizumab, including 1 each from probable streptococcal sepsis and MAS (other listed causes were traffic accident, pulmonary hypertension [2 cases], and pneumothorax) (*40*). Two deaths were reported in a trial of long-acting anti–IL-1 agent canakinumab, both from MAS (1 was previously treated with anakinra and tocilizumab) (*39*). All these patients had active disease and had been treated with a combination of multiple DMARDs before or at death; some were reported as part of the So-JIA cohort associated with pulmonary complications and high death rate (*19*). The most recent report from the United States for 2008–2012 highlighted 7 deaths (1 from an accident) in children with JIA treated with multiple DMARDs (methotrexate and steroids), including biologicals (4 anakinra, 1 each etanercept and infliximab), of which 3 were associated with severe infections (multiorgan failure from disseminated tuberculosis, viral illness, and sepsis) (*38*).

Conclusions

Three patients with refractory JIA reported here died with evidence of CVC-related bacterial sepsis caused by common pathogens (α-hemolytic *Streptococcus* and coagulase-negative *Staphylococcus*): 2 while receiving high-dose systemic corticosteroids; and methotrexate and after recent exposure to the anti–TNF-α biological DMARD infliximab; 1 during HSCT procedure. As has been previously reported, we observed unusual severity of septic shock and rapid progression to multiorgan failure despite timely and appropriate antimicrobial treatment (*31*–*33*). Patient 4 died of disseminated adenovirus and EBV infection during HSCT procedure.

Evidence from clinical trials facilitated by multicenter international collaborative research enabled introduction of biological DMARDs in the treatment of rheumatic diseases in children and unprecedented improvement in their care during the past 20 years (*1*–*4*,*41*,*42*). However, severe infections are emerging as an important risk factor for death among children with JIA treated with combined and multiple conventional and new biological DMARDs (*8*,*19*,*37*,*38*). Accurately reporting all cases of severe infections and especially deaths in these children is of paramount importance, as was highlighted a decade ago (*25*). Although monitoring safety and reporting side effects of new biological DMARDs is in place and improving, we note marked inconsistency in the current literature (*7*,*8*,*19*,*24*,*27*,*38*–*40*).

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Dr. Abinun is a senior consultant in pediatric immunology at Great North Children's Hospital and an honorary clinical senior lecturer at Newcastle University, Institute of Cellular Medicine, Primary Immunodeficiency Group. His research interests include primary immunodeficiency, autoimmune and auto inflammatory diseases, and treatment with hematopoietic stem cell transplantation.

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Address for correspondence: Mario Abinun, Great North Children's Hospital, Royal Victoria Infirmary, Queen Victoria Rd, Newcastle upon Tyne, NE1 4LP, UK; email: mario.abinun@ncl.ac.uk

Accuracy of Diagnosis of Human Granulocytic Anaplasmosis in China

Gary P. Wormser

In 2008, human granulocytic anaplasmosis (HGA) was reported from China. However, the clinical and laboratory findings, including reports of nosocomial transmission, were inconsistent with those reported for HGA in the United States. In 2012, it was demonstrated that the patients described in the 2008 report had all been infected with a newly discovered bunyavirus, severe fever with thrombocytopenia syndrome virus, which causes an illness with the same clinical features described for the patients in the 2008 report. This finding raises the question of HGA misdiagnosis in China and establishes the need for further studies to determine whether HGA occurs there.

The first clinical report of human granulocytic anaplasmosis (HGA) in China was published in 2008 (*1*). This publication was also the first and only report claiming human-to-human transmission of *Anaplasma phagocytophilum* (or, to my knowledge, of any rickettsial agent in the absence of a blood transfusion or a needlestick) and is frequently cited in articles on tickborne infections (*2*,*3*).

To determine the accuracy of the report from China, I compared certain clinical and laboratory features of the 9 laboratory-confirmed cases of HGA in that outbreak (all secondary case-patients were claimed to have been infected by the index patient) (*1*) with 44 culture-confirmed cases of HGA reported from a study conducted in the United States (*4*) (Table 1). The 9 China cases differed from the 44 US cases in that the patients from China were significantly less likely to report headache but significantly more likely to have diarrhea, leukopenia, severe leukopenia (≤3,000 leukocytes/mm^3), and thrombocytopenia (including more severe thrombocytopenia of ≤100,000 platelets/mm^3). As was pointed out in the editorial that accompanied the report of HGA in China (*5*), other noteworthy differences were observed between the HGA patients in China and those in the United States. One of these differences was relative bradycardia in all 9 patients in China (*1*), a finding never reported for HGA in the United States. Another difference was convalescent antibody titers against *A. phagocytophilum* of ≤1:256 for all 9 patients in China, with testing performed by using an IgG immunofluorescent antibody kit (Focus Diagnostics, Cypress, CA, USA) (*1*), whereas titers of ≥1:640 were documented for ≈95% of 44 culture-positive HGA patients in the United States (*4*). In addition, for none of the 9 China patients were morulae observed on blood smear, despite the fact that no patient had received a tetracycline antimicrobial drug, and all patients were said to be positive for *A. phagocytophilum* DNA according to PCR (*1*). In contrast, morulae were seen for 34 (77.3%) of the 44 culture-positive US HGA patients (*4*). Furthermore, in the China case series, sequencing of the *gro*EL gene product amplified by nested PCR indicated that the sequence was more similar to that of strains of A. *phagocytophilum* from the United States than from China, consistent with the occurrence of laboratory contamination. Nested PCR testing is prone to contamination, and the increased sensitivity afforded by this testing method is usually unnecessary when bacteria are numerous enough to be visualized by microscopy, as would be anticipated for patients with HGA (*6*).

The reported nosocomial transmission of *A. phagocytophilum*, in conjunction with the atypical clinical and laboratory test results, already raised questions as to whether the diagnosis of HGA was correct when the article was published (*5*). A potential breakthrough in understanding what type of infection the index patient and the 9 secondary case-patients may have actually had occurred in April 2011, when Yu et al. identified a novel bunyavirus in parts of China that coincided geographically with the earlier report of nosocomial acquisition of HGA (*7*). On the basis of clinical data reported in the article about the initial discovery of this bunyavirus (*7*), plus other studies that followed (*8*–*10*), this tickborne bunyavirus was found to be responsible for a febrile illness associated with leukopenia, thrombocytopenia, and gastrointestinal manifestations. In addition, this virus has been repeatedly shown to be transmissible from person to person through mucocutaneous exposure to the blood of an infected patient (*11*–*17*). Furthermore, relative bradycardia in persons with this infection has been reported (*8*). The bunyavirus is called severe fever with thrombocytopenia syndrome virus (SFTSV).

When the 9 HGA cases described in the initial report from China (*1*) were compared with the 81 cases of SFTSV infection described in the initial publication about this infection (*7*), no significant differences were found for any of the parameters assessed (Table 2). This similarity is in

Author affiliation: New York Medical College, Valhalla, New York, USA

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Table 1. Frequency of certain clinical and laboratory features of HGA in China versus the United States*

Characteristic	China, no. (%) patients†	United States, no. (%) patients‡	p value§
Headache	2 (22)	36 (82)	0.0011
Diarrhea	7 (78)	6 (14)	0.0003
Leukopenia			
<4,500 cells/mm^3	9 (100)	24 (55)	0.0097
≤3,000 cells/mm^3	8 (89)	11 (25)	0.0006
Thrombocytopenia			
<150,000 platelets/mm^3	9 (100)	27 (61)	0.0442
≤100,000 platelets/mm^3	8 (89)	13 (30)	0.0015

*HGA, human granulocytic anaplasmosis.
†Initial case series, n = 9.
‡Culture-confirmed case series, n = 44.
§By Fisher exact test.

marked contrast to the differences found when compared with the 44 culture-confirmed HGA cases from the United States (Table 1) (*4*). SFTSV infection, however, has many distinctive features when compared with HGA in the United States (Table 3).

Thus, it is logical to ask if the 9 HGA case-patients plus the index patient originally reported in China (*1*) actually had SFTSV infection. Liu et al. raised this same question and in their article published in 2012 demonstrated convincingly (i.e., by reverse transcription PCR, serologic testing, or both) that the index patient and the 9 secondary case-patients were infected with SFTSV (*11*). The article did not report, however, the results of retesting of the patient specimens for *A. phagocytophilum* DNA. Thus, there are only 2 potential hypotheses: 1) that all of the patients in the original China case series reported to have had HGA actually had SFTSV infection and not HGA, or 2) that all of the patients were co-infected with SFTSV and *A. phagocytophilum* (*18*,*19*).

The former hypothesis seems much more plausible because of the inconsistency of the laboratory test results for HGA in China with what would have been expected for this infection in the United States, in conjunction with the unprecedented and unlikely nosocomial transmission of *A. phagocytophilum* (*18*). In addition, the latter potential conclusion seems less likely in that it would mean that all 9 secondary case-patients were simultaneously infected with both infectious agents, without even 1 person having only 1 of the 2 infections, which is highly improbable. Considering that 23%–45% of the contacts became infected, depending on the time frame and proximity of the exposure to the index patient (*1*), and assuming that neither of these infections would per se affect the likelihood of person-to-person transmission of the other infection, it would be very unlikely that all secondary case-patients would have been co-infected with both pathogens. Assuming a binomial distribution, the likelihood that this would have occurred by chance alone is <0.0008 (Paul Visintainer, pers. comm., 2016 Jul 8). It also seems implausible that the frequency of headache would have been so low among these 9 case-patients if they were actually co-infected with HGA (Table 1). Why would simultaneous infection with SFTSV reduce the frequency of headache in HGA-infected patients?

To establish the recently proposed hypothesis that the index patient was co-infected with *A. phagocytophilum* and SFTSV by a single tick bite (*19*), several key pieces of information are required. One is whether the *Haemaphysalis longicornis* ticks (the primary tick vector for SFTSV) (*9*) found in the specific area of the Anhui Province of China where the index patient resided are infected with pathogenic strains of *A. phagocytophilum* (*5*); if so, the frequency of co-infection with SFTSV should be determined. In addition, it would be essential to establish whether this species of tick is a competent vector for *A. phagocytophilum* (*18*,*19*).

Since 2008, additional case series of patients with HGA in China have been reported (*20*–*22*). What is the accuracy of these HGA diagnoses? The clinical and laboratory features reported are rather atypical in comparison with those reported for US patients, including in certain case series a higher frequency of gastrointestinal symptoms, regional lymphadenopathy, hepatosplenomegaly, relative bradycardia, facial edema, proteinuria, elevated cardiac enzyme levels, bleeding, and death. Headache and myalgia were reported significantly less commonly in the case series of HGA in China than in the United States,

Table 2. Frequency of certain clinical and laboratory features of HGA versus SFTSV infection in China*

Characteristic	HGA, no. (%) patients†	SFTSV, no. (%) patients‡	p value§
Headache	2 (22)	10 (12)	0.3434
Diarrhea	7 (78)	34 (42)	0.0737
Leukopenia, <4,500 cells/mm^3	9 (100)	64/74 (86)	0.5910
Thrombocytopenia, <150,000 platelets/mm^3	9 (100)	69/73 (95)	1.0000

*HGA, human granulocytic anaplasmosis; SFTSV, severe fever with thrombocytopenia syndrome virus.
†Initial case series, n = 9.
‡Initial case series, n = 81.
§By Fisher exact test.

Table 3. Prominent differences between HGA in the United States and SFTSV infection in Asia*

Clinical sign	More common with SFTSV infection	More common with HGA
Bleeding	Yes	No
Death	Yes	No
Gastrointestinal symptoms	Yes	No
Headache	No	Yes
Leukopenia and lower leukocyte counts	Yes	No
Lymphadenopathy	Yes	No
Person-to-person transmission†	Yes	No
Proteinuria	Yes	No
Relative bradycardia†	Yes	No
Thrombocytopenia and lower platelet counts	Yes	No

*HGA, human granulocytic anaplasmosis; SFTSV, severe fever with thrombocytopenia syndrome virus. Blank cells indicate not more common.
†Never reported in the United States.

whereas leukopenia and severe thrombocytopenia were reported significantly more frequently (Table 4) (*4*,*20*–*22*). It is unlikely that the patients in these case series from China (*20*–*22*) were actually infected with the novel *Anaplasma* species provisionally named *A. capra* because cytopenia is infrequent among patients infected with *A. capra* (*23*,*24*).

If the cases in the original article about nosocomial transmission were misdiagnosed as HGA (*1*), several cautions should ideally be exercised regarding the diagnosis of HGA in China, as well as in other geographic areas where HGA has not been previously diagnosed. One is that reliance on the US Centers for Disease Control and Prevention case definition of HGA in the United States, intended for surveillance purposes (*25*), may not be sufficient to justify a diagnosis of HGA in China at this time. Validation and standardization of the PCR testing methods for *A. phagocytophilum* used in China should be a priority. Furthermore, serologic titers of <1:640 should not be considered indicative of HGA infection (*26*). Low positive titers are common in China (up to 20%) (*27*) and in the United States (*26*) and by no means establish current or prior infection with *A. phagocytophilum*. Locally cultivated strains of *A. phagocytophilum* might be a preferred source of antigens for serologic testing. In addition, given the numerous well-documented examples of clinically misdiagnosed HGA in China before recognition of SFTSV infection (*28*), individual patient assessments and all newly published case series of HGA should include testing for this virus as well as for other relevant infections included in the differential diagnosis. To date, testing for SFTSV has not been reported in many of the published case series of HGA in China (*20*–*22*). More emphasis should be placed on establishing the diagnosis of HGA by using the microbiological standard of culturing the organism (*4*), at least until other diagnostic modalities can be adequately validated. To my knowledge, a positive culture result has been obtained for only 4 reported cases of HGA in China (*20*).

In conclusion, HGA cases have been reported from China since 2008. The clinical and laboratory features, including the claim of nosocomial transmission, differ markedly from the overall features of this infection in the United States. In retrospect, some of the HGA case-patients in China seem to have been infected with the newly discovered bunyavirus, SFTSV. Thus, I recommend that further efforts be made to validate laboratory testing for HGA in China.

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Dr. Wormser is chief of the Division of Infectious Diseases, vice chairman of the Department of Medicine, and professor of medicine, microbiology and immunology, and pharmacology at

Table 4. Selected clinical and laboratory variables of patients with an HGA diagnosis in China and the United States*

Variable	China, no. (%) patients†	United States, no. (%) patients‡	p value§
Number	165	44	
Headache	38 (23.0)	36 (81.8)	<0.0001
Myalgias	44 (26.7)	33 (75.0)	<0.0001
Diarrhea	63 (38.2)	6 (13.6)	0.002
Leukopenia¶	144/145 (99.3)	24 (54.5)	<0.0001
≤100,000 platelets/mm^3	151 (91.5)	13 (29.5)	<0.0001

*HGA, human granulocytic anaplasmosis.
†3 case series of HGA published in 2011, 2013, and 2015 (*20*–*22*).
‡Case series of culture-confirmed HGA published in 2013 (*4*).
§Fisher exact test.
¶<4,500, ≤3,600, <4,000 leukocytes/mm^3 for references (*4*), (*20*), and (*22*), respectively.

New York Medical College. He is also chief of the Section of Infectious Diseases at Westchester Medical Center and director and founder of the Lyme Disease Diagnostic Center. His main research interests are tickborne infections.

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Address for correspondence: Gary P. Wormser, New York Medical College, Division of Infectious Diseases, 40 Sunshine Cottage Rd, Skyline Office #2N-F05, Valhalla, NY 10595, USA; email: gwormser@nymc.edu

Population-Level Effects of Human Papillomavirus Vaccination Programs on Infections with Nonvaccine Genotypes

David Mesher, Kate Soldan, Matti Lehtinen, Simon Beddows, Marc Brisson, Julia M.L. Brotherton, Eric P.F. Chow, Teresa Cummings, Mélanie Drolet, Christopher K. Fairley, Suzanne M. Garland, Jessica A. Kahn, Kimberley Kavanagh, Lauri Markowitz, Kevin G. Pollock, Anna Söderlund-Strand, Pam Sonnenberg, Sepehr N. Tabrizi, Clare Tanton, Elizabeth Unger, Sara L. Thomas

We analyzed human papillomavirus (HPV) prevalences during prevaccination and postvaccination periods to consider possible changes in nonvaccine HPV genotypes after introduction of vaccines that confer protection against 2 high-risk types, HPV16 and HPV18. Our meta-analysis included 9 studies with data for 13,886 girls and women ≤19 years of age and 23,340 women 20–24 years of age. We found evidence of cross-protection for HPV31 among the younger age group after vaccine introduction but little evidence for reductions of HPV33 and HPV45. For the group this same age group, we also found slight increases in 2 nonvaccine high-risk HPV types (HPV39 and HPV52) and in 2 possible high-risk types (HPV53 and HPV73). However, results between age groups and vaccines used were inconsistent, and the increases had possible alternative explanations; consequently, these data provided no clear evidence for type replacement. Continued monitoring of these HPV genotypes is important.

Author affiliations: Public Health England, London, UK (D. Mesher, K. Soldan, S. Beddows); London School of Hygiene and Tropical Medicine, London (D. Mesher, S.L. Thomas); University of Tampere, Tampere, Finland (M. Lehtinen); Imperial College London, London (M. Brisson); Centre de recherche du CHU de Québec, Quebec City, Quebec, Canada (M. Brisson, M. Drolet); Université Laval, Quebec (M. Brisson, M Drolet); Victorian Cytology Service, Melbourne, Victoria, Australia (J.M.L. Brotherton); University of Melbourne, Melbourne (J.M.L. Brotherton, S.M. Garland, S.N. Tabrizi); Melbourne Sexual Health Centre, Melbourne (E.P.F. Chow, C.K. Fairley); Monash University, Melbourne (E.P.F. Chow, C.K. Fairley); Indiana University School of Medicine, Indianapolis, Indiana, USA (T. Cummings); Murdoch Childrens Research Institute, Parkville, Victoria, Australia (S.M. Garland, S.N. Tabrizi); The Royal Women's Hospital, Melbourne (S.M. Garland, S.N. Tabrizi); Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA (J.A. Kahn); University of Cincinnati College of Medicine, Cincinnati (J.A. Kahn); University of Strathclyde, Glasgow, Scotland, UK (K. Kavanagh); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (L. Markowitz, E. Unger); Health Protection Scotland, Glasgow (K.G. Pollock); Division of Clinical Microbiology, Laboratory Medicine, Skåne, Lund, Sweden (A. Söderlund-Strand); University College London, London (P. Sonnenberg, C. Tanton)

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Persistent infection with a high-risk human papillomavirus (HPV) genotype is necessary for development of cervical cancer (*1*). Two high-risk types, HPV16 and HPV18, cause ≈70%–80% of cervical cancers (*2–4*). The HPV vaccines currently available commercially have been shown in trial settings to have ≈100% vaccine efficacy against cervical disease caused by vaccine-specific high-risk HPV types: bivalent and quadrivalent vaccines against HPV16 and HPV18 and the new nonavalent vaccine against HPV31, HPV33, HPV45, HPV52, and HPV58 (*5–7*). Clinical trial data for the bivalent and quadrivalent vaccines have shown low-to-moderate protection (i.e., cross-protection) against other high-risk HPV types that are phylogenetically related to HPV16 and HPV18 (*8,9*).

Many countries have now introduced HPV vaccination programs (*10*). A recently published systematic review and meta-analysis assessed population-level effects of HPV vaccination on vaccine HPV types and showed strong evidence that HPV vaccination is highly effective against infections with these vaccine-specific high-risk types (*11*). The review also examined closely related HPV types as a single group and found evidence of cross-protection overall in a population-based setting (*11*). However, assessment of changes in the prevalence of closely related HPV types combined may not provide full evidence of the effects of a national vaccination program because examining the types as a single group potentially conceals decreases or increases in the prevalence of individual types. Grouping HPV types together limits the possibility of examining

cross-protection provided by specific HPV types and of detecting changes in other individual nonvaccine types. For example, a theoretical concern is that reduced prevalences of infection with HPV16 and HPV18 could lead to other high-risk HPV types occupying those niches and becoming more common causes of disease. Although type replacement was not observed in the clinical trials (*12*), monitoring for possible type replacement in population-based settings after the introduction of national HPV vaccination programs is important. Furthermore, because nonvaccine HPV types are far less common than vaccine HPV types, a single study may have limited scope to determine whether type replacement has occurred. Combining data from several reports improves the ability to investigate type replacement. We aimed to investigate population-level effects of HPV vaccination programs that used bivalent or quadrivalent vaccines on type-specific prevalences of infection caused by individual nonvaccine high-risk HPV types.

Methods

Objectives

Using data from surveys conducted before an HPV vaccination program was introduced and data from surveys after the program was introduced, we compared HPV prevalences for similar populations within the same country. We conducted a systematic literature search to determine changes in HPV prevalence for each nonvaccine high-risk HPV type. At the time of our search, any eligible study would have considered vaccination that used bivalent or quadrivalent vaccines; consequently, high-risk HPV types used only in the nonavalent vaccine were considered nonvaccine HPV types. Each individual type was presented separately in our analysis. We included HPV types for which some cross-protection had been demonstrated in clinical trials (HPV31 and HPV33, which are phylogenetically related to HPV16, and HPV45, which is phylogenetically related to HPV18) (*8,9,13*); other high-risk HPV types included in the nonavalent vaccine (HPV52 and HPV58); other high-risk and probably high-risk HPV types (HPV35, HPV39, HPV51, HPV56, HPV59, and HPV68); and other possibly high-risk HPV types (HPV26, HPV53, HPV70, HPV73, and HPV82), as classified by the International Agency for Research on Cancer (*14*). This systematic review and meta-analysis was reported in accordance with PRISMA guidelines (*15*).

Search Strategy and Selection Criteria

Using Embase, Medline, LILACS, and African Index Medicus databases, we searched for eligible publications published from 2007, the year that the first HPV vaccination programs were introduced, through February 19, 2016. To identify relevant studies that mentioned both vaccination and HPV infection or a related disease (such as HPV-related precancerous lesions, cancers, and genital warts), the search strategy incorporated MeSH terms from the PubMed database (http://www.ncbi.nlm.nih.gov/mesh) and relevant words found in the title or abstract (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/16-0675-Techapp1.pdf). The search had no language restrictions.

Eligible studies were those that assessed population-level effects of HPV vaccination over time by comparing the prevalence of HPV infection (defined by the detection of HPV DNA in patient samples) during a prevaccination period with the prevalence during a postvaccination period. We excluded studies comparing HPV infection in vaccinated persons with HPV infection in unvaccinated persons as part of an individually randomized trial because such studies would not measure population-level effects. Similarly, we excluded studies in which HPV infection was compared only between unvaccinated and vaccinated persons in the postvaccination period. We also excluded studies in which only a small proportion (<2%) of the postvaccination study population was vaccinated (i.e., studies conducted in largely unvaccinated populations). One author (D.M.) initially reviewed titles and abstracts of studies for eligibility; we reviewed in full those studies that appeared to address changes in HPV prevalence after introduction of HPV vaccination programs. We also compared search results with those identified in a recent related review (*11*), which compared prevaccination and postvaccination periods for high-risk vaccine types (HPV16 and HPV18), cross-protected types (HPV31, HPV33, and HPV45), and all high-risk HPV nonvaccine types combined.

Data Extraction and Data Quality

For each study, we extracted data on study design and country of study. Then, for both prevaccincation and postvaccination periods, we extracted data on year(s) of sample collection, study setting and population, sample size, specimen type, assay used for HPV DNA testing, HPV genotypes included in the assay, demographic and sexual behavior data collected, and the measure of effect (and method used to determine any effect). For the postvaccination period, we also extracted data on the method used to ascertain estimated vaccination coverage.

In addition, we assessed the potential bias in each study by considering the comparability of the study populations in the prevaccination versus postvaccination periods (i.e., similar setting and population demographics); the extent of adjustment for potential confounders; the suitability of the specimen type to assess HPV DNA infection; the suitability of the assay used for accurate HPV DNA testing (and whether the suitability of assays differed between the prevaccination and postvaccination periods); and the method used to estimate HPV vaccination coverage. To assess

external validity, we considered whether the study samples were population based. Each of these factors was scored as either low risk or high risk.

When published data on HPV prevalence and prevalence ratios (PRs) for individual high-risk HPV types were unavailable, we contacted authors to request the HPV type-specific prevalences during the prevaccination and postvaccination periods and the PRs for the 2 periods for each nonvaccine high-risk HPV type. We requested PRs adjusted for demographic and sexual behavior data or the unadjusted PRs if data on confounders were unavailable; we calculated unadjusted PRs if authors provided raw data. By using data from a previously conducted validation study, 1 study included adjusted odds ratios rather than PRs to adjust for the change in assay used during the prevaccination and postvaccination periods (*16*).

Data Analysis

We used estimates weighted to account for selection processes if that data were available from authors unweighted numbers, as shown in online Technical Appendix Table 1). We also stratified data by age group (i.e., ≤19 and 20–24 years of age) because of expected lower rates of vaccination coverage and lower vaccine effectiveness in those vaccinated at older ages. Consequently, for each study, we requested data from authors for the same 2 age groups. One study included data for girls <13 years of age, so we requested data restricted to those 16–19 years of age (*17*).

To enable calculation of a PR for a prevalence of 0 during either the prevaccination or postvaccination period, we used a continuity correction of 0.5. When prevalence was 0 for both the prevaccination and postvaccination periods, we excluded the study from the meta-analysis for the relevant age group and HPV type. Results were further stratified by type of vaccine used (i.e., bivalent or quadrivalent). PRs within each subgroup were combined to obtain a summary PR by using a fixed-effects model if data were not shown to be heterogeneous; lack of heterogeneity was determined by a p value ≥0.10 calculated with the Cochrane Q test or by an I^2 value <25% (*18*). Sensitivity analyses were restricted to studies that used cervical, vulval, or vaginal swabs as specimen type because urine samples have lower sensitivity for detecting HPV DNA infection (*19*).

Results

Included Studies

After we eliminated duplications, we identified 4,648 unique articles in searches from all 4 databases (Figure 1). An initial search of title and abstracts of these articles excluded 4,508 (97.0%) because of ineligibility. For the remaining 140 articles, we examined the full text to determine compliance with eligibility criteria and identified 10 eligible studies (Figure 1). Of these 10 studies, 1 met all eligibility criteria, but the type-specific PRs were unavailable from authors (*20*). Therefore, we included 9 studies in the systematic review and meta-analysis (*16,17,21–27*). All eligible studies were repeat cross-sectional studies that compared changes in prevalence in populations before and after introduction of a national HPV vaccination program (online Technical Appendix Table 1). Because only 1 study considered changes in HPV infection among male and female populations, we considered only female populations in the analysis. Two studies were population-based national surveys (*23,26*); 3 studies were conducted among young women obtaining chlamydia screening (*16,17,27*); 2 studies comprised young women attending a primary care clinic, community health center, or hospital-based adolescent clinic (*21,22*); and 2 studies comprised women obtaining cervical screening (*24,25*) (online Technical Appendix Table 1). The included studies contained data on 13,886 girls and women ≤19 years of age and 23,340 women 20–24 years of age.

The studies varied in methodologic quality on the basis of potential bias (Table 1). Most studies collected some demographic and sexual behavior data to enable appropriate

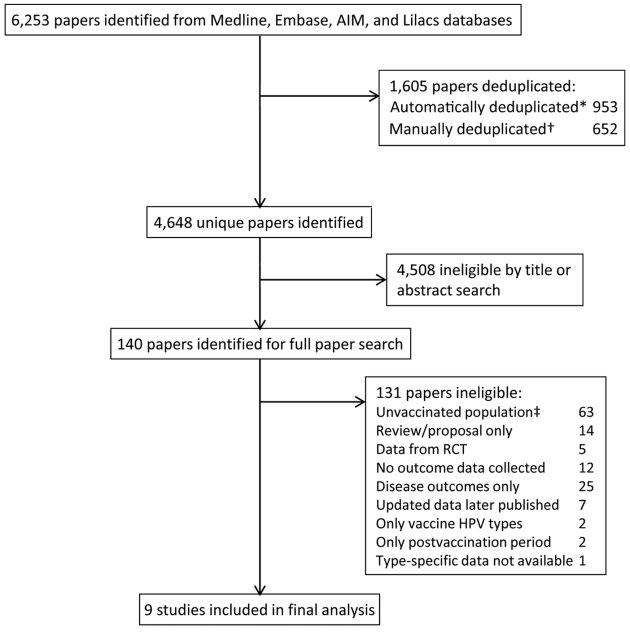

Figure 1. Flowchart for eligible studies included in systematic review and meta-analysis of changes in prevalences of nonvaccine human papillomavirus (HPV) genotypes after introduction of HPV vaccination. *100% title match, author's surname and initial, publication year, and periodical; †85% title match, and author surname; ‡includes studies in which the vast majority of the population were unvaccinated. RCT, randomized controlled trials.

Table 1. Potential bias and external validity of studies included in meta-analysis of changes in prevalences of nonvaccine HPV genotypes*

Potential bias factors	Study, authors (reference no.)								
	Mesher et al. (*16*)	Söderlund-Strand et al. (*17*)	Cummings et al. (*21*)	Kahn et al. (*22*)	Sonnenberg et al. (*23*)	Tabrizi et al. (*24*)	Cameron et al. (*25*)	Markowitz et al. (*26*)	Chow et al. (*27*)
Population-based samples†	**H**	**H**	**H**	**H**	L	L	L	L	**H**
Comparative populations†	**H**	**H**	L	L	L	L	L	L	**H**
Risk factor data collected and adjusted for	**H**	**H**	L	L	L	**H**	**H**	L	L
Samples suitable for assessing HPV	L	L	L	L	**H**	L	L	L	L
Assay with suitable accuracy	L	L	L	L	L	L	L	L	L
Identical HPV assays†	**H**	L	L	L	L	L	L	L	L
Vaccination status collected	**H**	**H**	L	L	**H**	L	L	**H**	**H**

*HPV, human papillomavirus; H (in bold), high risk of bias; L, low risk of bias.
†For both prevaccination and postvaccination periods.

adjustment of the relative risks, although the number of factors collected was limited in some studies (*16,17,24,25*) (Table 1; online Technical Appendix Table 1).

HPV Types Included in Nonavalent HPV Vaccines

HPV Types with Prior Evidence for Cross-Protection

We found evidence of reduced prevalence of HPV31 (Figure 2; Table 2) among girls and women ≤19 years of age (PR 0.73, 95% CI 0.58–0.91) but found little evidence of changed prevalences for HPV33 or HPV45 among this age group (PR 1.04, 95% CI 0.78–1.38 for HPV33; PR 0.96, 95% CI 0.75–1.23 for HPV45). Results were heterogeneous for HPV31, HPV33, and HPV45 in women 20–24 years of age; consequently, we did not calculate summary PRs (Figure 2; Table 2).

Other HPV Types

We found evidence of increased prevalence of HPV52 in those ≤19 years of age (PR 1.34, 95% CI 1.13–1.59) (Figure 3; Table 2), but because of heterogeneity, we did not calculate summary PRs for those 20–24 years of age. We found no evidence of a changed prevalence for HPV58 among the younger age group (PR 1.01, 95% CI 0.80–1.26) but found borderline evidence of an increase for those 20–24 years of age (PR 1.14, 95% CI 0.99–1.31).

Other High-Risk and Possibly High-Risk HPV Types

No consistent patterns appeared across the studies for other HPV vaccine types not used in the nonavalent vaccine (Table 2; online Technical Appendix Figure 1). We found evidence of increased prevalences from the prevaccination period to the postvaccination period in those ≤19 years of age for HPV39 (PR 1.27, 95% CI 1.05–1.54), HPV53 (PR 1.51, 95% CI 1.10–2.06), and HPV73 (PR 1.36, 95% CI 1.03–1.80). For women 20–24 years of age, evidence indicated increased prevalence for HPV39 (PR 1.13, 95% CI 1.00–1.28).

Sensitivity Analysis

As a sensitivity analysis, we performed 3 additional analyses, all stratified by age group: by type of vaccine used (i.e., bivalent or quadrivalent); by potential bias of the original study (i.e., relatively low potential bias, defined as <3 factors indicating high risk of bias; or relatively high potential bias, defined ≥3 factors indicating high risk of bias) (Table 1); and by vaccination coverage (i.e., low <50%; high ≥50%). For studies in settings that used the bivalent vaccine, we found evidence of increased prevalence between the prevaccination period and postvaccination periods among those ≤19 years of age for HPV52, HPV53, HPV56, and HPV70 (online Technical Appendix Table 2, Figures 2–4). Prevalence of HPV53 among women 20–24 years of age also increased. For the quadrivalent vaccine, evidence showed increased prevalences of HPV39, HPV51, and HPV59 for those ≤19 years of age. Among those 20–24 years of age, evidence indicated increased prevalence of HPV52 and HPV70 (online Technical Appendix Table 2, Figures 2–4).

Many of our analyses that were stratified by potential bias of the included studies had results similar to those in the unstratified analyses (online Technical Appendix Table 3). However, among those ≤19 years of age, studies with a relatively low potential bias showed no evidence of increased prevalence for HPV52 or HPV39, although evidence existed when the studies were unstratified. For studies with relatively high potential bias, among this younger age group, evidence showed increased prevalences of HPV51 and HPV70, although these increases were not present in the unstratified analysis. In women 20–24 years of age, evidence showed decreased prevalence for HPV33 in those studies with a relatively low potential bias. No

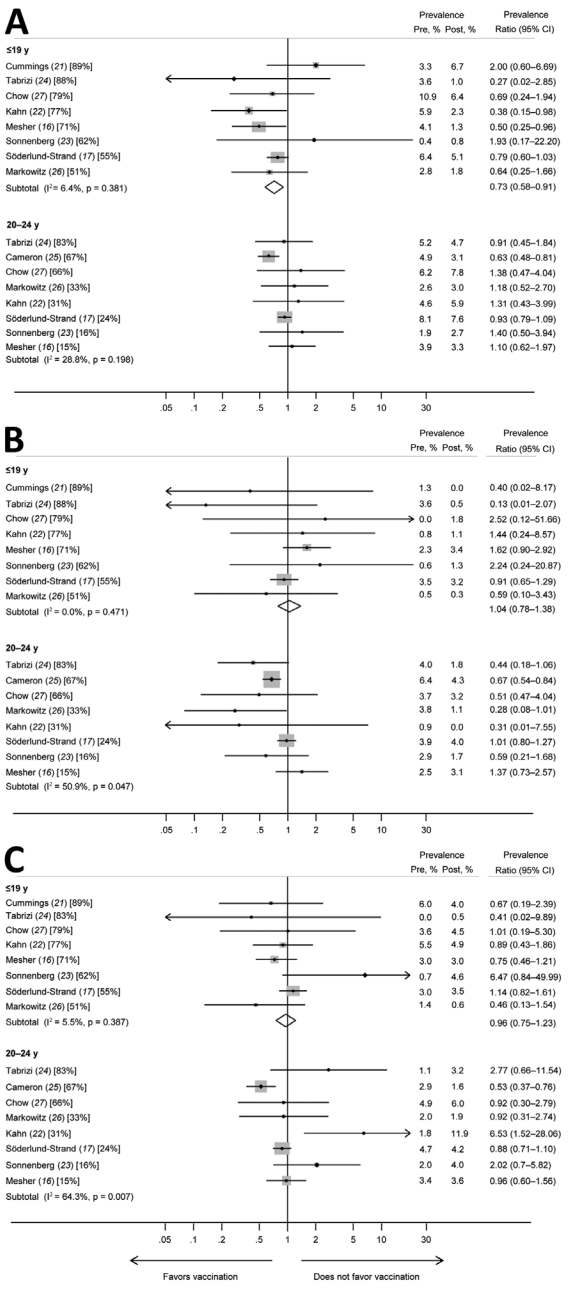

Figure 2. Prevalence ratios and 95% CIs for high-risk human papillomavirus (HPV) types (HPV31, HPV33, and HPV45) that had evidence of cross-protection for girls and women ≤19 years of age and women 20–24 years of age in studies included in a meta-analysis of changes in prevalences of nonvaccine HPV genotypes after introduction of HPV vaccination. A) HPV31; B) HPV33; C) HPV45. Percentages in brackets represent vaccination coverage (≥1 dose) for each study and age group. The size of the gray boxes around the plot points indicates the relative weight given to each study in the calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for the group ≤19 years of age. The study by Cummings et al. (*21)* is omitted from analyses for women 20–24 years of age because this study included no data for this age group. Pre, prevaccination; post, postvaccination.

summary estimate was provided in the unstratified analysis because of heterogeneity of data. Studies with a relatively high potential bias showed evidence of increased prevalences of HPV52 and HPV58 among women 20–24 years of age. Among this older age group, evidence existed for decreased prevalence of HPV82 in those studies with both relatively high potential bias and relatively low potential bias, although those studies with relatively high potential bias had a larger decrease. Again, no summary estimate was provided in the unstratified analysis because of heterogeneity.

Vaccination coverage was high for the younger age group in all studies (online Technical Appendix Table 4). For the older age group, studies with high vaccination coverage showed decreased prevalence for HPV31. No summary estimate was provided for the unstratified analysis because of heterogeneity. For the older age group, we found evidence of increased prevalences for HPV39 and HPV58 (similar to results from the unstratified analysis) but only in studies with low vaccination coverage. Although not seen in the unstratified analysis, we also found evidence of an increased prevalence for HPV70 in low-coverage studies and borderline evidence of an increased prevalence for HPV26 in high-coverage studies. No summary estimates were provided for the unstratified analyses because of heterogeneity.

Discussion

Comprehensive postvaccination surveillance should not only consider reductions of vaccine type–specific infection and associated disease but should also assess any other potential effects of reductions of targeted infections. We assessed changes in nonvaccine HPV types to determine evidence of cross-protection for individual HPV types and to investigate the potential concern that reductions in certain HPV types after the introduction of HPV vaccination in a population could create a niche that enables other nonvaccine high-risk HPV types to become more common (i.e., type replacement). We found evidence of a reduction in the prevalence of HPV31 among girls and women ≤19 years of age. Our main analysis showed increases in other nonvaccine HPV types (HPV39, HPV52, HPV53, HPV58, and HPV73), but these increases were inconsistent for the 2 age groups examined and the vaccines used.

A previous systematic review evaluated changes in high-risk HPV types combined and found evidence of a reduction in the prevalence of HPV types closely related to vaccine types (HPV31, HPV33, and HPV45) when they were considered as a single group (PR 0.72, 95% CI 0.54–0.96 for girls and women 13–19 years of age) (*11*). Our review provides evidence of reduced prevalence for HPV31 but little evidence of reduced prevalence for HPV33 or HPV45.

Table 2. Summary prevalence ratios for meta-analysis of changes in nonvaccine high-risk HPV types among girls and women, by age group*

Population age group, y, and HPVtype	No. studies†	Heterogeneity		Prevalence ratio (95% CI)
		I^2, %	p value	
≤19				
HPV types in nonavalent vaccine	8			
HPV31		6.4	0.381	0.73 (0.58–0.91)
HPV33		0	0.471	1.04 (0.78–1.38)
HPV45		5.5	0.387	0.96 (0.75–1.23)
HPV52		24.0	0.238	1.34 (1.13–1.59)
HPV58		0	0.727	1.01 (0.80–1.26)
Other high-risk HPV types	8			
HPV35		25.1	0.229	–
HPV39		0	0.984	1.27 (1.05–1.54)
HPV51		43.6	0.088	–
HPV56		74.3	<0.001	–
HPV59		66.8	0.004	–
HPV68		0	0.690	1.26 (0.88–1.81)
Other possibly high-risk HPV types	6			
HPV26		0	0.478	1.63 (0.84–3.16)
HPV53		3.6	0.394	1.51 (1.10–2.06)
HPV70		23.6	0.257	1.34 (0.75–2.39)
HPV73		0	0.961	1.36 (1.03–1.80)
HPV82		49.0	0.081	–
20–24				
HPV types in nonavalent vaccine	8			
HPV31		28.8	0.198	–
HPV33		50.9	0.047	–
HPV45		64.3	0.007	–
HPV52		31.0	0.180	–
HPV58		0	0.806	1.14 (0.99–1.31)
Other high-risk HPV types	8			
HPV35		7.9	0.369	1.07 (0.85–1.34)
HPV39		0	0.522	1.13 (1.00–1.28)
HPV51		49.8	0.052	–
HPV56		82.6	<0.001	–
HPV59		63.6	0.007	–
HPV68		35.6	0.145	–
Other possibly high-risk HPV types	6			
HPV26		44.3	0.110	–
HPV53		30.8	0.204	–
HPV70		25.1	0.246	–
HPV73		59.2	0.032	–
HPV82		38.3	0.151	–

*HPV, human papillomavirus; –, prevalence ratio not calculated because of heterogeneity of data.
†Number of studies was the same for all HPV types within each category.

Comparing HPV prevalence in a prevaccination period to prevalence in a similar population in a postvaccination period enables consideration of population-level effects of HPV vaccination on HPV prevalence. However, these repeat cross-sectional study designs have limitations. Although all studies addressed similar populations in the prevaccination and postvaccination periods, these populations may have undergone temporal changes that are independent of HPV vaccination over time and that possibly affect HPV prevalence. For example, increases in diagnoses of other sexually transmitted infections have occurred during the same period as that of HPV vaccination programs (*28*). Furthermore, incidence of genital warts increased in many countries before vaccine introduction (*29–31*) and has continued to increase postvaccination in persons ineligible for vaccination (*11*). Such findings suggest that the increases we observed in some HPV types are possibly associated with broad increases in sexual risk over time. We considered changes in demographics and sexual behavior for the populations over time when information was available, but unrecorded population changes or other temporal changes affecting the relative proportions of high-risk HPV types likely occurred over time (*32,33*). Also, more geographic variation exists in the relative frequency of nonvaccine HPV types in populations compared with the prevalence of HPV16, which, before the vaccination programs, was the most frequent high-risk type observed in almost all populations (*34*).

Furthermore, the change in assay used during the prevaccination and postvaccination periods was a potential source of bias in 1 study (*16*), which calculated odds ratios (ORs) adjusted for differences in diagnostic accuracy. This adjusted OR could not be converted to a PR by using the log-binomial model and was included as an OR. However,

given the low prevalence of individual HPV types, the use of an OR instead of a PR for this study was unlikely to have affected the results substantially.

Another limitation is that the broad-spectrum assays used in these studies (and in baseline prevaccination evaluations globally) can lack sensitivity for detecting individual HPV types when multiple types are present, particularly if another HPV type with a higher viral load is present. In the postvaccination period, in the absence of HPV16 and HPV18, this lack of sensitivity could lead to an apparent but artificial increase in nonvaccine types because these types were underestimated in the prevaccine period because of the predominance of HPV16 or HPV18. Studies have shown this potential unmasking effect (*35,36*); some increases in nonvaccine types that we observed could result from unmasking.

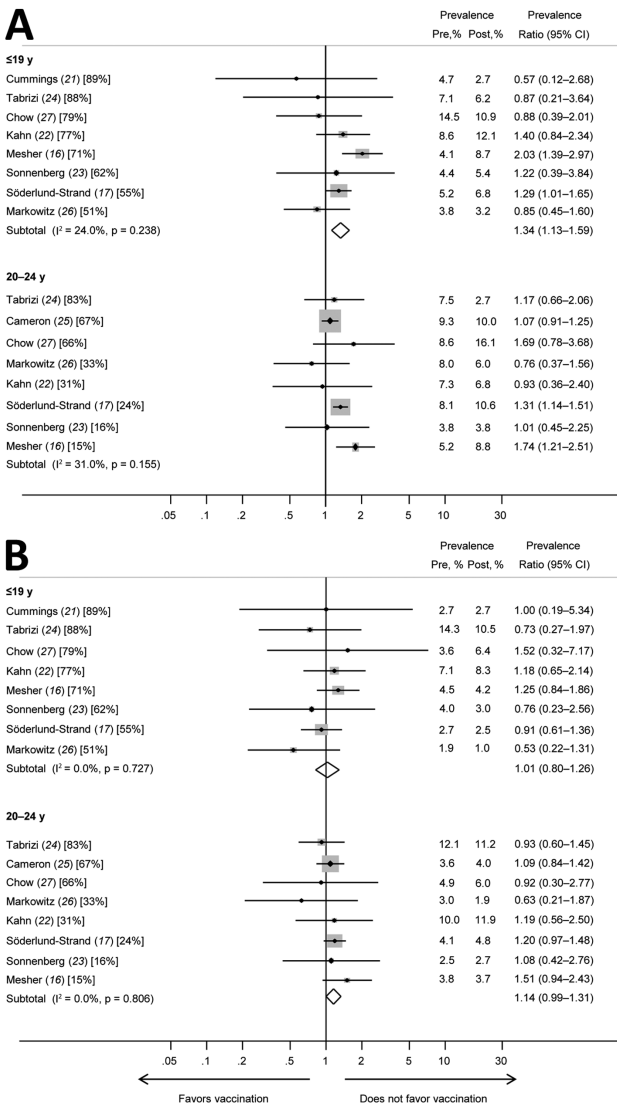

Figure 3. Prevalence ratios and 95% CIs for other high-risk human papillomavirus (HPV) types (HPV52 and HPV58) included in the nonavalent vaccine for girls and women ≤19 years of age and women 20–24 years of age in studies included in a meta-analysis of changes in prevalences of nonvaccine HPV genotypes after introduction of HPV vaccination. A) HPV52; B) HPV58. Percentages in brackets represent vaccination coverage (≥1 dose) for each study and age group. The sizes of the gray boxes around the plot points indicates the relative weight given to each study in the calculation of the summary estimate. The study by Cameron et al. (*25*) is omitted from analyses for the younger age group because this study included no data for persons ≤19 years of age. The study by Cummings et al. (*21*) is omitted from analyses for women 20–24 years of age because the study included no data for this age group. Pre, prevaccination; post, postvaccination.

Given the low prevalence of some nonvaccine HPV types, assessing changes in prevalence for individual types since the introduction of HPV vaccination has been challenging. By combining data from several studies, we enhanced our power to consider changes in individual HPV types. However, even with data from 13,886 girls and women ≤19 years of age and 23,340 women 20–24 years of age, we still had limited power to consider changes in very rare HPV types or to investigate reasons for the heterogeneity in findings for some HPV types because of inconsistent evidence for increases of specific nonvaccine types between age groups and the 2 (i.e., bivalent and quadrivalent) vaccines. Conversely, type 1 errors can occur with multiple testing, so modest evidence for increases should be interpreted with caution.

We decided against performing random-effects meta-analyses in the presence of between-study heterogeneity because, in most instances, inconsistency occurred in the direction of effect, making a summary estimate (i.e., the average value of these opposing effects) uninformative (*37*). Exploring the causes of heterogeneity could provide further insight into the reasons for these increases, so we performed 3 subgroup analyses by vaccine used, potential bias, and vaccine coverage. Results of the stratification by potential bias suggested that increased PRs for some HPV types may have been reported more often in the studies with relatively high potential bias. However, for all 3 subgroup sensitivity analyses, the small number of studies in each stratum limited the interpretation of the analyses. Similarly, we were limited to only 8 studies for each age group and had insufficient ability to perform meta-regression analyses (because meta-regression should generally not be considered for <10 studies) (*37*). As further data accrue, a useful future analysis would be exploring the association between reductions in the HPV vaccine types and any increases (not resulting from unmasking) in nonvaccine HPV types. If increases result from type replacement, then we would expect to see increasing prevalences of nonvaccine HPV types as prevalences of vaccine HPV types decrease.

Our confirmation of reductions in a cross-protected HPV type is encouraging. However, the results of this systematic review and meta-analysis provide no clear evidence

for type replacement because the data are unclear about the extent to which any observed increases result from other temporal changes, changes in the study populations, or an unmasking effect of broad spectrum HPV assays. Large-scale epidemiologic analyses that use various designs have not detected evidence of any interactions between high-risk types, and the known high evolutionary stability of these viruses lessens the risk that type replacement will be a problem (*38,39*).

Most women included in the surveillance studies were those vaccinated at older ages (i.e., potentially vaccinated after HPV exposure), and some studies included populations with relatively low coverage, compared with nationally reported vaccination coverage for routine cohorts. Future studies should continue to monitor population-level prevalences of these HPV types. In particular, studies should consider populations vaccinated at young ages and having high vaccination coverage and, perhaps more important, should examine the absolute prevalence of cervical intraepithelial neoplasia 3 lesions attributed to each high-risk HPV type.

Acknowledgments

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Mr. Mesher is a senior scientist at Public Health England. His work involves monitoring and evaluating the population-level effects of the national HPV vaccination program.

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Address for correspondence: David Mesher, HIV and STI Department, National Infection Service, Public Health England, 61 Colindale Ave, London NW9 5EQ, UK; email: david.mesher@phe.gov.uk

Cat-Scratch Disease in the United States, 2005–2013

Christina A. Nelson, Shubhayu Saha, Paul S. Mead

Cat-scratch disease (CSD) is mostly preventable. More information about the epidemiology and extent of CSD would help direct prevention efforts to those at highest risk. To gain such information, we reviewed the 2005–2013 MarketScan national health insurance claims databases and identified patients <65 years of age with an inpatient admission or outpatient visit that included a CSD code from the International Classification of Diseases, Ninth Revision, Clinical Modification. Incidence of CSD was highest among those who lived in the southern United States (6.4 cases/100,000 population) and among children 5–9 years of age (9.4 cases/100,000 population). Inpatients were significantly more likely than outpatients to be male and 50–64 years of age. We estimate that each year, 12,000 outpatients are given a CSD diagnosis and 500 inpatients are hospitalized for CSD. Prevention measures (e.g., flea control for cats) are particularly helpful in southern states and in households with children.

Cat-scratch disease (CSD) is a zoonosis caused by *Bartonella henselae,* a fastidious, hemotropic, gram-negative bacterium. *B. henselae* is maintained and spread among cats—the principal mammal reservoir species—by the cat flea (*Ctenocephalides felis*); transmission to humans occurs via scratches, and possibly bites, from cats. CSD occurs throughout the United States and worldwide wherever cats and their fleas are found (*1*). Knowledge of this emerging pathogen continues to expand; additional *Bartonella* species, such as *B. clarridgeiae*, and mammal hosts, including dogs, have also been linked to CSD (*2–4*).

The predominant clinical feature of CSD is lymphadenopathy proximal to the site of a cat scratch or bite; in many patients, a papule develops at the initial wound site before onset of lymphadenopathy. Some patients with *B. henselae* infection experience more serious manifestations, such as neuroretinitis, Parinaud oculoglandular syndrome, osteomyelitis, encephalitis, or endocarditis (*1*). *B. henselae* infection can be particularly severe for patients with immunocompromising conditions, such as AIDS, in whom vascular proliferative lesions (bacillary angiomatosis and bacillary peliosis) may develop (*5*).

Author affiliations: Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (C.A. Nelson, P.S. Mead); Emory University, Atlanta, Georgia, USA (S. Saha)

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In the United States, CSD is not a notifiable condition; therefore, information on the epidemiology of this disease has been limited to clinical case series and analyses of hospital discharge databases. Jackson et al. used records from the Commission on Professional and Hospital Activities (1983–1989) and the National Hospital Discharge Survey (1978–1989) to estimate incidence of CSD hospitalizations to be 0.77–0.86/100,000 population/year. Most hospitalized patients were ≤18 years of age (55%) and male (60%); highest incidence was in the southern United States. Although the authors also extracted outpatient records from the National Ambulatory Medical Care Survey, only 10 cases of CSD were identified, limiting analysis and extrapolation (*6*).

Reynolds et al. analyzed the Kids' Inpatient Database for the year 2000 and estimated the incidence of CSD hospitalizations to be 0.60/100,000 children <18 years of age/year. In this study, incidence was also highest in southern states but was slightly higher among girls and women (*7*).

Although the syndrome of CSD was first defined in 1950, identification of the etiologic agent and development of diagnostic tests did not occur until the mid-1980s and later (*8*). Therefore, the epidemiology of CSD may have changed in the past few decades because of improved diagnostic tests for CSD and other conditions that mimic CSD (*9*). Patients with typical signs of CSD and compatible exposure history can be given a presumptive clinical diagnosis; diagnostic tests such as serology, PCR, and culture can be useful for confirming typical CSD or for diagnosing atypical CSD (*1*).

To better define the current epidemiology and burden of CSD in the United States, we analyzed a large medical claims database to 1) describe national patterns of clinician-diagnosed CSD among inpatients and outpatients, 2) evaluate changes in disease patterns during the study period, and 3) identify demographic groups at higher risk for CSD. Improved understanding of CSD may facilitate recognition by clinicians and identification of risk groups for whom education about cats and flea control is particularly helpful.

Methods

We conducted a retrospective analysis of the Truven Health MarketScan Commercial Claims and Encounters database (Truven Health Analytics, Ann Arbor, MI, USA) for 2005–2013. This database contains information on

employer-sponsored private health insurance claims from employees, their spouses, and their dependents from all 50 states. The database contains records for persons <65 years of age only.

The MarketScan database includes information about inpatient admissions, outpatient clinic visits, and emergency department visits with associated billing codes from the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). A case was defined as illness in any patient with an insurance claims record for inpatient or outpatient care that included the ICD-9-CM code for cat-scratch disease (078.3) as either a principal or secondary diagnosis. Only the first record with the 078.3 code was counted and considered the incident event. Patients who had been hospitalized and had also had outpatient visits were counted as inpatients.

Because some persons in the database were covered by an included health insurance plan for only part of each year, we calculated the denominators for incidence calculations by summing the total number of person-months by year, region, age, and sex. We then divided these sums by 12 to yield the number of person-years for each category. To assess changes in the epidemiology of CSD over time, we divided the dataset into two 3-year periods (2005–2007 and 2011–2013), with a washout period of 3 years in between.

The cost of hospitalization and outpatient visits was calculated by summing total payments for the initial encounter plus any subsequent encounter with the 078.3 code up to 1 year afterward. Total costs included the primary insurance payment, co-insurance, and patient copayment. Costs for procedures such as phlebotomy and laboratory testing were included if they had an associated 078.3 code. Costs of inpatient medications were incorporated into the total payments for hospital stay. Outpatient prescriptions were included in the total cost calculation if they were for an antimicrobial drug recommended for treatment of CSD and prescribed within 30 days before or after the initial outpatient 078.3 encounter (*10*,*11*).

To estimate the total annual number of US patients <65 years of age with a CSD diagnosis, we first calculated age-specific and US census region–specific rates of CSD within the MarketScan database. We then performed direct standardization by multiplying these rates by the US population by age and region. Population estimates were obtained from the 2010 US Census Bureau Population Survey (*12*).

Descriptive statistics and comparisons were performed by using SAS version 9.3 (SAS Institute, Cary, NC, USA). To compare categorical data between inpatients and outpatients and between the periods 2005–2007 and 2011–2013, we calculated risk ratios (RRs).

The protocol underwent human subjects review at the Centers for Disease Control and Prevention and was determined not to be research involving human subjects. As such, institutional review board approval was not required.

Results

Study Population

During 2005–2013, the MarketScan database contained information on a median of 39,970,145 enrollees for each year (range 16,159,068–53,131,420). A total of 280,522,578 person-years were analyzed during the 9-year study period.

Incidence and Geographic Distribution

During the study period, we identified 13,273 patients with a diagnosis of CSD: 12,735 outpatients and 538 inpatients. Average annual incidence was 4.5 outpatient diagnoses/100,000 population (range 4.0–5.7/100,000) and 0.19 inpatient admissions/100,000 population (range 0.17–0.22/100,000).

Annual incidence of outpatient CSD diagnoses was highest in 2005 (5.7/100,000 population) then steadily declined to a low of 4.0/100,000 population in 2013 (Figure 1). The decrease in incidence over time occurred primarily in southern states. For inpatient admissions, annual incidence remained relatively stable during the study period and peaked slightly in 2008 (0.22/100,000 population).

Incidence was highest in the West South Central, East South Central, and South Atlantic divisions (6.1–6.4 cases/100,000 population) and lowest in the more arid Mountain division, where cat fleas are less common (2.2 cases/100,000 population) (Figure 2). The highest overall proportion of cases occurred in the South Atlantic division (26.3%), followed by the West South Central division (19.7%).

Distribution by Age and Sex

Highest average annual CSD incidence for outpatients and inpatients was among children 5–9 years of age (9.0 cases/100,000 patients and 0.4 cases/100,000 patients, respectively) (Figure 3). Children ≤14 years of age accounted for 32.5% of diagnoses overall. Among adults, highest

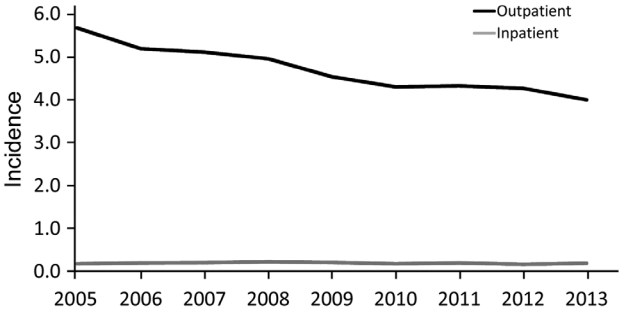

Figure 1. Average annual incidence (cases/100,000 population) of cat-scratch disease outpatient diagnoses and inpatient admissions by year, United States, 2005–2013.

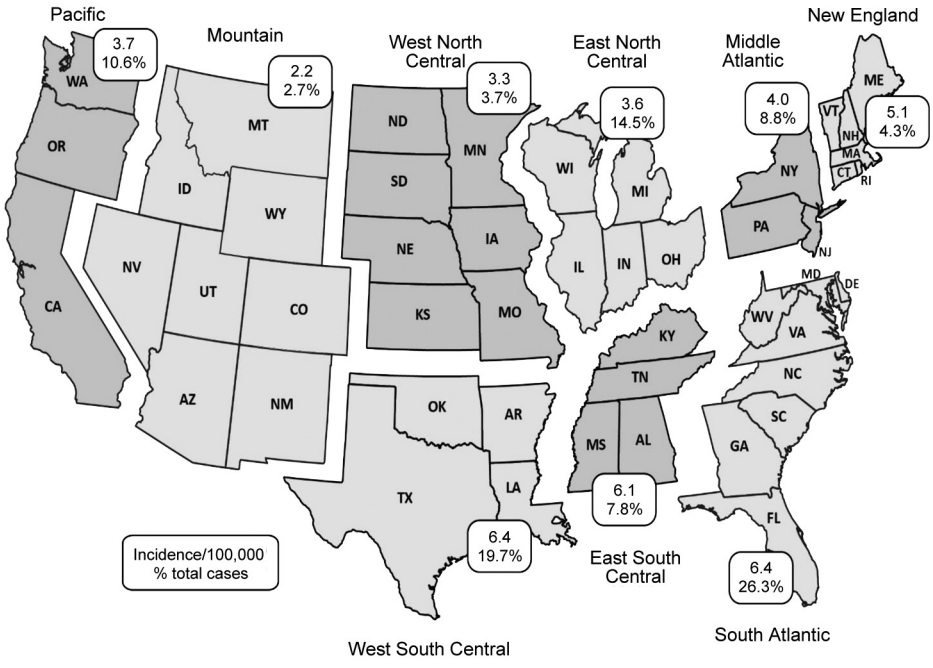

Figure 2. Geographic distribution of cat-scratch disease cases by US census division, United States, 2005–2013. Rates are reported as average incidence per 100,000 population per year. During the study period, there were <10 cases in Alaska and <10 cases in Hawaii.

incidence for outpatients and inpatients was among women 60–64 years of age (6.6 cases/100,000 and 0.3 cases/100,000, respectively) (Figure 3).

Women and girls accounted for 62.0% of outpatient diagnoses and 55.6% of inpatient diagnoses. Although most inpatients were female, inpatients were significantly more likely than outpatients to be male (RR 1.17, 95% CI 1.06–1.29) (Table). Incidence among female patients was higher than that among male patients in all age groups with the notable exceptions of inpatients 0–4, 25–29, and 40–49 years of age. The incidence difference between adult female and male patients widened as age increased.

Seasonality

The largest proportion of diagnoses was made during January (10.2%), followed by August–November (9.1%–9.6%/month) (Figure 4). Diagnoses were significantly more likely to be made during August–November for inpatients than for outpatients (Table). Of note, when data were stratified by region, a spike in January was apparent for all regions but most pronounced in the North Central region and least pronounced in the West region (Figure 5).

To account for differences in enrollment by time of year, we also compared incidence by using monthly insurance enrollment figures and incidence among the subset of persons enrolled for the entirety of each year. A similar monthly pattern remained, including the notable spike in January. When stratified by age group, patients 10–49 years of age were most commonly given their diagnosis in January, whereas patients ≤9 years and ≥50 years of age were most commonly given their diagnosis during August–November.

Comparisons over Time

During the 3-year period of 2005–2007, a total of 2,881 patients were given a CSD diagnosis (incidence 5.5 cases/100,000 population/year). During 2011–2013, a total of 5,522 patients received a CSD diagnosis (incidence 4.4 cases/100,000 population/year). The MarketScan database included records from substantially more patients during the latter period, hence the lower overall incidence despite a greater number of patients with CSD.

The proportion of patients with CSD who were hospitalized increased from 3.5% in 2005–2007 to 4.2% in 2011–2013. Patient sex or residence in a rural area did not differ significantly between the 2 periods. Patients with CSD in 2011–2013, as opposed to 2005–2007, were significantly less likely to be ≤14 years of age (RR 0.79, 95% CI 0.74–0.84), to have been given their diagnosis during August–November (RR 0.92, 95% CI 0.87–0.97), and to be from southern states (RR 0.76, 95% CI 0.73–0.79).

Estimated Cost and Extrapolation to the US Population

The mean cost of care for outpatients with CSD was $244/patient (median $100; interquartile range [IQR] $68–$169). The mean cost of an inpatient admission plus follow-up care for CSD was $13,663 (median $8,525; IQR $5,535–$15,273). Median length of stay for inpatient admissions was 3 days (IQR 2–5 days).

After directly standardizing age- and region-specific CSD incidence rates to the US population, we estimated that each year 12,000 outpatient diagnoses are made among patients <65 years of age. In addition, each year ≈500 patients <65 years of age are hospitalized for CSD. Using

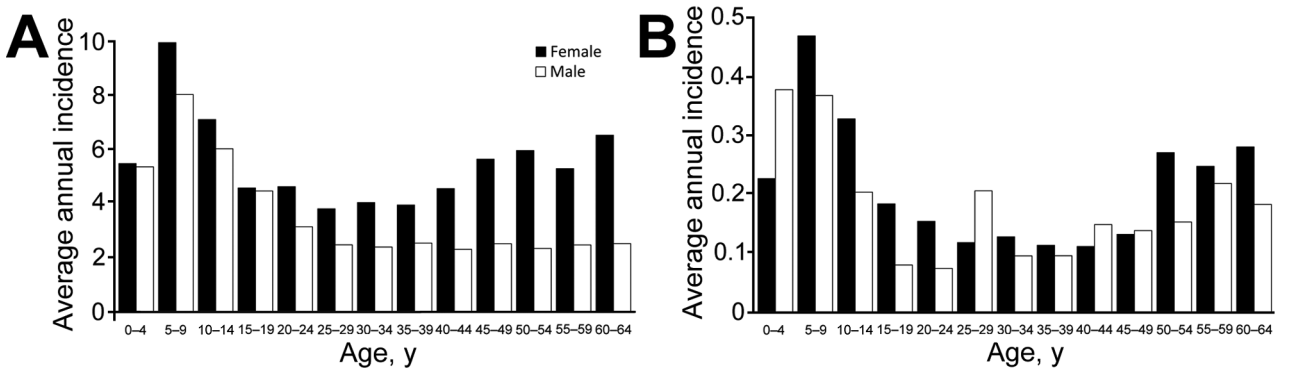

Figure 3. Age- and sex-specific incidence (cases/100,000 population) of cat-scratch disease outpatient diagnoses (A) and inpatient admissions (B), United States, 2005–2013.

these estimates, we calculated the total annual cost of CSD among persons <65 years of age to be $2,928,000 for outpatients and $6,832,000 for inpatients. Thus, in the United States, the total direct medical costs for CSD are estimated to be $9,760,000/year.

Discussion

Using data from a large national health insurance claims database, we estimated that the annual incidence of CSD is 4.7 per 100,000 persons <65 years of age and that a total of 12,500 patients in this age group receive a CSD diagnosis each year in the United States. The highest rates of outpatient diagnoses and inpatient admissions for CSD occur among children 5–9 years of age.

The epidemiology of CSD inpatient admissions described in this study was in some regards similar to that found by previous studies of national hospitalization databases (*6*,*7*). For example, we found that cases occurred predominantly in southern states and during the late summer and fall. Furthermore, incidence of outpatient diagnoses and inpatient admissions in the MarketScan database was highest among children ≤14 years of age. We also found, however, that children ≤14 years of age accounted for a smaller proportion of inpatient admissions (35.5%) than that found by the Jackson et al. analysis of hospital databases (45%–50%) (*6*). This difference is surprising given that the MarketScan databases include information on insured persons <65 years of age only, so the proportion of children with CSD in our analysis is artificially inflated.

Although very little data on incidence of CSD in outpatients have been published, our estimate (4.5 cases/100,000 population) is similar to that reported from an outpatient survey conducted in the Washington, DC, metropolitan area (3.3 cases/100,000 population) (*13*). Although Jackson et al. reported a higher estimated incidence (9.3 cases/100,000 population), this extrapolation was based on only 10 patients identified in an ambulatory care database (*6*). The incidence of outpatient diagnoses—but not inpatient admissions— in the MarketScan database clearly and steadily decreased during the study period and should be further evaluated by use of other data sources.

Incidence of inpatient admissions reported in this study (0.19/100,000 population) is substantially lower than that reported in 2 studies of national hospitalization databases (0.60–0.86/100,000). Although Jackson et al. and Reynolds et al. used different data sources, these studies relied on the same basic method of using ICD-9-CM code 078.3 to identify cases. The reported incidences in both previous studies, however, had wider variation and were limited by smaller sample sizes (*6*,*7*).

The lower incidence of inpatient admissions found by our study is surprising, given that the number of US

Table. Demographic characteristics of patients with cat-scratch disease, United States, 2005–2013*

Characteristic	Inpatients, no. (%) n = 538	Outpatients, no. (%) n = 12,735	Risk ratio (95% CI)
Male sex	239 (44.4)	4,842 (38.0)	**1.17 (1.06–1.29)**
Age, y			
Child, ≤14	191 (35.5)	4,128 (32.4)	1.10 (0.97–1.23)
Older adult, 50–64	157 (29.2)	2,971 (23.3)	**1.25 (1.09–1.43)**
Month of diagnosis			
Aug–Nov (late summer and fall)	233 (43.3)	4,764 (37.4)	**1.16 (1.05–1.29)**
Jan	54 (10.0)	1,305 (10.2)	0.98 (0.76–1.27)
Residence in southern state†	314 (58.4)	6,828 (53.6)	**1.09 (1.01–1.17)**
Residence in rural area‡	118 (21.9)	3,056 (24.0)	0.91 (0.78–1.08)

*Boldface indicates statistical significance between comparison groups.
†Includes states in the South Atlantic, East South Central, and West South Central divisions (Figure 2).
‡Defined as residence of primary beneficiary outside of a Metropolitan Statistical Area.

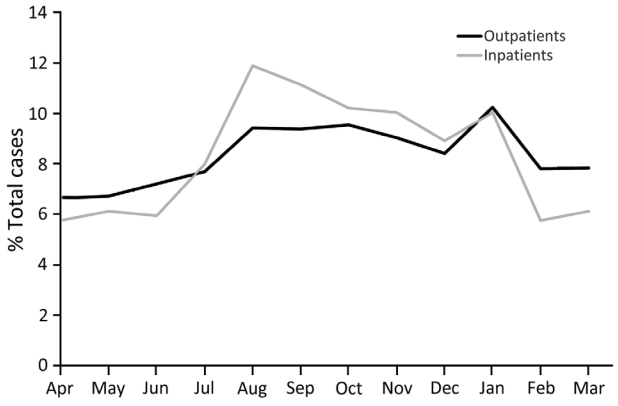

Figure 4. Seasonal variation of cat-scratch disease outpatient diagnoses and inpatient admissions, United States, 2005–2013.

households with cats has increased in recent decades to an all-time high of 45 million (*14*). The overall proportion of CSD patients who were hospitalized may be artificially low because our analysis did not include patients ≥65 years of age, who typically have more comorbidities and thus are more likely to be hospitalized. It is also possible that patients with atypical manifestations of CSD were given ICD-9-CM codes for specific clinical conditions (e.g., encephalitis) but not the underlying etiology and so were not included according to our search criteria.

Greater availability and efficacy of flea control products may have reduced risk for *B. hensalae* transmission to humans. Furthermore, while laboratory diagnosis of CSD has improved in recent decades—which may bolster the calculated incidence of CSD—diagnostic capability for other causes of lymphadenopathy (e.g., neoplasms) has also advanced and may have prevented overdiagnosis of CSD.

It is unclear why an unusually high number of cases occurred in January. Jackson et al. also observed an increase in hospitalizations during January, although the increase was not as striking (*6*). A study of Lyme disease, which used the same MarketScan database, did not show an unusual peak in January, suggesting that it is not artifact from changes in insurance coverage at the beginning of each year (*15*). Kittens are typically born in the spring and adopted during the summer (*16*), and kittens 0–6 months of age have been shown to be at greatest risk for *B. henselae* bacteremia. Conversely, *C. felis* flea abundance peaks during fall and winter, and the highest risk for *B. henselae* bacteremia among cats is during winter (*17*,*18*). These seasonal variations elucidate the reasons for increased incidence of CSD during the fall but do not fully explain the decrease in December and subsequent peak in January.

One hypothesis to explain the January peak is that cats are adopted from shelters more often during the holiday season, for sentimental reasons or as gifts. However, we are unaware of any comprehensive data on cat adoption statistics by month, so assessing this relationship is difficult. Of note, stratified analysis showed that the January peak occurred primarily among patients 10–49 years of age. Another hypothesis is that teenagers and middle-aged adults, who typically spend a great deal of time at school or work, spend more time during the holidays at home in contact with their cats or traveling to other houses with cats. Also, cats spend more time indoors as temperatures decrease during the winter. These and other potential explanations for the unexpected January peak in disease should be explored further.

In contrast to several previous studies that reported an overall predilection of CSD among male patients (*6*,*7*,*19*,*20*), our analysis indicated that only 44.4% of inpatients and 38.0% of outpatients were male. The reasons for this discrepancy are unclear and should be explored further. Compared with outpatients, inpatients were more likely to be male (although a minority of inpatients were male), be 50–64 years of age, and reside in the South. These differences could results from risk factors for severe disease that should be explored further. Differences could also result from more accurate diagnosis for inpatients, rather than true epidemiologic differences.

Our study has several limitations. First, the case definition relies on diagnosis by clinicians and subsequent coding by clinicians or billing specialists, both of which are subject to error. For example, the 078.3 code could have been inappropriately used for care of a cat-scratch wound but not actual CSD. Also, in some cases, the 078.3 code may have been recorded as a rule-out diagnosis when CSD was not actually confirmed. To our knowledge, there are no data on the sensitivity and specificity of the 078.3 code for CSD. Furthermore, the MarketScan database is a convenience sample and does not include insurance claims from

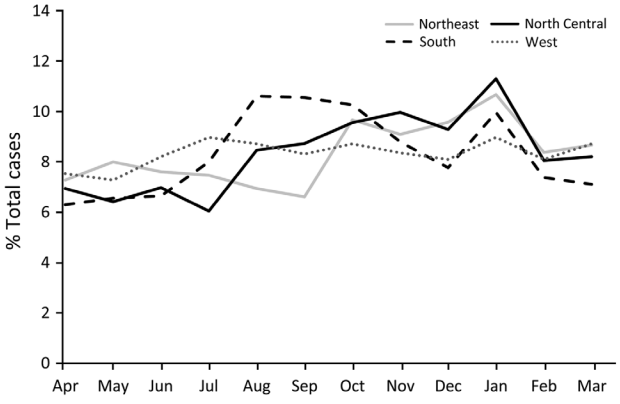

Figure 5. Seasonal variation of cat-scratch disease diagnoses by region, United States, 2005–2013. Northeast region = New England and Middle Atlantic divisions; North Central = East North Central and West North Central; South = South Atlantic, East South Central, and West South Central; West = Mountain and Pacific (divisions shown in Figure 2).

persons ≥65 years of age, military personnel, uninsured persons, or Medicaid/Medicare enrollees, among whom risk for CSD may differ. Last, the calculated medical cost of CSD is probably an underestimate because some CSD-related visits and procedures may have been missing the 078.3 code and we did not account for indirect costs such as time away from work.

CSD causes a substantial burden of disease nationwide and disproportionately affects children. Because CSD is a zoonotic infection that is maintained and spread among cats by fleas, comprehensive flea control for cats can help reduce the risk for human infection. Risk may also be reduced by washing hands after contact with cats, to remove potentially infectious flea feces that could enter breaks in the skin. Furthermore, because cats that hunt outdoors are at substantially greater risk for *B. henselae* bacteremia (*17*), limiting hunting activity of cats may reduce risk for human infection. Educational efforts should focus on cat owners, particularly those with children in the household or those with immunocompromising conditions. Additional research is warranted to elucidate the reasons for epidemiologic differences noted in this study and risk factors for severe disease.

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Dr. Nelson is a medical epidemiologist at the Bacterial Diseases Branch, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado. Her primary research interests are the epidemiology and clinical manifestations of Lyme disease, *Bartonella* infections, tularemia, and plague.

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Address for correspondence: Christina Nelson, Centers for Disease Control and Prevention, 3156 Rampart Rd, Mailstop P02, Fort Collins, CO 80521, USA; email: wje1@cdc.gov

Community- and Healthcare-Associated *Clostridium difficile* Infections, Finland, 2008–2013[1]

Saara M. Kotila, Silja Mentula, Jukka Ollgren, Anni Virolainen-Julkunen, Outi Lyytikäinen

We evaluated incidence, case-fatality rate, and trends of community-associated (CA) and healthcare-associated (HA) *Clostridium difficile* infections (CDIs) in Finland during 2008–2013. CDIs were identified in the National Infectious Disease Register, deaths in the National Population Information System, hospitalizations to classify infections as CA or HA in the National Hospital Discharge Register, and genotypes in a reference laboratory. A total of 32,991 CDIs were identified: 10,643 (32.3%) were CA (32.9 cases/100,000 population) and 22,348 (67.7%) HA (69.1/100,000). Overall annual incidence decreased from 118.7/100,000 in 2008 to 92.1/100,000 in 2013, which was caused by reduction in HA-CDI rates (average annual decrease 8.1%; p<0.001). The 30-day case-fatality rate was lower for CA-CDIs than for HA-CDIs (3.2% vs. 13.3%; p<0.001). PCR ribotypes 027 and 001 were more common in HA-CDIs than in CA-CDIs. Although the HA-CDI incidence rate decreased, which was probably caused by increased awareness and improved infection control, the CA-CDI rate increased.

Clostridium difficile is a common cause of antimicrobial-associated diarrhea in Finland (*1*) and elsewhere in Europe and Northern America (*2*,*3*). Dissemination of *C. difficile* genotypes with different virulence properties contributes to *C. difficile* infection (CDI) epidemiology (*4*–*6*). PCR ribotype 027 has been associated with more severe CDI outcomes (*7*–*9*), but not all studies have confirmed this finding (*10*). In Finland, hospitalizations associated with CDIs doubled during 1996–2004 (*11*). CDI laboratory-based surveillance was initiated in Finland in 2008, simultaneously with strengthening of infection control according to the European recommendations in several regions (*8*,*12*). During 2008–2010, a 24% reduction was observed in overall CDI incidence in Finland (*13*).

CDI is typically a healthcare-associated (HA) disease, but there are indications that a notable proportion of cases are not associated with recent healthcare exposure (*14*–*16*). Some studies have shown that the incidence and severity of community-associated (CA) CDIs have been increasing (*17*). In Finland, the proportion of CA-CDIs among hospitalized patients in 16 acute-care hospitals was 16% during 2008–2010 (*13*).

The purpose of this study was to compare CA-CDI with HA-CDI in terms of population-based incidence, case-fatality rates, and trends in Finland during 2008–2013. We obtained data from national registers and genotyping results from a reference laboratory.

Methods

In Finland (population 5.5 million), the national healthcare system is organized into 21 geographically and administratively defined healthcare districts, which have populations ranging from 28,700 to 1.6 million. Sixteen healthcare districts have primary-care and secondary-care hospitals, and 5 provide tertiary-care services.

Since 2008, CDI reporting has been mandatory and all microbiology laboratories in Finland report *C. difficile* findings (positive cultures, toxin production, presence of toxin genes) for stool samples electronically to the National Infectious Disease Register (NIDR) (*8*). Each notification includes specimen date, each person's unique national identity code, date of birth, sex, and place of residence. In 2008, all laboratories used methods for detecting both TcdA and TcdB and 87% (20/23) used culture of *C. difficile*; 3 laboratories had started to use nucleic acid amplification tests (NAATs) for primary diagnostics (*18*). During 2011–2013, five laboratories were using NAATs as primary diagnostics tests.

The National Hospital Discharge Register is a civil register comprising comprehensive healthcare records provided by all hospitals and primary-care wards in Finland, including outpatient surgery (i.e., day surgery). Each record includes the patient's national identity code, admission and discharge dates, healthcare provider code, type of service, specialty, and place (home or institution) from which the patient came to the hospital.

Since 2008, clinical microbiology laboratories have been requested to send *C. difficile* isolates from severe cases (CDI-related intensive care, colectomy, or death)

Author affiliation: National Institute for Health and Welfare, Helsinki, Finland

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(*4*,*12*,*19*) and persistent outbreaks to the national reference laboratory for genotyping. All isolates received by the reference laboratory during 2008–2013 were PCR ribotyped. PCR ribotyping was performed according to the protocol of the Anaerobe Reference Laboratory (Cardiff, UK) (*20*) and by using the Cardiff-European Centre for Disease Prevention and Control (Solna, Sweden) culture collection as reference strains. After gel electrophoresis, band patterns were analyzed by using BioNumerics 3.0–6.6 software (Applied Maths NV, Sint-Martens-Latem, Belgium).

For this study, all notifications of toxin-positive *C. difficile* accompanied by an appropriate national identity code during 2008–2013 were extracted from the NIDR. Using a 3-month time interval, we merged multiple notifications for the same person as a single episode. A total of 32 reports without an appropriate national identity code and 312 reports for persons <1 year of age were excluded. Data from the National Population Information System for 2008–2013 were used as denominators to calculate annual incidence rates and age- and sex-specific average annualized incidence rates, including incidence rate ratios with 95% CIs. Dates of deaths were obtained from the National Population Information System by using the national identity code. Case-fatality rates were calculated by dividing all deaths from any cause ≤30 days after a positive diagnostic result for CDI was obtained by the total number of CDIs.

We considered as significant p values <0.05 without Bonferroni corrections, as per Fisher exact test and χ^2 test for comparing proportions of PCR ribotypes in CA-CDIs and HA-CDIs. Poisson regression was used to assess whether secular trends in the incidence rates were significant.

On the basis of specimen date for *C. difficile* and national identity code, data for hospitalizations before the *C. difficile*-positive specimen date were obtained from the hospital discharge register. An episode of CDI was classified as HA if the positive specimen was obtained >2 days after admission to a hospital or ≤4 weeks after discharge and as CA otherwise (obtained outside a hospital, >4 weeks after hospital discharge, or ≤2 days after admission). Episodes of CDI among residents in long-term care facilities (LTCFs) could be classified as HA only if residents were transferred to a hospital and the positive specimen was obtained ≤2 days after admission. PCR ribotyping data were linked to NIDR data by using the patient's date of birth and healthcare district if the date of the specimen was ≤3 months of the date used for statistics reported to NIDR.

Permission to analyze and link data from the NIDR and the National Hospital Discharge Register was granted by the Ethics Research Committee of the National Institute for Health and Welfare. Because data were already anonymous, informed consent of patients was waived.

Results

During the 6-year study period, a total of 32,991 incident episodes of CDI (range by year 5,021–6,320) were identified among 29,577 persons. Of the 32,991 CDIs, 10,643 (32.3%) were classified as CA (32.9/100,000 population) and 22,348 (67.7%) as HA (69.1/100,000 population, 3.2/10,000 patient-days).

Of the 10,643 CA-CDIs, 3,166 (29.7%) were among patients whose positive *C. difficile* specimen date was ≤2 days after admission. Of the 22,348 HA-CDIs, 16,319 (73.0%) were hospital onset (positive specimen date >2 days after hospital admission) and 4,813 (21.5%) were community onset (positive specimen date ≤4 weeks after hospital discharge). The remaining 1,216 (5.4%) HA-CDIs were in patients transferred from another healthcare institution. For hospital-onset HA-CDIs, median time from hospital admission to positive specimen date was 13 days (range 3–3,785 days), which was similar to that for community-onset HA-CDIs, for which median time from hospital discharge was 13 days (range 1–28 days). Of 4,813 community-onset HA-CDIs, 2,730 (56.7%) were among patients whose positive *C. difficile* specimen date was ≤2 days after hospital admission.

The average annualized incidence rate for CA-CDIs among persons 15–44 years of age was higher than that for HA-CDIs in the same age group (rate ratio 0.5, 95% CI 0.4–0.7). HA-CDI was most common among persons >45 years of age (Table 1). Overall, the CA-CDI rate for female patients was 1.5 times higher than that for male patients (rate ratio 1.5, 95% CI 1.5–1.6). For persons 15–44 years of age, this difference by sex was ≈2-fold (rate ratio 1.8, 95% CI 1.7–2.0). Although the overall HA-CDI rate was higher for female patients (rate ratio 1.3, 95% CI 1.2–1.3), for persons 45–84 years of age, the rate was higher for male patients.

The overall annual incidence rate of CDI decreased significantly from 118.7/100,000 population in 2008 to 92.1/100,000 in 2013 (average annual decrease 4.2%; p<0.01) (Figure 1). The reduction was caused by the decreasing rate of HA-CDI (annual decrease 8.1%; p<0.001). Regionally, the HA-CDI rate decreased for 6 of the 21 healthcare districts and increased in 1 small healthcare district, from 100.3/100,000 population in 2008 to 150.0/100,000 in 2013. The annual incidence rate of CA-CDI increased slightly, from 30.8/100,000 population in 2008 to 37.5/100,000 in 2013 (average annual increase 4.3%; p<0.01). The increase was caused mostly by the increasing trend in persons >74 years of age (Figure 2). The CA-CDI rate increased in 12 healthcare districts, including the healthcare district that showed an increasing HA-CDI trend, and decreased in 1 healthcare district.

Of all CDI episodes during 2008–2013, a total of 3,318 (10.1%) resulted in death within 30 days. The 30-day

Table 1. Incidence of community-associated and healthcare-associated *Clostridium difficile* infections in patients, by age and sex, Finland, 2008–2013*

	Community-associated						Healthcare-associated					
	Female sex			Male sex			Female sex			Male sex		
Patient age, y	No.	No. person-years	Rate	No.	No. person-years	Rate	No.	No. person-years	Rate	No.	No. person-years	Rate
1–14	252	2,612,610	9.6	248	2,729,947	9.1	126	2,612,610	4.8	152	2,729,947	5.6
15–44	1,551	5,881,383	26.4	886	6,163,161	14.4	578	5,881,383	9.8	533	6,163,161	8.6
45–64	1,655	4,593,089	36.0	1,188	4,554,108	26.1	1,524	4,593,089	33.2	2,047	4,554,108	44.9
65–74	950	1,682,708	56.5	755	1,472,378	51.3	1,917	1,682,708	113.9	2,232	1,472,378	151.6
75–84	1,195	1,176,883	101.5	680	767,551	88.6	4,325	1,176,883	367.5	3,140	767,551	409.1
>84	944	510,899	184.8	339	187,511	180.8	4,224	510,899	826.8	1,550	187,511	826.6
All	6,547	16,457,572	39.8	4,096	15,874,656	25.8	12,694	16,457,572	77.1	9,654	15,874,656	60.8

*Rate is the average annualized incidence rate (episodes/100,000 population).

case-fatality rate was lower for CA-CDIs than for HA-CDIs (3.2% vs. 13.3%; p<0.001), and a difference was observed for all age groups (Table 2). The case-fatality rate for CA-CDI among patients 45–64 years of age was higher for male patients than for female patients, and the case-fatality rate for HA-CDI among patients >64 years of age was higher for male patients than for female patients. The 30-day case-fatality rate was highest for patients with hospital-onset HA-CDIs (15.5%) and lowest for patients with CA-CDIs (2.0%) who had no prior hospitalization since the start of the study in 2008. The 30-day case-fatality rates for CA-CDI and HA-CDI were 3.1% and 14.5%, respectively, in 2008, and 3.7% and 12.7%, respectively, in 2013. The decrease in the case-fatality rate for HA-CDI was significant (p = 0.001), but the case-fatality rate for CA-CDI remained constant.

During 2008–2013, a total of 16/21 healthcare districts sent 1,523 *C. difficile* isolates for PCR ribotyping. A total of 1,193 *C. difficile* isolates could be linked to CDI episodes in the NIDR data (3.6%), of which 283 were CA and 910 were HA. Among CA and HA isolates, 67 and 99 PCR ribotype patterns were identified, respectively. In both groups, the most frequently identified PCR ribotype was 027. Among the 10 most common PCR ribotypes for the 1,193 isolates (Table 3), PCR ribotypes 027 and 001 were more common among HA isolates, and ribotype 078 was more common among CA isolates. Reasons for requesting typing (severe case or persistent outbreak) were not systematically indicated for isolates, and only 56 were designated to originate from severe cases, of which 43 were HA and 13 were CA. In this subgroup, the most commonly implicated PCR ribotypes were 001 and 027.

Discussion

Our nationwide population-based study aimed to estimate CA-CDI incidence and case-fatality rates for Finland. One third of all CDIs were CA. The overall CDI rate decreased during the study, driven by the decreasing rate of HA-CDI. The CA-CDI rate increased slightly, mostly for elderly persons.

As reported by Lessa et al. in a recent study that assessed CDI burden in the United States (*21*), the comparability of current results with previously published CDI rates is limited by several factors, including differences in CDI definitions and emergence of high-sensitivity NAATs. In our study, an episode of CDI was classified as HA if the positive specimen was obtained ≤4 weeks after hospital discharge or >2 days after admission and as CA otherwise, in accordance with the European CDI surveillance protocol (*22*). CDIs for which the positive specimen was obtained >4 weeks but <12 weeks after hospital discharge were considered to be CA. If, for better comparability with other population-based CA-CDI studies, the time frame of HA-CDI definition were expanded from 4 weeks to 12 weeks after hospital discharge, the CA-CDI rate would be 24.3 cases/100,000 population (23.8% of all CDIs).

However, our results cannot be compared directly with those of most other studies, which have either separated LTCF residents or combined them with the HA-CDI category. The proportion of CA-CDIs in Finland was lower

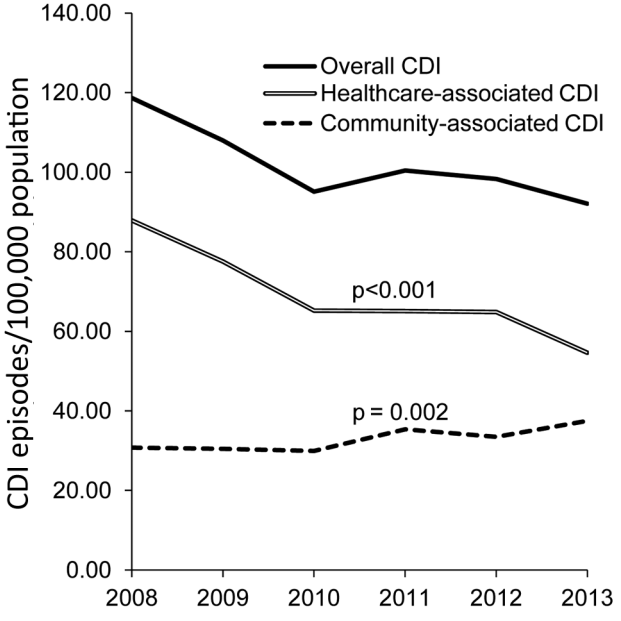

Figure 1. Annual incidence rates of community-associated, healthcare-associated, and overall CDI, Finland, 2008–2013. CDI, *Clostridium difficile* infection.

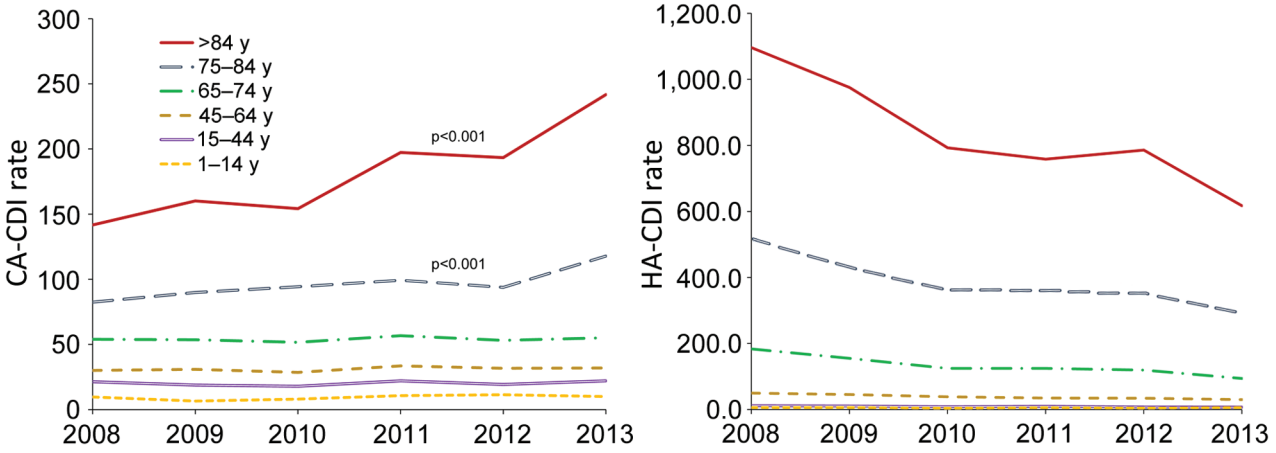

Figure 2. Trends in CDI rates by age group, Finland, 2008–2013. A) CA-CDI; B) HA-CDI. The decrease in the HA-CDI rate was statistically significant (p<0.001) for all age groups except persons 1–14 years of age. CA, community-associated; CDI, *Clostridium difficile* infection; HA, healthcare-associated.

than in a population-based study that included 10 geographic areas across the United States that participated in the Emerging Infections Program (total population of 11.2 million persons [34.0%] in 2011) (*21*) and in a study in Manitoba, Canada, in 2005–2006 (27%) (*15*). The proportion of CA-CDIs in Finland would have been even lower if we had been able to classify all CDIs of LTCF residents as HA. The CA-CDI rate was at a comparable level in Finland as in Manitoba, where the rate was 23.4 cases/100,000 population. In 10 geographic areas across the United States, the pooled mean crude incidence of CA-CDI was considerably higher (48.2 cases/100,000 population).

In the United States, ≈50% of the 121 laboratories participating in the Emerging Infections Program were using NAATs in 2011 (*21*,*23*). In microbiology laboratories in Finland, the large-scale transition to NAATs took place after the study period in 2014; the proportion of cases diagnosed by using NAATs for CDIs reported to the NIDR increased from <6% in 2013 to 33% in 2014 (*1*). Before use of NAATs, culture and antigen tests were most commonly used in parallel, which indicates that the change in sensitivity has been less drastic than if the antigen tests had been used alone previously. The later transition to NAATs in laboratories in Finland might partially explain the difference in CDI rates compared with those for the United States, but not the difference between proportions of CA-CDIs and HA-CDIs.

Lower levels of antimicrobial drug use could explain to some extent why the CDI rate is lower in Finland (*11*,*24*). It is also likely that awareness of the CDI problem started earlier in the United States than in Finland, which would have influenced diagnostic activity (*11*,*18*). In Finland, ≤3-fold differences still exist between healthcare districts (unpub. data). Data for the Netherlands and Denmark suggest that current estimations of CA-CDI incidence are largely underestimated because of low diagnostic activity (*25*–*27*).

As reported in previous studies in other countries (*14*,*28*), patients with CA-CDIs in Finland were younger and more likely to be female. In other countries, increased antimicrobial drug use and different use patterns (e.g., treatment of urinary tract infections) have been observed for women (*29*,*30*), which potentially explains the high rate of CA-CDIs in young women. In Finland, the level of fluoroquinolone use has been associated with regional differences in CDI rates; these drugs are used mostly in outpatient

Table 2. Thirty-day case-fatality rates for patients with community-associated and healthcare-associated *Clostridium difficile* infections, by age and sex, Finland, 2008–2013*

	Community-associated					Healthcare-associated				
	Female sex		Male sex			Female sex		Male sex		
Age, y	CFR, % (no.)	No. episodes	CFR, % (no.)	No. episodes	p value	CFR, % (no.)	No. episodes	CFR, % (no.)	No. episodes	p value
1–14	0.4 (1)	252	0.0 (0)	248	1.0	0.8 (1)	126	3.3 (5)	152	0.2
15–44	0.1 (1)	1,551	0.1 (1)	886	1.0	2.1 (12)	578	3.2 (17)	533	0.3
45–64	0.9 (15)	1,655	2.5 (30)	1,188	0.001	7.0 (106)	1,524	8.6 (177)	2,047	0.07
65–74	2.6 (25)	950	4.0 (30)	755	0.1	7.9 (152)	1,917	13.0 (291)	2,232	<0.001
75–84	4.8 (57)	1,195	6.2 (42)	680	0.2	13.3 (574)	4,325	16.5 (518)	3,140	<0.001
>84	10.7 (101)	944	9.7 (33)	339	0.7	17.9 (758)	4,224	23.9 (371)	1,550	<0.001
All	3.1 (200)	6,547	3.3 (136)	4,096	0.5	12.6 (1,603)	12,694	14.3 (1,379)	9,654	<0.001

*Values in parentheses are total no. deaths in category. CFR, case-fatality rate.

Table 3. Ten most common PCR ribotypes for *Clostridium difficile* strains with known community or healthcare associations, Finland, 2008–2013

Ribotype	No. (%) strains		
	Community-associated, n = 283	Healthcare-associated, n = 910	All, N = 1,193
027	30 (10.6)	237 (26.0)	267 (22.4)
001	25 (8.8)	154 (16.9)	179 (15.0)
014	19 (6.7)	76 (8.4)	95 (8.0)
023	24 (8.5)	50 (5.5)	74 (6.2)
002	21 (7.4)	42 (4.6)	63 (5.3)
020	20 (7.1)	41 (4.5)	61 (5.1)
078	18 (6.4)	27 (3.0)	45 (3.8)
005	13 (4.6)	25 (2.7)	38 (3.2)
018	12 (4.2)	25 (2.7)	37 (3.1)
011	9 (3.2)	19 (2.1)	28 (2.3)
Other	92 (32.5)	214 (23.5)	306 (25.6)

care (*31*). Several possible risk factors for CA-CDIs have been suggested, including use of proton pump inhibitors, food contaminated with *C. difficile*, person-to-person and zoonotic transmission, and outpatient healthcare exposure (*32*–*35*). The increase in the CA-CDI rate in Finland was caused mostly by infections in persons >64 years of age, which might represent elderly persons living in the community or in LTCFs. However, the current trend in Finland is to move elderly patients from LTCFs and nursing homes to different types of home care services.

Since 2000, the burden of CDI has increased in North America and in many parts of Europe (*4*,*36*). However, in England and Ontario, Canada, this increasing trend has been overcome by a reduction in HA-CDI rates because of enhanced surveillance and improved control measures (*37,38*). We also observed a decrease in the HA-CDI rate in Finland since 2008.

In our study, the 30-day case-fatality rate for CA-CDIs was 3.2%. This rate is higher than the 30-day mortality rate estimated for CA-CDIs in the United States (1.3%) (*21*), most likely because our CA-CDI category included episodes in LTCF residents who probably have several concurrent illnesses and are of an advanced age, but lower than the case-fatality rate (4%) in Örebro, Sweden, earlier during 1999–2000 (*16*). In the study in the United States, CDI was considered to be HA ≤12 weeks after discharge. In the study in Sweden, patients were followed up for 6–18 months, and case-patients who were not hospitalized in the preceding 60 days were classified as having CA-CDI.

One third of the CA-CDIs in our study were detected in hospitals ≤2 days after admission. However, we do not know whether CDI was the reason for hospitalization. In Olmsted, Minnesota, USA (*14*) and Connecticut, USA (*28*), hospitalization rates of 40% and 46%, respectively, were observed for CA-CDI patients. However, in both studies the definition of CA-CDI was more exclusive than in our study; this definition considered community-onset case-patients who were not hospitalized in the preceding 3 months (i.e., ≈12 weeks) as having CA-CDIs.

In Finland, the molecular surveillance of CDI aims to support nosocomial outbreak investigations and identify PCR ribotypes that cause severe disease. Thus, only a fraction of isolates are PCR ribotyped. For this study, PCR ribotype was known for only a small, unrepresentative proportion of CDIs, especially with regard to CA-CDI. As in the United States (*21*), hypervirulent PCR ribotype 027 was detected in HA-CDIs and CA-CDIs but was more commonly found in HA-CDIs. PCR ribotype 001 was the second most common ribotype in Finland and was also prevalent in many other countries in Europe during 2008 (*39*). PCR ribotype 078, which has similar genetic properties to hypervirulent type 027 (deletions in the *tcdC* toxin regulator gene), was more common among CA-CDIs than HA-CDIs. This type has been associated with CA-CDI and has severity similar to PCR ribotype 027 in the Netherlands (*6*).

Our study has several limitations. First, there is no national register of all LTCFs in Finland like that for hospitals. Thus, we were not able to classify all LTCF residents with CDIs as HA-CDI; this classification could be made only if residents were transferred to a hospital. This limitation made comparison of the CA-CDI proportion, rate, and case-fatality rate with those of other studies difficult. Second, we did not have data for concurrent conditions and their severity, which would have been needed to evaluate attributable mortality rates, which are known to be more appropriate measurement of CDI outcome. We assessed only 30-day case-fatality rates and compared them with those of previous studies that reported the same measurement. Third, we could ascertain only inpatient healthcare exposure and day surgery, and not whether patients had visited outpatient healthcare facilities. Fourth, it is probable that not all patients with CDIs, especially persons without traditional risk factors, are tested for CDI, which would lead to an underestimation of CA-CDI incidence. Furthermore, the sensitivity of the CDI diagnosis depends largely on the test and algorithm used (*2*). Conversely, it is possible that patients without diarrhea have been tested for CDI, which would result in erroneous inclusion of asymptomatic *C. difficile* carriers as having cases of CDI and overestimation of CDI incidence rate. Moreover, we used the positive specimen date for CDI as a proxy indicator for date of symptom onset, which is also used to determine the origin of CDI cases in the interim CDI case definition for surveillance (*4*). Fifth, the definition used for a CDI episode in our National Infectious Disease Register, which combines multiple reports with a 3-month time interval, might include recurrences and relapses.

One third of CDIs in Finland diagnosed during the 6-year study were CA. Although the HA-CDI rate decreased at the national level, probably in response to

improved infection control measures and increased awareness, the CA-CDI rate increased slightly. Prudent use of antimicrobial drugs in outpatient settings, especially for elderly persons, is necessary to reduce the CA-CDI burden, and preventive efforts, such as antimicrobial stewardship campaigns, should also cover long-term care and outpatient settings.

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Ms. Kotila is a research scientist at the National Institute for Health and Welfare, Helsinki, Finland. Her research interests include epidemiology and molecular typing of infectious agents, particularly *Clostridium difficile* and foodborne and waterborne pathogens.

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Address for correspondence: Saara M. Kotila, National Institute for Health and Welfare, Mannerheimintie 166, 00271 Helsinki, Finland; email: saara.kotila@gmail.com

Carbapenem Resistance in Clonally Distinct Clinical Strains of *Vibrio fluvialis* Isolated from Diarrheal Samples

Goutam Chowdhury, Gururaja Perumal Pazhani, Anirban Sarkar, Krishnan Rajendran, Asish K. Mukhopadhyay, Mihir K. Bhattacharya, Amit Ghosh, Thandavarayan Ramamurthy

Carbapenems have been used for many years to treat severe nosocomial *Enterobacteriaceae* infections. The spread of resistance to these drugs among other bacterial families is an emerging problem worldwide, mostly caused by New Delhi metallo-β-lactamase (NDM-1). We screened for the prevalence of NDM-1–expressing enteric pathogens from hospitalized patients with acute diarrhea in Kolkata, India, and identified 27 *Vibrio fluvialis*–harboring bla_{NDM-1} (NDM-VF) strains. These isolates were also resistant to all the tested antimicrobial drugs except doxycycline. The large plasmid of *V. fluvialis* harboring bla_{NDM-1} could be easily transferred to other enteric pathogens. Genes flanking the bla_{NDM-1} were found to be identical to the reported sequence from an *Escherichia coli* isolate. Analyses showed that the *V. fluvialis* possessing the NDM-VF region belonged to different clones. The pathogenicity of *V. fluvialis* to humans and its ubiquitous presence in the environment call for constant monitoring of this species for emerging antimicrobial drug resistance.

The increasing incidence of carbapenem-resistant bacterial infection is a major public health concern (*1*). Several species of carbapenemase-producing bacteria also display co-resistance to most, if not all, available antibiotic drugs used against different infections, thereby limiting the medication options (*1*). The novel carbapenemase New Delhi metallo-β-lactamase (NDM-1), encoded by the gene bla_{NDM-1}, has been identified in many pathogenic members of the family *Enterobacteriaceae*, which are capable of colonizing hosts and also transfer the bla_{NDM-1} gene region to other bacteria. Several of these bacteria have been associated with contaminated hands, food, and water in hospitals, community settings, and in the environment (*1*). However, reports on the prevalence of bla_{NDM-1} among enteric pathogens are relatively fewer.

NDM-1–producing *Klebsiella pneumoniae* was first identified in 2008 in a urine sample from a traveler from Sweden who acquired a urinary tract infection in India (*2*). Investigations by Kumarasamy et al. (*3*) led to the initial report of widespread prevalence of NDM-1 in *Escherichia coli* and *K. pneumoniae* strains isolated from several clinical settings in India, Pakistan, and the United Kingdom. Numerous studies in subsequent years reported NDM-1–producing *Enterobacteriaceae* and other bacteria, including *Vibrio cholerae* in many countries (*4*–*7*). Recently, several reports on carbapenemase-producing *Enterobacteriaceae* in India have been published (*8*–*10*). In unrelated gram-negative bacteria, the presence of the bla_{NDM-1} gene has been reported to be associated with several plasmid incompatibility types (e.g., IncA/C, IncF, IncL/M, IncH, or untypeable) or was found integrated into the chromosomes (*11*). Because the gene bla_{NDM-1} located on plasmids is also carrying bacterial growth promoter regions, the possibility of gene transfer to other gram-negative bacteria is very high (*12*).

V. fluvialis is known to be commonly present in many aquatic environments and seafood (*13*). This organism has been reported as an emerging pathogen associated with cholera-like diarrhea in India and China (*14*,*15*). We report the identification and characterization of NDM-1–producing *V. fluvialis* strains isolated from diarrheal fecal samples from patients admitted to the 2 hospitals in Kolkata, India.

Materials and Methods

Using systematic active surveillance, we enrolled every fifth hospitalized patient at the Infectious Diseases Hospital (IDH) and B.C. Roy Memorial Hospital for Children (BCH) in Kolkata who had diarrhea or dysentery on 2 randomly selected days of the week during May 2009–September 2013. Diarrhea was defined as ≥3 episodes of loose or liquid stools with or without blood within 24

Author affiliations: National Institute of Cholera and Enteric Diseases, Kolkata, India (G. Chowdhury, G.P. Pazhani, A. Sarkar, K. Rajendran, A.K. Mukhopadhyay, M.K. Bhattacharya, A. Ghosh, T. Ramamurthy); National Institute of Pharmaceutical Education and Research, Kolkata (G.P. Pazhani); Translational Health Science and Technology Institute, Faridabad, India (T. Ramamurthy)

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hours, accompanied by dehydration, nausea, vomiting, abdominal cramping, fever, chills, muscle aches, and fecal urgency. A questionnaire that collected demographic information, illness onset and symptoms, medical care sought, and food/drink consumed was completed by the patient or a family member. Patients with other associated illness and who used antibiotic drugs before hospitalization were not included in this study.

Fecal specimens were collected in McCartney bottles (KM Enterprises, Kolkata, India) by using sterile catheters or rectal swabs in Cary Blair medium (Difco, Sparks, MD, USA) and were examined within 2 hours for enteric pathogens comprising bacterial, viral, and parasitic pathogens by using a combination of conventional, immunological, and molecular methods (*16*). Patients were observed until their discharge from the hospital. The patients lived in different areas of the Kolkata Municipal region.

We screened for carbapenem resistance in multidrug-resistant isolates of diarrheagenic *E. coli*, *V. cholerae*, *V. parahaemolyticus*, *V. fluvialis*, *Salmonella* spp., and *Shigella* spp. isolated from these patients. We detected *V. fluvialis* and $bla_{\text{NDM-1}}$ by using simplex PCR with previously described methods, lysed cells as templates (*17*,*18*), and Taq DNA polymerase (Roche, Mannheim, Germany). Amplicons were purified by using a QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany) and sequenced by using the ABI BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) in an automated DNA sequencer (ABI 3730; Applied Biosystems). Sequences were edited with Lasergene software (DNASTAR, Inc., Madison, WI, USA) and analyzed by using BLAST (http://www.ncbi.nlm.nh.gov/blast).

We tested antibiotic susceptibility according to Clinical Laboratory Standards Institute (CLSI) guidelines (*19*) using commercially available antibiotic discs (Becton-Dickinson, Sparks, MD, USA) for ampicillin, cefuroxime, ceftriaxone, cefotaxime, cefotaxime/clavulanic acid, ceftazidime, ceftazidime/clavulanic acid, chloramphenicol, erythromycin, gentamicin, nalidixic acid, ciprofloxacin, ofloxacin, norfloxacin, imipenem, streptomycin, azithromycin, tetracycline, and trimethoprim/sulfamethoxazole. We used ceftazidime and cefotaxime to confirm production of extended-spectrum β-lactamase by double-disk synergy test. We determined MICs of imipenem, ciprofloxacin, norfloxacin, ceftazidime, cefotaxime, and cefepime using Etest strips (bioMérieux, Marcy l'Étoile, France) following the CLSI interpretive criteria for *Vibrio* spp. (*20*). For non-cholera *Vibrio* spp., the CLSI guidelines lack interpretive criteria for some antibiotic drugs; hence, we used breakpoints for *E. coli* ATCC 25922, which was used as a control in antimicrobial drug susceptibility testing.

We performed the modified Hodge test on Mueller–Hinton agar (Difco) plates, using *E. coli* ATCC 25922 as the indicator organism and a 10-μg imipenem disk (*21*). The modified Hodge test is a phenotypic assay for the detection of carbapenemase enzyme–producing bacteria. This assay is based on the inactivation of a carbapenem by carbapenemase-producing test isolates that facilitate a carbapenem-susceptible indicator strain (*E. coli* ATCC 25922) to spread its growth toward a carbapenem-containing disc along the streak of inoculum of the test isolate. A positive test result produces a cloverleaf-like hollow.

We used the Kado and Liu method (*22*) to extract plasmid DNA from donors, recipients, and transconjugants and analyzed it by gel electrophoresis using 0.8% agarose. We used a PCR-generated DNA probe by the chemoluminescent method (ECL nucleic acid detection system; GE Healthcare Life Sciences, Buckinghamshire, UK) to make Southern hybridization to confirm the presence of $bla_{\text{NDM-1}}$ in the plasmids. Plasmid-mediated transfer of antibiotic resistance from a NDM-1–positive *V. fluvialis* isolate (IDH 04744) to *E. coli* J53 (having dual resistant markers for nalidixic acid and sodium azide [Na-Az^{R}]) was tested on MacConkey agar plates (Difco) containing sodium azide (100 mg/L) and meropenem (5 mg/L). Another plasmid-mediated transfer of antibiotic resistance from a NDM-1–positive *V. fluvialis* isolate (IDH 04744) has also been tested with diarrheagenic *E. coli*, *Salmonella* spp., and *Shigella* spp. and *V. parahaemolyticus* on meropenem (5 mg/L) supplemented MacConkey, xylose lysine deoxycholate, and thiosulfate citrate bile sucrose agar (Difco) plates.

Presence of $bla_{\text{NDM-1}}$ in the transconjugants was confirmed by PCR. We used PCR and amplicon sequencing to identify other antibiotic resistance genes (*aadB*, *aadA1*, *strA*, *aphA1–1a*, *catA1*, $bla_{\text{TEM-9}}$, $bla_{\text{OXA-1}}$, $bla_{\text{OXA-7}}$, $bla_{\text{OXA-9}}$, bla_{SHV}, $bla_{\text{PSE-4}}$, $bla_{\text{CTX-M-3}}$, *aac[6']-1b-cr*, and *floR*) using lysed cells, primers, and previously described conditions (*23*). We used published primers to determine integrons and resistance gene cassettes in *V. fluvialis* isolates by PCR (*24*). The PCR amplicons were purified and directly sequenced. The identities of the sequences were established through a database search by using BLAST and matched with the reference *dfrA1* sequence of *V. fluvialis* (GenBank accession no. AY605688).

We determined the replicon types of $bla_{\text{NDM-1}}$ harboring plasmids from the wild isolates and transconjugants by PCR using published methods (*25*). Sequencing of the $bla_{\text{NDM-1}}$ and its flanking regions were made from a wild isolate of *V. fluvialis* (IDH 05720) by primer walking. The DNA sequence reported in this study has been deposited in GenBank (accession no. KR733543).

Pulsed-field gel electrophoresis (PFGE) analysis of *Not*I-digested genomic DNA of $bla_{\text{NDM-1}}$–harboring *V. fluvialis* isolates (NDM-VF) was performed by using a CHEF-Mapper (Bio-Rad Laboratories, Hercules, CA, USA) according to the PulseNet standardized protocol for subtyping of *V.*

cholerae (*26*). The PFGE image was captured by using a Gel Doc XR system (Bio-Rad). The PFGE image was normalized by aligning the peaks of the *Xba*I size standards of *Salmonella enterica* serovar Braenderup (H9182) in each gel and was analyzed by using BioNumerics software version 4.0 (Applied Maths, Sint-Martens-Latem, Belgium). The similarities between isolates were evaluated by using the cluster analysis with the UPGMA method and the Dice correlation coefficient with a position tolerance of 1.5%.

Results

A total of 115 *V. fluvialis* were isolated from the acute diarrheal patients (each isolate represent a case), of which 27 (23.5%) were resistant for carbapenem and harbored $bla_{\text{NDM-1}}$. The first $bla_{\text{NDM-1}}$–positive *V. fluvialis* was isolated on May 16, 2011. The isolation rate of NDM-VF was highest in 2012 (14 isolates), followed by 7 in 2011 and 6 in 2013. The NDM-VF was not detected during 2009–2010. The rest of the pathogens tested in this study were susceptible to carbapenem.

Of the 27 NDM-VF strains, 13 (48.1%) were isolated as the sole pathogen; the remaining were co-pathogens isolated with any other pathogen, such as diarrheagenic *E. coli*, *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Giardia lamblia*, and rotavirus. None of the enteric bacteria identified as co-pathogens had $bla_{\text{NDM-1}}$. Most of the NDM-VF were resistant to ampicillin, ceftriaxone, cefuroxime, cefotaxime, nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, and streptomycin (100% each), followed by trimethoprim/sulfamethoxazole (96.2%), imipenem (88.8%), gentamycin (74.0%), chloramphenicol (70.3%), and tetracycline (14.8%). However, most of the isolates were susceptible to azithromycin (85%) and doxycycline (100%). Higher MICs were observed for cefotaxime (≥16 mg/L), ceftazidime (≥32 mg/L), cefepime (≥16 mg/L), and cefotetan (≥32 mg/L). In 50% of the NDM-VF, ciprofloxacin MIC was ≥32 mg/L, and for norfloxacin and imipenem the MIC values ranged from 4–32 mg/L (Table 1).

The $bla_{\text{NDM-1}}$–harboring *V. fluvialis* isolates carried multiple plasmids ranging from 5 kb to 150 kb. In Southern hybridization, the large plasmids extracted from the transconjugants were positive for $bla_{\text{NDM-1}}$. In the transconjugants, only a single plasmid of ≈80–90 kb was detected. The transconjugant (TC-J53) also showed resistance to ampicillin, erythromycin, streptomycin, ceftriaxone, cefotaxime, cefuroxime, and imipenem (MIC 2 mg/L), indicating the possibility that the NDM-1 plasmid also harbored genes encoding resistance to these antibiotics. The transconjugant was susceptible to ciprofloxacin, tetracycline, trimethoprim, chloramphenicol, and azithromycin, suggesting that the genes encoding resistance to these drugs are not carried by the $bla_{\text{NDM-1}}$–harboring plasmid. Most of the other enteric pathogens used as transconjugants showed resistance to ampicillin, ceftriaxone, cefotaxime, and sulfamethoxazole. The transfer frequencies ranged from 1.4×10^3 to 8.7×10^5 (Table 2).

Class-1 integron was identified in all the NDM-1–positive isolates. In 9 isolates, a 1.6-kb PCR amplicon was obtained with the dihydrofolate reductase gene cassette (*dfrA1*), which encodes resistance for trimethoprim. Overall, 9 different resistance gene profiles were identified (Table 1). All of the 27 NDM-VF isolates were positive for β-lactamase–encoding genes $bla_{\text{OXA-1}}$, $bla_{\text{OXA-7}}$, and $bla_{\text{OXA-9}}$; streptomycin-encoding gene *aadA1*; gentamycin-encoding gene *aadB*; and ciprofloxacin-modifying enzyme-encoding gene *aac(6')Ib-cr* (amino glycoside actetyltransferase). Most NDM-VF isolates had *sul1*, conferring resistance to sulfonamides (96.2%); *strA*, conferring resistance to streptomycin (92.6%); and *sul3*, conferring resistance to sulfonamides (88.8%). The *floR* gene that encodes resistance to chloramphenicol was found in 20 (74%) of NDM-VF isolates. The other β-lactamase encoding genes, $bla_{\text{TEM-9}}$ and $bla_{\text{CTX-M-3}}$, which confer resistance to ceftriaxone, were detected in 15 (55.5%) isolates. The tetracycline resistance marker gene *tet(B)* was detected in only 4 isolates (14.8%).

In replicon typing, plasmids of NDM-VF isolates were untypeable. To gain insight into the genetic background of $bla_{\text{NDM-1}}$, the flanking regions of this gene were examined in a representative *V. fluvialis* isolate (IDH 04744). $bla_{\text{NDM-1}}$ flanking sequences of IDH 04744 *V. fluvialis* were identical to the ones reported in the *E. coli* isolates from Hong Kong, China (pNDM-HK; GenBank accession no. HQ451074), and from a Spanish traveler returning from India (DVR22; GenBank accession no. JF922606.1) (Figure 1). The left junction of the sequences starts upstream of the $bla_{\text{NDM-1}}$ with a truncated IS*Aba125* region, whereas the right junction possessed different genes such as ble_{MBL} (bleomycin-resistance encoding gene), *trpF*, $bla_{\text{DHA-1}}$, and *ampR*.

Eighteen different patterns that could be grouped into 2 distinct clusters (A–C; Figure 2) were obtained in the PFGE analysis. Most of the isolates in cluster B had ≈90%–100% similarity. Nearly identical PFGE profiles were obtained for 11 isolates (cluster B). These isolates were isolated over a span of 1 year (May 2011–May 2012), without any epidemiologic link. We also found no correlation between the PFGE and antimicrobial drug resistance patterns.

Discussion

Since its discovery, global distribution of $bla_{\text{NDM-1}}$ in different bacterial species has been extensively documented (*27*). NDM-1 producers are reported not only from patients epidemiologically linked to the Indian subcontinent but also from several indigenous cases all over the world with no such link. Previously, we reported on the emerging trend of *V. fluvialis* among the diarrheal cases in the Kolkata region (*14*). However, NDM-VF emerged in Kolkata during

Table 1. Antimicrobial drug resistance genes and MICs of *Vibrio fluvialis* isolates in study of diarrheal fecal samples from patients in Kolkata, India, May 2009–September 2013*†

Isolate no.	Resistance gene profile	MIC, μg/mL							
		IPM	CTX	TAZ	PM	CIP	NOR	CN	TET†
IDH 03626	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, aadB,aac(6´)-Ib-cr, sul1*	8	>16	>32	>16	32	16	>32	ND
IDH 03631	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	16	>16	>32	>16	32	16	>32	ND
IDH 03645	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	16	>16	>32	>16	>32	48	>32	ND
IDH 03671	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	16	>16	>32	>16	>32	16	>32	ND
IDH 03679	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	16	>16	>32	>16	32	24	>32	ND
IDH 03893	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, tetB, sul1, sul3*	4	>16	>32	>16	8	12	>32	24
BCH01733	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, tetB, sul1, sul3*	32	>16	>32	>16	8	12	>32	24
IDH 04022	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	8	>16	>32	>16	>32	24	>32	ND
IDH 04149	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3*	4	>16	>32	>16	8	12	>32	ND
IDH 04166	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, aadB, aac(6´)-Ib-cr, sul1*	8	>16	>32	>16	>32	16	>32	ND
IDH 04169	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	16	>16	>32	>16	32	16	>32	ND
IDH 04228	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3*	8	>16	>32	>16	>32	32	>32	ND
IDH 04252	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr*	32	>16	>32	>16	32	24	>32	ND
IDH 04325	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	8	>16	>32	>16	16	16	>32	ND
IDH 04326	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	16	>16	>32	>16	>32	16	>32	ND
IDH 04382	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	8	>16	>32	>16	32	16	>32	ND
IDH 04414	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	8	>16	>32	>16	>32	32	>32	ND
BCH02360	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	8	>16	>32	>16	32	24	>32	ND
IDH 04568	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, tetB, sul1, sul3, floR*	32	>16	>32	>16	12	4	>32	16
IDH 04607	*bla*$_{NDM-1}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	32	>16	>32	>16	32	24	>32	ND
IDH 04744	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, tetB, sul1, sul3, floR*	32	>16	>32	>16	>32	32	>32	24
IDH 05335	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	8	>16	>32	>16	>32	24	>32	ND
IDH 05715	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	2	>16	>32	>16	>32	32	>32	ND
IDH 05720	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	8	>16	>32	>16	8	6	>32	ND
IDH 05733	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	32	>16	>32	>16	32	24	>32	ND
IDH 05799	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{CTX-M-3}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	24	>16	>32	>16	32	32	>32	ND
IDH 05818	*bla*$_{NDM-1}$, *bla*$_{TEM-9}$, *bla*$_{OXA-1}$, *bla*$_{OXA-7}$, *bla*$_{OXA-9}$, *aadA1, strA, aadB, aac(6´)-Ib-cr, sul1, sul3, floR*	32	>16	>32	>16	32	32	>32	ND

*All isolates were positive in the modified Houge test and susceptible for imipenem with EDTA. CIP, ciprofloxacin; CN, cefotetan; CTX, cefotaxime; IPM, imipenem; ND, not done; NOR, norfloxacin; PM, cefepime; TET, tetracycline; TAZ, ceftazidime.
†MIC assay was not done for TET-susceptible isolates.

2011, and 25%–50% of the *V. fluvialis* isolates harbored *bla*$_{NDM-1}$ each year until 2013. It is difficult to epidemiologically link the isolates because of the wide difference in the dates of isolation of NDM-VF, lack of common food sources, and variation in the proximity of the residential area of the patients; antibiogram and PFGE patterns are also widely divergent.

NDM-1 producers have been found to be highly resistant to several classes of antibiotics (*28*–*30*), related to their unusual genetic assemblage, which helps in the

Table 2. Antimicrobial drug resistance profiles before and after transfer of NDM-1–encoding plasmid from *Vibrio fluvialis* strains to other bacterial species in study of diarrheal fecal samples from patients in Kolkata, India, May 2009–September 2013*

Isolate no.	Test isolate	Resistance profile of wild type	Resistance profile of transconjugant	MIC IPM, μg/mL†	Frequency of transfer
J53-Na-Azide	*Escherichia coli*	–	**AMP**, **CRO**, **SXT**, **CXM**, **CTX**	3	8.7×10^{5}
BCH 04216	EAEC	AMP, ERY, SXT, NA, CXM, CTX	AMP, E, SXT, NA, CXM, CTX, **CRO**	3	5.9×10^{5}
IDH 04184	EPEC	AMP, ERY, OFX, NOR, SXT, NA, CIP	AMP, E, OFX, NOR, SXT, NA, CIP, **CRO**, **CXM**, **CTX**	1.5	2.7×10^{5}
IDH 06412	ETEC	AMP, ERY, NA, SXT	AMP, E, NA, SXT, CRO, **CXM**, **CTX**	3	7.5×10^{5}
BCH 0704	*Salmonella* Bareilly	–	**AMP**, **CRO**, **SXT**, **CXM**, **CTX**	3	1.7×10^{3}
570764	*Salmonella* Newport	–	**AMP**, **CRO**, **SXT**, **CXM**, **CTX**	3	1.4×10^{3}
IDH 06370	*Shigella dysenteriae* 12	AMP, STR, NA	AMP, STR, NA, **CRO**, **SXT**, **CXM**, **CTX**	1.5	2.4×10^{3}
IDH 06498	*Shigella flexneri* 4	AMP, E, STR, SXT	AMP, E, STR, SXT, **CRO**, **CXM**, **CTX**	3	4.4×10^{3}
IDH 06342	*Shigella flexneri* 1b	AMP, E, NA, STR, SXT	AMP, E, NA, STR, SXT, **CRO**, **CXM**, **CTX**	0.75	1.9×10^{3}
IDH 03988	*V. parahaemolyticus*	AMP	AMP, CRO, **SXT**, **CXM**, **CTX**	0.50	5.7×10^{5}

*Antibiogram in bold among transconjugants denote acquired resistance phenotypes from *V. fluvialis* IDH05720 after conjugation. AMP, ampicillin; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; CXM, cefuroxime; EAEC, enteroaggressive *E. coli*; EPEC, enteropathogenic *E. coli*; ERY, erythromycin; ETEC, enterotoxigenic *E. coli*; NA, nalidixic acid; NDM-1, New Delhi metallo-β-lactamase; NOR, norfolxacin; OFX, ofloxacin; STR, streptomycin, SXT, sulfamethoxxozole.
†MIC for imepenium. MIC denotes values of imepenium across different pathogens that were used as transconjugants.

acquisition and transfer of many resistance genes. Environmental strains of *Aeromonas caviae* and *V. cholerae* were found to carry bla_{NDM-1} on the chromosomes (*12*). In contrast, we found that, in NDM-VF, bla_{NDM-1} is present on the large plasmids.

Generally, the emergence of NDM-1 producers is associated with excessive use of carbapenems in patients with nonintestinal infections that necessitate a prolonged stay in a hospital. However, none of the patients in this study had a history of using carbapenem drugs. Most NDM-VF isolates remained susceptible to azithromycin, which is currently used in the treatment of diarrheal patients in Kolkata.

We found that a large plasmid from NDM-VF was effectively transferred to *E. coli* J53 and other enteric pathogens.

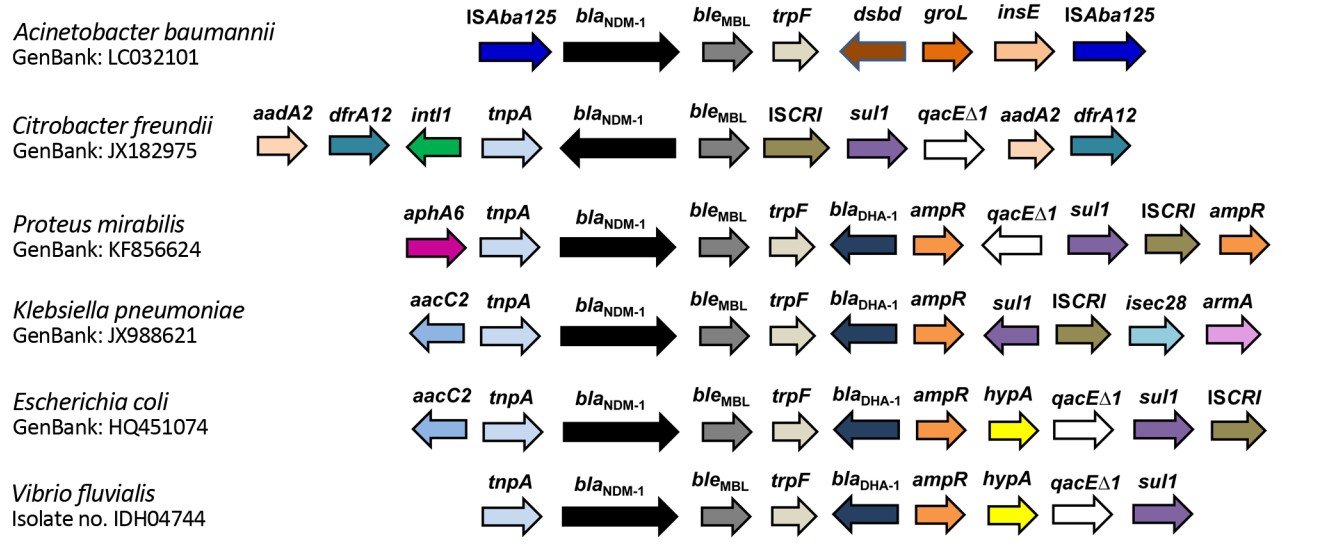

Figure 1. Structural features of bla_{NDM-1} flanking regions of *Vibrio fluvialis* and other bacterial species in study of diarrheal fecal samples from patients in Kolkata, India, May 2009–September 2013. Arrow lengths are proportionate to the lengths of the genes or open reading frames. GenBank accession numbers are shown. Gene names: *ISAba125*, insertion sequence bla_{NDM-1}, New Delhi metallo-β-lactamase; ble_{MBL}, bleomycin resistance protein; *trpF*, phosphoribosylanthranilate isomerase; *dsbd*, cytochrome c-type biogenesis protein; *groL*, chaperonins; *insE*, transposase insertion sequence; *aadA2*, aminoglycoside adenyltransferase; *dfrA12*, dihydrofolate reductase; *IntI1*, class I integron integrase; *tnpA*, transposition transposase; *ISCRI*, insertion sequence common region; *sulI*, dihydropteroate synthase; *qacEΔ1*, ethidium bromide resistance protein; *aphA6*, aminoglycoside phosphotransferase; bla_{DHA-1}, Class C β-lactamase; *ampR*, transcriptional regulator; *aacC2*, aminoglycoside acetyltransferase; *isec28*, transposase; *armA*, 16S rRNA methylase; *hypA*, putative hydrogenase nickel incorporation protein.

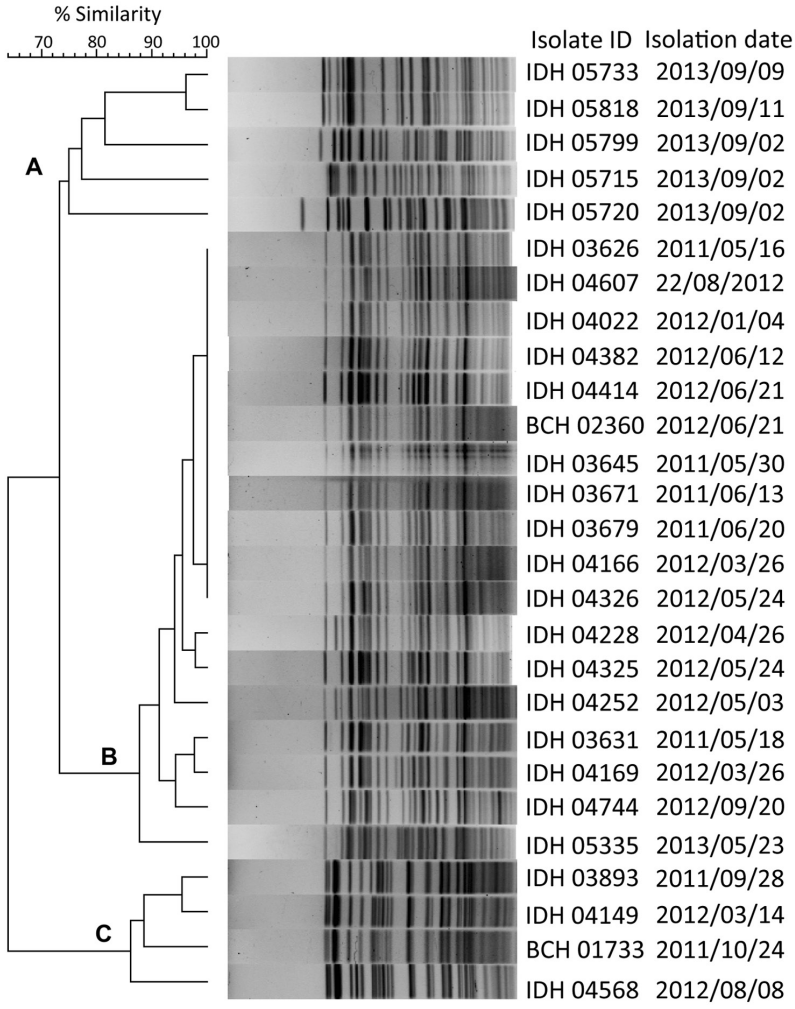

Figure 2. Pulsed-field gel electrophoresis analysis of *Not*I-digested genomic DNA of bla_{NDM-1} harboring *V. fluvialis* isolates in study of diarrheal fecal samples from patients in Kolkata, India, May 2009–September 2013. In the dendrogram, 3 distinct clusters (A–C) formed on the basis of the band similarity. Isolate identification (ID) includes name of associated hospital: IDH, Infectious Diseases Hospital; BCH, B.C. Roy Memorial Hospital for Children. bla_{NDM-1}, New Delhi metallo-β-lactamase.

Even though we demonstrated the in vitro transfer of bla_{NDM-1} in other enteric bacteria, these bacteria are not completely resistant to carbapenems, as is *V. fluvialis*. Multiple NDM-1–producing pathogens belonging to different species from a patient have been reported (*31*). Although in our study, 14 of 27 patients were infected with other pathogens (enteroaggressive *E. coli* [EAEC], enterotoxigenic *E. coli* [ETEC], *V. cholerae*, *V. parahaemolyticus*, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp.), only patients with *V. fluvialis* were found to harbor bla_{NDM-1}. The controlling factors that may prevent such transfer in the gut milieu should be explored further.

The resistance profiles of ampicillin, ceftriaxone, trimethoprim/sulfamethoxazole, cefuroxime, and cefotaxime have been transferred to all the transconjugants. This indicates that the bla_{NDM-1}–positive isolates may carry similar plasmids with the uniform resistance genes and, hence, confer the same resistance phenotype. Generally, the conjugative plasmids carrying bla_{NDM-1} have been classified into several replicon types, including IncA/C, $IncFII_Y$, IncHI1b, IncX3, and IncT (*32*). However, the NDR-VF isolates were negative for all the NDM-1 plasmids in the PCR-based replicon typing. These results suggest that the NDM-1–encoding genes move with several plasmid scaffolds or as the same Inc type, which might not be covered by the currently used replicon typing scheme of *Enterobacteriaceae*. In many bacterial species from India, the bla_{NDM-1}–harboring plasmids were found to belong to A/C-type, an uncommon group for conferring multidrug-resistant phenotypes (*3*).

Analysis of the genes adjoining the bla_{NDM-1} in *V. fluvialis* isolate IDH 04744 revealed a high homology with *E. coli* NDM-HK and DUR-22 (GenBank accession nos. HQ451074 and JF922606) (*33*,*34*). Insertion sequences (IS) IS*26* and IS*Aba125* have been identified upstream of the bla_{NDM-1} gene, and these sequences have been reported in other organisms. In most of the NDM-1–positive bacteria, the IS elements are detected in the flanking regions of bla_{NDM-1}. We detected the IS*26* and IS*Aba125* in the upstream of the bla_{NDM-1} gene. The presence of IS*26*, IS*CR1*, and transposases have been increasingly implicated in interspecies and intraspecies dissemination of antimicrobial

drug resistance genes (*35*,*36*). These IS elements probably help in the mobility of $bla_{\text{NDM-1}}$.

We also identified the ble_{MBL} gene downstream of $bla_{\text{NDM-1}}$. In most of the *Enterobacteriaceae*, $bla_{\text{NDM-1}}$ has been detected between a truncated IS*Aba125* located upstream and ble_{MBL} at the downstream. This genetic arrangement suggests an en bloc acquisition of $bla_{\text{NDM-1}}$ and ble_{MBL} through the IS*Aba125*-related mobilization system. The presence of ble_{MBL} appears to be an added advantage to the $bla_{\text{NDM-1}}$–positive bacteria, because both genes are expressed under the control of single promoter; therefore, the presence of ble_{MBL} may help the $bla_{\text{NDM-1}}$–bearing plasmids to spread in other bacterial species (*37*).

V. fluvialis is increasingly being detected in our setting and among diarrheal patients (*14*). These *V. fluvialis* isolates are capable of readily acquiring antibiotic resistance genes through mobile genetic elements (*38*). Our findings indicate that *V. fluvialis* might acquire the $bla_{\text{NDM-1}}$ gene without any antibiotic selective pressure. This pathogen also has the potential to transfer this gene to other enteric pathogens. PCR-based identification of the NDM-1 regions in suspected pathogens will be very useful. The *V. fluvialis* isolates harboring $bla_{\text{NDM-1}}$ are mostly susceptible to doxycycline and azithromycin. Considering the pathogenicity of *V. fluvialis* to humans and its ubiquitous presence in the environment, the need for constant monitoring of this *Vibrio* species is ongoing.

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G.C., G.P.P., A.G., and A.K.M. isolated and identified the pathogens and performed phenotypic characterization and all genetic analysis. K.R. and M.K.B. analyzed the clinical data and provided the specimens. T.R.M. and A.G. conceived the study and wrote the manuscript. All authors were involved in the compilation of the report and approved the final version.

Dr. Chowdhury is a postdoctoral researcher at the Division of Bacteriology, National Institute of Cholera and Enteric Diseases, Kolkata, India. His research interest includes the molecular epidemiology of antimicrobial drug resistance, with emphasis on enteric pathogens.

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Address for correspondence: Thandavarayan Ramamurthy, Center for Human Microbial Ecology, Translational Health Science and Technology Institute, NCR Biotech Science Cluster, 3rd Milestone, Faridabad-Gurgaon Expressway, Faridabad 121001, Haryana, India; email: tramu@thsti.res.in

Whole-Genome Characterization of Epidemic *Neisseria meningitidis* Serogroup C and Resurgence of Serogroup W, Niger, 2015

Cecilia B. Kretz,[1] Adam C. Retchless,[1] Fati Sidikou, Bassira Issaka, Sani Ousmane, Stephanie Schwartz, Ashley H. Tate, Assimawè Pana, Berthe-Marie Njanpop-Lafourcade, Innocent Nzeyimana, Ricardo Obama Nse, Ala-Eddine Deghmane, Eva Hong, Ola Brønstad Brynildsrud, Ryan T. Novak, Sarah A. Meyer, Odile Ouwe Missi Oukem-Boyer, Olivier Ronveaux, Dominique A. Caugant, Muhamed-Kheir Taha, Xin Wang, and the Niger Response Team[2]

In 2015, Niger reported the largest epidemic of *Neisseria meningitidis* serogroup C (NmC) meningitis in sub-Saharan Africa. The NmC epidemic coincided with serogroup W (NmW) cases during the epidemic season, resulting in a total of 9,367 meningococcal cases through June 2015. To clarify the phylogenetic association, genetic evolution, and antibiotic determinants of the meningococcal strains in Niger, we sequenced the genomes of 102 isolates from this epidemic, comprising 81 NmC and 21 NmW isolates. The genomes of 82 isolates were completed, and all 102 were included in the analysis. All NmC isolates had sequence type 10217, which caused the outbreaks in Nigeria during 2013–2014 and for which a clonal complex has not yet been defined. The NmC isolates from Niger were substantially different from other NmC isolates collected globally. All NmW isolates belonged to clonal complex 11 and were closely related to the isolates causing recent outbreaks in Africa.

Neisseria meningitidis commonly causes meningitis in the African meningitis belt, where periodic meningococcal epidemics have contributed to the highest reported incidence of meningococcal meningitis in the world (*1*). Most meningococcal disease historically has been caused by *N. meningitidis* serogroup A (NmA); however, NmA disease dramatically decreased after the preventative MenAfriVac vaccination campaign was initiated in 2010 (*2*). Serogroup W (NmW) has been the major cause of meningococcal disease in the region since then (*2*).

N. meningitidis serogroup C (NmC) disease has rarely been reported in the meningitis belt; it has not been detected in many molecular studies of invasive isolates (*3*,*4*) and is rarely found in carriage studies (*5*,*6*). The last large NmC epidemic in Africa occurred in Burkina Faso (then Upper Volta) in 1979 (*7*). During 2013 and 2014, NmC outbreaks were reported in Nigeria (*8*). The Nigerian outbreaks were caused by a novel NmC strain with a previously undescribed sequence type, 10217 (ST-10217), which does not belong to a defined clonal complex. In 2015, an epidemic of 9,367 meningococcal meningitis cases occurred in Niger, with NmC disease comprising most laboratory-confirmed cases (*9*).

NmW disease has been reported in the meningitis belt since the 1980s (*10*,*11*), and NmW from clonal complex 11 (CC11) has been a major concern in the region since 2001 (*12*). The first large epidemic of disease caused by CC11 NmW occurred during 2002 in Burkina Faso (*13*). Subsequently, NmW disease outbreaks were reported in Niger during 2010 and 2011, both involving CC11 (*14*). These outbreaks were followed by another large epidemic caused by CC11 NmW in Burkina Faso during 2012 (*15*). Whole-genome sequencing (WGS) analysis of diverse NmW isolates from around the world has demonstrated that a clone within CC11, commonly associated with NmC, became globally dispersed after it switched to serogroup W (*16*,*17*). WGS analyses also provided sufficient resolution to assign isolates from the meningitis belt to a long-standing regional

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (C.B. Kretz, A.C. Retchless, S. Schwartz, A.H. Tate, R.T. Novak, S.A. Meyer, X. Wang); Centre de Recherche Médicale et Sanitaire, Niamey, Niger (F. Sidikou, B. Issaka, S. Ousmane, O.O.M. Oukem-Boyer); Agence de Médecine Préventive, Paris (B. Njanpop-Lafourcade); World Health Organization, Niamey (I. Nzeyimana, A. Pana, R. Obama Nse); Institut Pasteur, Paris, France (A.-E. Deghmane, E. Hong, M.-K. Taha); Norwegian Institute of Public Health, Oslo, Norway (O.B. Brynildsrud, D.A. Caugant); World Health Organization, Geneva, Switzerland (O. Ronveaux)

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

[2]Members of the Niger Response Team who contributed to this study are listed at the end of this article.

population and to a clone that became globally dispersed after an outbreak during the 2000 Hajj pilgrimage (*16*,*17*; A. Retchless, unpub. data).

In addition to distinguishing among closely related strains, WGS provides information about allelic variation in genes that may affect antibiotic susceptibility and the coverage of protein-based vaccines. Two vaccines designed for serogroup B meningococcus have been approved for use in the United States and Europe: Trumenba and Bexsero. Trumenba targets the factor H–binding protein (FHbp), and includes components belonging to FHbp subfamilies A and B (*18*). Bexsero includes 4 different components: an FHbp of variant 1 (subfamily B); a *Neisseria* adhesion A protein (NadA); a neisserial heparin-binding antigen (NhbA); and outer membrane vesicles from a serogroup B strain containing PorA P1.4 (*19*). Recognizing the diversity of these genes among strains can aid in evaluating whether these vaccines may provide protection. Likewise, whole-genome sequences can be rapidly screened for indications of antibiotic resistance when the genetic determinants are well characterized, as with genes *penA*, *gyrA,* and *rpoB*, which are involved in reduced susceptibility to penicillin, ciprofloxacin, and rifampin, respectively. To clarify the meningococcal population in Niger during the 2015 epidemic season, we completed genomic analysis on the 102 NmC and NmW invasive isolates collected during this period.

Materials and Methods

Strain Collection

A total of 102 isolates from the Centre de Recherche Médicale et Sanitaire (CERMES; Niamey, Niger; online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/10/16-0468-Techapp1.pdf) were received at the World Health Organization Collaborating Centres for Meningitis at the following sites: Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA), the Institut Pasteur (Paris, France); and the Norwegian Institute of Public Health (Oslo, Norway). These isolates originated from 12 districts (Figure 1). Species and serogroup of 102 viable isolates were confirmed by culture, PCR, and slide agglutination (*20*) at the 3 World Health Organization Collaborating Centres. The results were 100% in concordance with the laboratory testing performed at CERMES. Conventional PCR-based molecular typing was performed on 9 cerbrospinal fluid specimens for which no associated isolates were available (3 NmW, 6 NmC); all ST, PorA, FetA, and *penA* sequences matched those of isolates that were fully sequenced. An additional 30 NmC isolates from 20 countries and 94 NmW isolates from 15 countries, representing the diversity of the 2 serogroups in the CDC culture collection, were selected and sequenced to compare with the Niger isolates (online Technical Appendix Table 1).

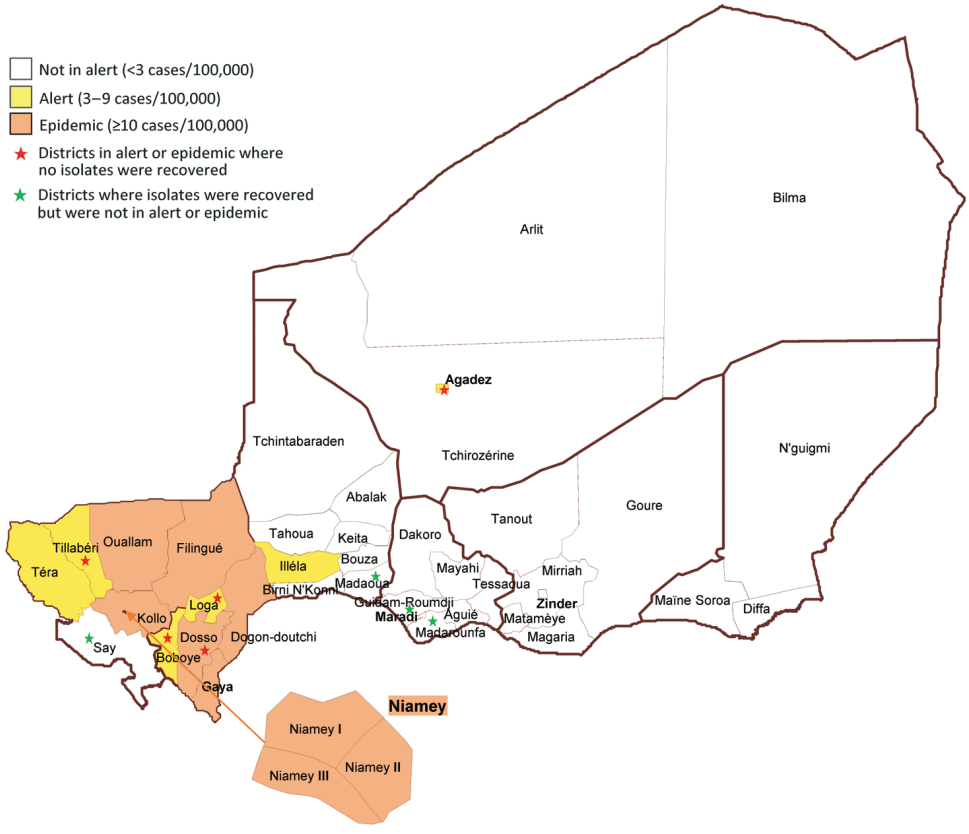

Figure 1. Distribution of *Neisseria meningitidis* isolates by district in Niger during the 2015 epidemic. Dogon-doutchi: 23 (NmC 15; NmW 8); Filingue: 2 (NmC 2); Gaya 2 (NmC 1; NmW 1); Guidan-Roumji: 1 (NmW 1); Illela: 1 (NmC 1); Kollo: 10 (NmC 5; NmW 5); Madaoua: 1 (NmW 1); Madarounfa: 1 (NmW 1); Niamey: 39 (NmC 37; NmW 2); Ouallam: 3 (NmC 2; NmW 1); Say: 3 (NmC 2; NmW 1); Téra: 11 (NmC 11).

Genome Sequencing

Genome sequencing data for each isolate were generated with both the Pacific Biosystems (PacBio; Menlo Park, CA, USA) RSII instrument and the Illumina HiSeq 2500 (San Diego, CA, USA). DNA was extracted from plated isolates by using ArchivePure DNA purification kit (5prime, Gaithersburg, MD, USA). PacBio sequences were generated by using P4-C2 sequencing chemistry and assembled using PacBio's Hierarchical Genome Assembly Process version 3 (HGAP) (*21*). HGAP produces linear DNA sequences, so we identified circular, complete chromosome sequences on the basis of the existence of reads that bridged the 2 ends of the chromosome after 1 copy of the terminal repeat produced by the assembler was removed. These assemblies were corrected with 250-bp, paired-end Illumina read data generated with TruSeq Rapid SBS chemistry (Illumina) from 600-bp libraries prepared with NEBNext Ultra DNA library preparation kits (New England BioLabs, Ipswich, MA, USA). The Illumina reads were trimmed with Trim Galore version 0.3.7 (Babraham Bioinformatics, Cambridge, UK) to remove reads below Q28, 100 bp, and an error rate of 0.03, then mapped with bowtie version 2.1.0 (*22*) and used to identify base-calling errors and indels by using freebayes version 0.9.16 (https://github.com/ekg/freebayes) with base quality >20, alternate count >20, and coverage >100. The PubMLST (http://pubmlst.org/neisseria/) identifier for the sequences are in online Technical Appendix Table 1 and genome coverage information and statistics on each genome in online Technical Appendix Table 2.

Molecular Characterization

We identified multilocus sequence typing (MLST) alleles on the basis of a BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) search of the assembled genomes compared with the PubMLST allele lists (*23*). We also identified potential antibiotic susceptibility based on PubMLST alleles for *gyrA, penA,* and *rpoB* genes. Protein sequences were likewise typed according to PubMLST sequence collection. PorA, PorB, and FetA were classified according to their respective variable regions, NadA was categorized by the Novartis convention of variant and peptide identifier (*24*), NhbA was identified by PubMLST peptide identifier, and FHbp was identified by the PubMLST peptide identifier and the Pfizer peptide identifier (subfamilies A and B).

Comparative Genomics

For each comparison of genome-wide similarity, we identified single nucleotide polymorphisms (SNPs) using kSNP version 3 software (*25*), with a kmer length of 25. We then built a maximum-likelihood phylogenic tree based on the core SNPs using MEGA6 (*26*), with the Tamura-Nei substitution model and 500 bootstrap iterations.

Results

Genomic Characterization and Diversity of NmC Isolates

We sequenced the genomes of 81 Niger NmC isolates using PacBio and Illumina sequencing. PacBio sequencing allowed reconstruction of the complete circular chromosome for 68 isolates. All 81 isolates had the same molecular profile (PorA P1.21–15,16, PorB 3–463, FetA F1–7, and ST-10127, which is not assigned to a known clonal complex; Table). The genome of the ST-10217 isolates were compared with the genomes of NmC isolates from 8 different clonal complexes, collected from countries in North and South America, Europe, Asia, and Africa as far back as 1976. We identified 13,746 core SNPs, with a difference of 0–32 core SNPs between the ST-10217 isolates and a difference of >4,400 core SNPs between ST-10217 and other NmC isolates. The ST-10217 isolates formed a distinct phylogenetic cluster, relative to the other NmC isolates (Figure 2).

Genomic Characterization and Diversity of NmW Isolates

We sequenced the genomes of 21 Niger NmW isolates; 20 were complete circular chromosomes. They all belonged to CC11/ST-11 and had a PorA P1.5,2 (Table). However, they differed in the PorB and FetA sequences. Fourteen NmW isolates had PorB 2–2, six had PorB 2–277, and 1 had PorB 2–60. Fifteen isolates had FetA F1–1, and 6 had FetA F1–84. When we compared the genomes of a collection of African

Table. Summary of molecular typing and serogroups of *Neisseria meningitidis* isolates, Niger, 2015*

Serogroup	No. isolates	ST/CC	PorA†	PorB†	FetA†	NadA‡	NhbA§	FHbp¶	*gyrA*§	*penA*§	*rpoB*§
C	81	ST-10217/NA	P1.21–15,16	3–463	1–7	Not present	798	27/A106	2	22	1
W	14	ST-11/CC11	P1.5,2	2–2	1–1	2/3.6	96	9/B45	4	1	9
W	6	ST-11/CC11	P1.5,2	2–277	1–84	2/3.6	96	9/B45	4	1	9
W	1	ST-11/CC11	P1.5,2	2–60	1–1	2/3.6	96	841/B#	4	1	9

*ST and CC are derived from multilocus sequence typing. CC, clonal complex; NA, not assigned; ST, sequence type.
†PorA, PorB, and FetA are typed according to their respective variable regions.
‡NadA is categorized by Novartis conventions of variant and peptide identifier.
§The alleles for *gyrA*, *penA*, and *rpoB* are identified by PubMLST DNA allele identifiers (http://pubmlst.org/). NhbA is identified by PubMLST peptide identifier.
¶FHbp is identified by the PubMLST peptide identifier and the Pfizer peptide identifier (subfamilies A and B). Assignment of Pfizer peptide identifier is pending for peptide 841.

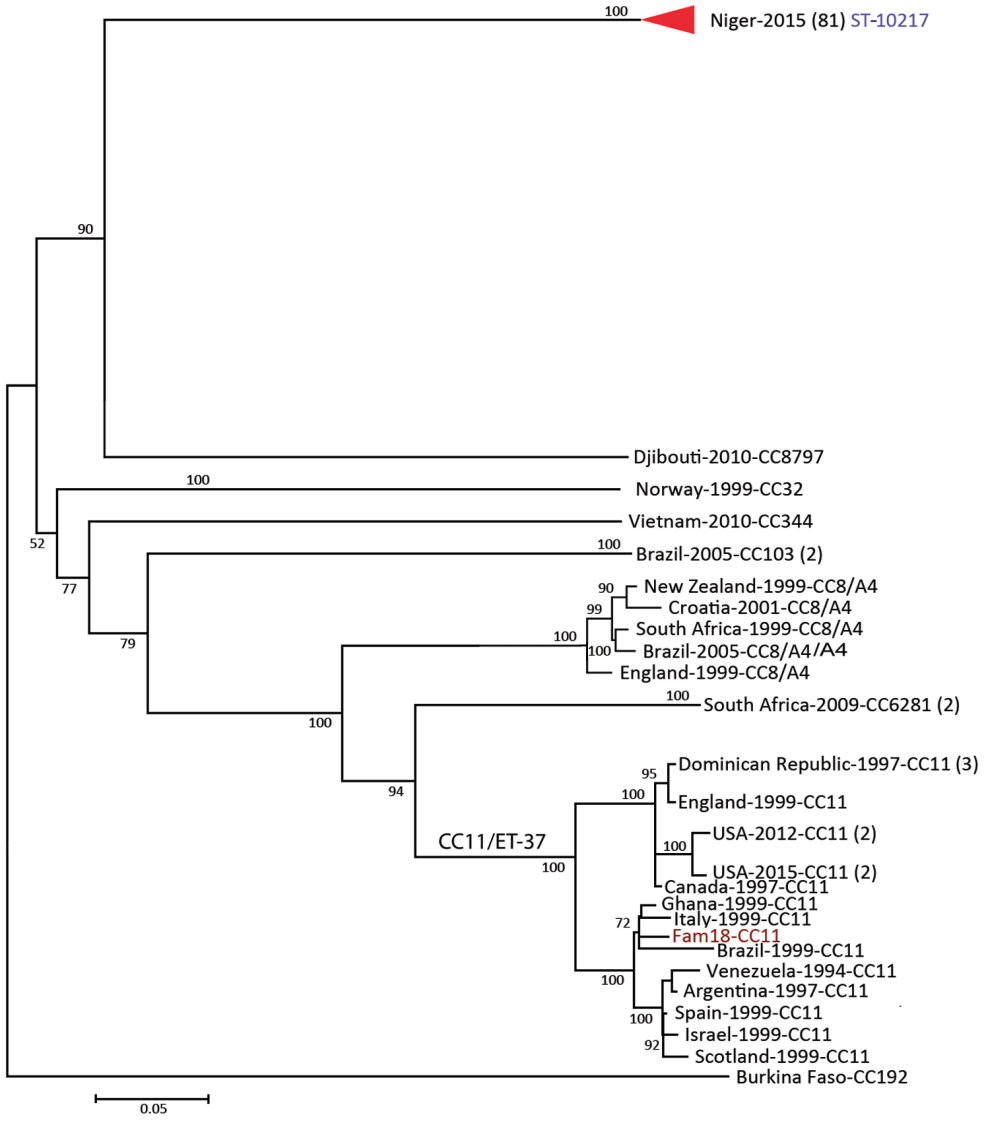

Figure 2. Phylogenetic tree of the *Neisseria meningitidis* serogroup C isolates, labeled with country of origin, year of isolation, and multilocus sequence typing (MLST) group (clonal complex or sequence type). Internal nodes are labeled with bootstrap values. The scale bar is based on the 13,746 positions in the core single nucleotide polymorphism (SNP) matrix and indicates nucleotide substitutions per site.

NmW isolates, including CC22 and CC175, we identified 11,324 core SNPs, with a difference of 0–122 SNPs among the isolates from Niger 2015. These isolates were closely related to NmW isolates collected from Burkina Faso and Mali in 2012, with 1–147 SNP differences (Figure 3). Moreover, the Niger CC11 NmW isolates showed 93–157 SNP differences when compared with an isolate collected in Saudi Arabia during the Hajj-related outbreak in 2000.

Antibiotic-Resistance Genes and Meningococcal Antigen-Encoding Genes

All NmC isolates had *gyr*A allele 2, *pen*A allele 22, and *rpo*B allele 1, whereas all NmW genomes contain *gyr*A 4, *pen*A 1, and *rpo*B 9 (Table). None of these alleles had the mutations associated with resistance to the respective antibiotic (*27*,*28*). The NmC ST-10127 isolates contained FHbp peptide 27, belonging to subfamily A and with 5 aa substitutions relative to peptide 19, against which Trumenba is likely effective on the basis of serum bactericidal activity using human complement (hSBA) (*18*). The NmW isolates contained FHbp peptides 9 or 841 of subfamily B, with 1 aa difference between them and another 13 aa differences relative to peptide 1 (B24), which was susceptible to hSBA (*18*) and is also the FHbp component of the Bexsero vaccine (*19*). None of the NmC isolates contained a *nadA* gene, but the NmW isolates contained NadA peptide 6, which belongs to variant group 2/3 and has 4 aa differences from the Bexsero vaccine component (peptide 8 belonging to variant group 2/3). The NhbA-encoding gene was found in ST-10127 NmC (peptide 798) and ST-11 NmW (peptide 96). Peptide 798 had 68 aa differences relative to the Bexsero component (peptide 2), in addition to being 69 aa longer. Peptide 96 had 85 aa differences, 13 aa missing, and an additional 8 aa relative to peptide 2. No isolate from

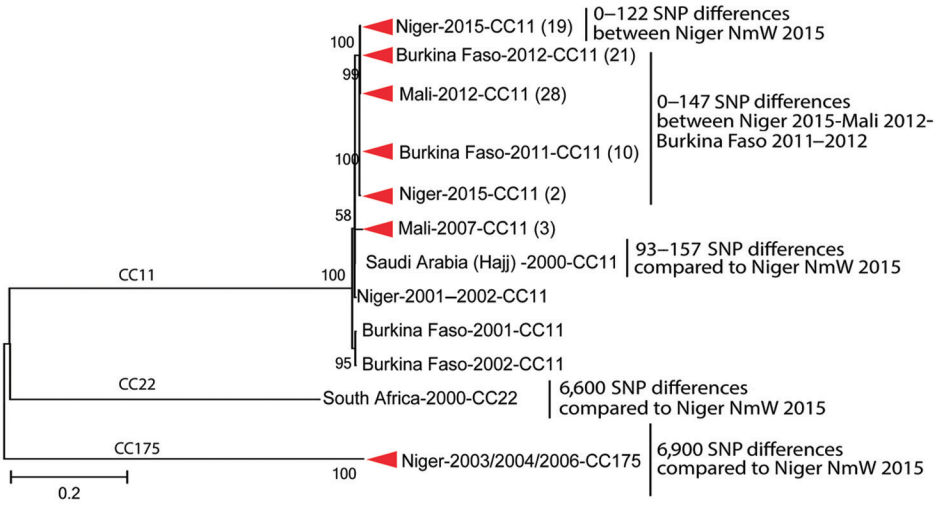

Figure 3. Phylogenetic tree of a subset of the *Neisseria meningitidis* serogroup W (NmW) isolates, labeled with country of origin, year of isolation, and clonal complex (CC). Clades comprising isolates from a single country and year are collapsed, with the isolate count in parentheses. Internal nodes are labeled with bootstrap values, and the number of single nucleotide polymorphisms (SNPs) distinguishing different groups is provided at right. The scale bar is based on the 11,324 positions in the core SNP matrix and indicates nucleotide substitutions per site.

this epidemic contained PorA P1.4, one of the components of Bexsero.

Discussion

This study provides a genomic analysis of 102 invasive NmC and NmW strains collected from Niger during a large epidemic in 2015. The isolates within each serogroup (C and W) were closely related and formed a distinct phylogenetic cluster, with identical ST and little variability in the rest of the genome, suggesting a recent emergence, recent clonal expansion, or both. No mutations involved in reduced antibiotic susceptibility were found, suggesting that these isolates are likely susceptible to penicillin, ciprofloxacin, and rifampin. The NmC isolates were not closely related to the reference NmC strain FAM18 or to any of the NmC isolates that were selected from the United States and 20 countries but had the same ST as the strain that caused the outbreaks in Nigeria during 2013–2014 (*8*). The NmW isolates were closely related to isolates collected in the neighboring countries Burkina Faso (2011 and 2012) and Mali (2012). All of these isolates belonged to a clade defined by an isolate from the Hajj-related outbreak in Saudi Arabia in 2000 (A. Retchless, unpub. data), suggesting that the Niger NmW strains may have recently diverged from prior circulating strains in the region. Recent WGS studies have shown that NmW isolates from CC11 form several clades (with 1 harboring the Hajj-related isolates), suggesting a multifocal emergence of the CC11 NmW strains (*16*,*17*; A. Retchless, unpub. data). Researchers may need to analyze larger numbers of NmW isolates from several countries of the meningitis belt to gain knowledge regarding the recent emergence and spread of these strains.

The scale of the epidemic in Niger (>8,500 cases), along with recent NmC outbreaks and sporadic cases in neighboring countries, highlights the risk for resurgent meningococcal meningitis in the meningitis belt, in the form of a newly emergent lineage (*2*). The novelty of this serogroup C lineage is especially concerning, raising questions about how long it has been present in the meningitis belt and why it has not been associated with prior outbreaks. Examination of the PubMLST database revealed only 2 observations of meningococcus with similar profiles: the same ST was observed in serogroup C strains from Nigeria during 2013–2014, and a similar ST (ST-9367, matching at 6 of 7 MLST loci) was represented by a nongroupable isolate from a carriage study in Burkina Faso during 2011. Although comparison between the ST-10127 NmC isolates from Niger 2015 and Nigeria 2013–2014 would reveal recent evolution of ST-10127 lineage, close comparison between ST-10127 and ST-9367 may illuminate recent evolution of the capsule locus. The origin of ST-10127 is unclear due to the limited number of genetically closely related strains. Additional invasive and carriage meningococcal strains that were collected from Africa and other countries in the past few decades should be examined at the genomic level to identify closely related strains and assess the genetic variations that have led to the emerging ST-10127 NmC.

This resurgence of meningococcal disease is not solely due to the novel NmC lineage; laboratory-confirmed NmW cases in Niger increased from 10 in 2013 (*29*) and 14 in 2014 (*2*) to 206 in 2015. The recurrence of non-NmA meningococcal disease after mass vaccination against NmA disease raises questions regarding whether serogroup replacement has occurred and is somehow related to vaccination against NmA, similar to the serotype replacement that was observed after the implementation of pneumococcal vaccines (*30*). Although NmW meningococcal disease continued to resurge after the MenAfriVac campaign in meningitis belt countries began in 2010 (*29*), and NmC subsequently emerged in Nigeria in 2013,

neither of these occurrences were likely to be a side-effect of mass vaccination because NmW epidemics had occurred before MenAfriVac (*14*,*31*), and the NmC outbreaks occurred in districts that had not yet been vaccinated. Reemergence of NmC epidemics may have been fueled by the population being immunologically naive to the causative strain. Evaluation of the serogroup replacement hypothesis, and of competing hypotheses such as variation in environmental characteristics (*32*), will greatly benefit from the routine collection of representative molecular surveillance data across the region.

The epidemiologic changes in the meningitis belt underscore the importance of continuous effort to develop vaccines against infectious disease caused by nonvaccine serogroups. Although polysaccharide-based vaccines should provide protection against the NmW and NmC strains, protection may also be provided by recently approved serogroup B meningococcus vaccines that are used in Europe and the United States and target surface proteins that are also found in non-B meningococcal strains (*33*). FHbp-based vaccines can provide protection against strains expressing alleles from the same subfamily of the protein (*34*). The ST-10127 NmC strain contain a FHbp of subfamily A, which is included in the Trumenba bivalent FHbp vaccine, but not the Bexsero multicomponent vaccine (*18*,*19*). This strain lacks NadA but does include a gene encoding NhbA. The ST-11 NmW genomes contain an FHbp of subfamily B, which is included in both Trumenba and Bexsero. Of interest, 3 of the polymorphic residues in FHbp are involved in hydrogen bonding of peptide 1 to human factor H (*35*,*36*). NadA and NhbA are also found in the ST-11 NmW isolates, which suggests that these vaccines may provide protection against ST-11 NmW disease. The antigenicity of these vaccine targets remains to be analyzed to precisely evaluate the coverage of these strains by Trumenba and Bexsero, as has been effectively done for emerging clonal complex 181 serogroup X isolates from the meningitis belt (*37*).

In addition to vaccination, natural immunity could be conferred by prior exposure to *N. meningitidis* strains carrying similar antigens. Because NmC has, until now, been very rare in Africa, immunity against serogroup C is unlikely to exist among the African population, which highlights the urgent need to prepare a response for potential NmC outbreaks and epidemics in the upcoming seasons.

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Dr. Kretz is a molecular microbiologist and bioinformatician in the Bacterial Meningitidis Laboratory in the Meningitis and Vaccine Preventable Diseases Branch, Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia. Her research focuses on the molecular characterization and comparative genomics, using whole genome sequencing, of meningitis causing pathogens and more specifically *N. meningitidis*.

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Address for correspondence: Xin Wang, Centers for Disease Control and Prevention. 1600 Clifton Rd NE, Mailstop D11, Atlanta, GA 30329-4027, USA; email: gqe8@cdc.gov

Ebola Virus Disease in Children, Sierra Leone, 2014–2015

Felicity Fitzgerald, Asad Naveed, Kevin Wing, Musa Gbessay, J.C.G. Ross, Francesco Checchi, Daniel Youkee, Mohammed Boie Jalloh, David Baion, Ayeshatu Mustapha, Hawanatu Jah, Sandra Lako, Shefali Oza, Sabah Boufkhed, Reynold Feury, Julia A. Bielicki, Diana M. Gibb, Nigel Klein, Foday Sahr, Shunmay Yeung

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

Learning Objectives

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

- Assess mortality for patients with pediatric Ebola virus disease (EVD), based on a retrospective cohort study
- Distinguish morbidity and laboratory findings for patients with pediatric EVD
- Identify characteristics associated with outcomes in patients with pediatric EVD

CME Editor

Karen L. Foster, MA, Technical Writer/Editor, *Emerging Infectious Diseases. Disclosure: Karen L. Foster has disclosed no relevant financial relationships.*

CME Author

Laurie Barclay, MD, freelance writer and reviewer, Medscape, LLC. *Disclosure: Laurie Barclay, MD, has disclosed the following relevant financial relationships: owns stock, stock options, or bonds from Pfizer.*

Authors

Disclosures: ***Felicity Fitzgerald, MBBS, MSc, MRCPCH; Asad Naveed, MBChB; Kevin Wing, PhD; Musa Gbessay, MBChB; J.C.G. Ross, BSc (Hons); Francesco Checchi, PhD; Daniel Youkee, MBBS, MPH; Mohammed Boie Jalloh, MBChB; David Baion, MPed, MBChB; Ayeshatu Mustapha, MBChB; Hawanatu Jah, MBChB; Sandra Lako, MD, DTMH; Shefali Oza, MSc; Sabah Boufkhed, MSc; Reynold Feury, BSc; Nigel Klein, MBBS, MRCP, PhD, FRCPH; Foday Sahr, BSc (Hon), MBChB, MSc, DSc;*** *and* ***Shunmay Yeung, PhD, MBBS, DTM&H, h****ave disclosed no relevant financial relationships.* ***Julia Bielicki, MD, MPH,*** *has disclosed the following relevant financial relationships: other (spouse) is Senior Legal Counsel at Novartis; owns stocks and stock options from Novartis.* ***Diana M. Gibb, MD,*** *has disclosed the following relevant financial relationships: served as an advisor or consultant for ViiV Healthcare Cabotegravir Advisory Committee.*

Author affiliations: Save the Children, London, UK (F. Fitzgerald, J.C.G. Ross, F. Checchi); University College of London Great Ormond Street Institute of Child Health, London (F. Fitzgerald, N. Klein); Save the Children, Freetown, Sierra Leone (A. Naveed, M. Gbessay); London School of Hygiene & Tropical Medicine, London (K. Wing, F. Checchi, S. Oza, S. Boufkhed, S. Yeung); Kings Sierra Leone Partnership—Connaught Hospital, Freetown (D. Youkee); Republic of Sierra Leone Armed Forces, Freetown (M.B. Jalloh, F. Sahr); Ola During Children's Hospital—Sierra Leone Ministry of Health, Freetown (D. Baion, A. Mustapha); Cap Anamur (German Emergency Doctors)—Ola During Children's Hospital, Freetown (H. Jah); Welbodi Partnership—Ola During Children's Hospital, Freetown (S. Lako); Western Area Emergency Response Centre, Freetown (R. Feury); St. George's University of London, London, UK (J.A. Bielicki); MRC Clinical Trials Unit at University College of London, London (J.A. Bielicki, D.M. Gibb)

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Little is known about potentially modifiable factors in Ebola virus disease in children. We undertook a retrospective cohort study of children <13 years old admitted to 11 Ebola holding units in the Western Area, Sierra Leone, during 2014–2015 to identify factors affecting outcome. Primary outcome was death or discharge after transfer to Ebola treatment centers. All 309 Ebola virus–positive children 2 days–12 years old were included; outcomes were available for 282 (91%). Case-fatality was 57%, and 55% of deaths occurred in Ebola holding units. Blood test results showed hypoglycemia and hepatic/renal dysfunction. Death occurred swiftly (median 3 days after admission) and was associated with younger age and diarrhea. Despite triangulation of information from multiple sources, data availability was limited, and we identified no modifiable factors substantially affecting death. In future Ebola virus disease epidemics, robust, rapid data collection is vital to determine effectiveness of interventions for children.

The Ebola virus disease (EVD) outbreak in West Africa during 2014–2016 comprised ≈28,600 cases and claimed ≈11,300 lives (*1*). The case-fatality rate (CFR) was high for Ebola virus (EBOV)–infected children <5 years of age (*2*–*4*). Outbreak conditions with overstretched health systems and paucity of data has meant there is little understanding of how modifiable clinical management, operational-, and response-specific factors might have affected outcomes of children with EVD (*3*,*5*).

During the outbreak, several different health service models for managing suspected EVD cases evolved. In the highly populated Western Area of Sierra Leone, including Freetown, on-site Ebola holding units (EHUs) were set up at health facilities. Their goal was to enable provision of normal healthcare to continue by screening patients before entry (*6*). Patients fulfilling screening criteria were admitted to the on-site EHUs for EVD testing. Persons testing positive for EBOV were transferred to Ebola treatment centers (ETCs). At the height of the outbreak, laboratory, ambulance, and bed capacities were overwhelmed, leading to substantial delays throughout the pathway, limited provision of clinical care, and long transfer distances (*7*,*8*). As the response scaled up, delays shortened and transfer distances decreased as more ETCs opened locally (*7*,*8*). These changing health systems factors possibly affected death and are amenable to modification in future outbreaks.

Patient data were collected on paper forms at EHUs and ETCs. Basic demographic and initial symptom information was telephoned to regional control centers for entry into electronic databases and transfer to international nongovernment organizations for epidemiologic surveillance (*3*,*5*,*9*,*10*). However, data on clinical management and key factors, such as caregiver accompaniment, were not included. Furthermore, communication between EHUs and ETCs was limited, meaning EHUs had no information about patient outcome after transfer, and data from ETCs were subject to substantial survivorship bias (*11*–*13*). Attempts to link clinical management data between sites were limited.

We describe the clinical features, management, and outcomes of children with EVD from initial presentation to final outcome. We also explore risk factors for death, in particular factors amenable to modification.

Methods

Study Population and Setting

All children <13 years of age admitted to 11 EHUs in the Western Area from August 14, 2014, through March 31, 2015, were eligible for inclusion, anticipating that the disease phenotype in adolescents might be similar to that in adults and that some factors (e.g., caregiver accompaniment) were more relevant for younger children (*3*,*14*). Patients were screened for EVD symptoms or contact with EBOV on entry to healthcare facilities. Patients whose signs and symptoms fulfilled the World Health Organization definition for a suspected case were admitted to on-site EHUs to have blood taken for EBOV PCR testing (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/10/16-0579-Techapp1.pdf) (*15*). Basic demographic data were reported to the Western Area Emergency Response Command Centre (WAERC). Samples were transported to specialist laboratories, which were usually off-site. Patients testing negative for EVD were discharged home or admitted to a routine (non-Ebola) hospital bed (Figure 1) (*8*). Patients testing positive were transferred to an ETC coordinated by the WAERC. Occasionally, if ETCs were full, a patient might stay at an EHU for the duration of illness and be discharged home directly.

Medical care varied among EHUs according to staffing capabilities and facilities available. The Sierra Leone Ministry of Health recommended that all patients admitted to an EHU receive antimalarial drugs and broad-spectrum antimicrobial therapy (*15*,*16*). Until late December 2014, most EHUs abstained from taking blood for tests other than EBOV PCR (WAERC, November 2014, unpub. data). Treatment was primarily supportive; some units operated a no-needle policy, providing only oral rehydration salt (ORS) solution and oral medications. Most units did not allow asymptomatic caregivers to enter an EHU high-risk area (i.e., Red Zone) with their children, because of the potential risk for nosocomial EBOV infection, so many children were admitted alone.

Data Collection

Data stored electronically in the WAERC database included demographics and symptoms at presentation. To obtain more detailed data, we visited 11 EHUs, Ola

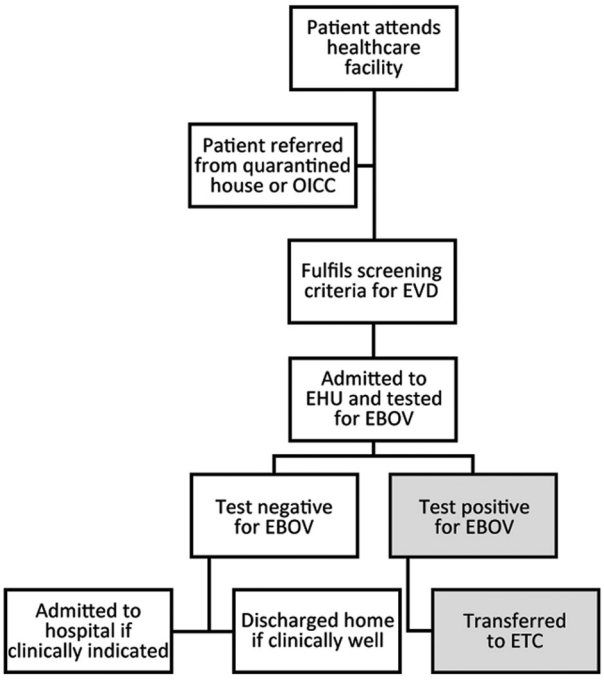

Figure 1. Patient care pathway of EHUs, Western Area (including Freetown), Sierra Leone, August 2014–March 2015. The OICC was set up to care for children with substantial Ebola virus exposure (usually a first-degree relative with confirmed EVD) and without relatives to care for them during the 21-day incubation period. Figure reproduced from (*8*) (Creative Commons License). EHU, Ebola holding unit; EVD, Ebola virus disease; OICC, observational interim care center.

During Children's Hospital, and the Western Area ETCs (Figure 2) to extract data from paper records, including demographic data; contact histories; clinical features at and during admission; treatment received; laboratory results; and health system factors, such as duration of transfer to ETC and outcome (death or survival to discharge) (online Technical Appendix Table 1). This information was supplemented by interviews with staff members, in particular about caregiver accompaniment, which was routinely documented only at 1 site. Information about outcomes after discharge was obtained from 2 survivor clinics in the Western Area and telephone calls to guardians. Data were cross-referenced with the WAERC database, test results from regional laboratories, child protection records, districtwide burial records, and the telephone service set up for community ambulance notification (online Technical Appendix Table 1). We developed a scheme to ensure consistency in matching records from different sources (online Technical Appendix). Data were entered directly into a password-protected database (Epi Info version 7.1.4; Centers for Disease Control and Prevention, Atlanta, GA, USA). Personal identifiers were removed before analysis.

Outcome and Potential Risk Factors

Possible outcomes were death (recorded at EHU or ETC) or survival (recorded as discharge from ETC or EHU). We considered variables encoding potential risk factors for outcome, including demographics (age, sex); duration from symptom onset to EHU arrival; symptoms at presentation; receipt of antimicrobial drugs, antimalarial drugs, or intravenous fluids at EHU; specific EHU attended; period in the epidemic (before or on/after January 9, 2015, when bed capacity first outstripped demand in the Western Area [*8*]); and whether the child was admitted alone, unaccompanied by a caregiver (online Technical Appendix).

We restricted a separate analysis to children transferred to ETCs. Variables were days from EHU admission to ETC transfer, transfer distance, ETC attended, and receipt of medications or intravenous fluids at the ETC. If health information was available for survivors after discharge, it was collected but was not a primary focus of this study.

Sample Size

At study conception in December 2014, we estimated 300 children would have sought care in Western Area EHUs. We aimed to obtain data on all these children (see calculations in online Technical Appendix).

Statistical Analysis and Ethical Considerations

We conducted a descriptive analysis on all children admitted to EHUs. Among children with known outcome, we conducted univariable and multivariable analyses to explore factors potentially affecting outcome (online Technical Appendix). We accounted for missing data with multiple imputation using chained equations (online Technical Appendix) (*17*). All analyses were conducted by using Stata version 14.0 (StataCorp LP, College Station, TX, USA). We obtained approval from the Sierra Leone Ethics and Scientific Review Committee and the London School of Hygiene and Tropical Medicine Ethics committee (ref. 8924).

Results

Overall Outcomes

Our study comprised all 309 children 2 days–12 years of age admitted to EHUs and testing positive for EBOV (median age 6 years, interquartile range [IQR] 3–10 years; 158 [51%] female). Outcome (death or discharge) was available for 282 (91%) children; CFR was 57% (95% CI 51%–63%) (Table 1, http://wwwnc.cdc.gov/EID/article/22/10/16-0579-T1.htm). Eighty-six (28%) children died at EHUs, and 223 (72%) were transferred to ETCs, where an additional 74 (24%) died. Therefore, 55% of deaths occurred at EHUs and 45% at ETCs (Figure 3, panel A). Of children transferred to ETCs, 116 (38%) were discharged home and 3 (1%) were

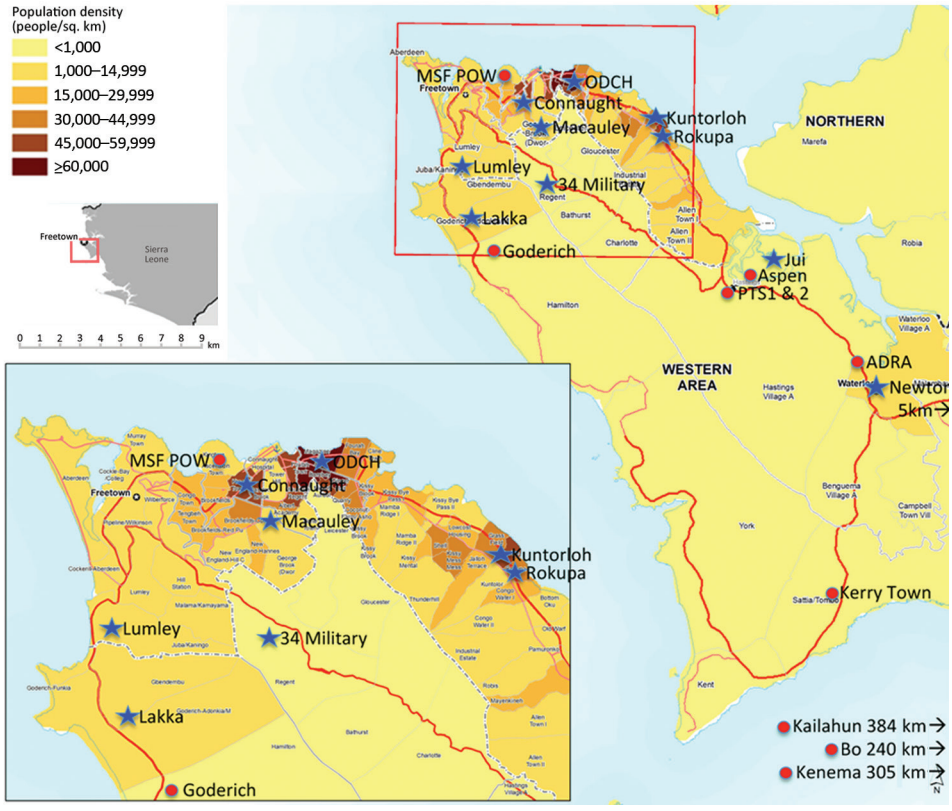

Figure 2. Location of Ebola holding units (blue star) and Ebola treatment centers (red circle), Western Area, Sierra Leone, January 2015. Population density map source: MapAction (cited 2015 Nov 8); reproduced with permission. Population figures are projected for 2014 from the 2004 census (http://www.mapaction.org/?option=com_mapcat&view=mapdetail&id=3589)..

discharged to a hospital (Figure 3, panel A). Three children were discharged home from EHUs without transfer. Median duration of EHU/ETC admission among children who died was 3 days (IQR 1–5 days) and for survivors was 17 days (IQR 11–20 days). Outcomes were missing for 27 (9%) children, all of whom had been transferred out of EHUs to ETCs (Figure 3, panel A). The CFR was highest in September and October 2014, a period of intense EBOV transmission, and peaked again in January 2015 (Figure 3, panel B).

Clinical Features

Data were available on symptoms at presentation for ≈70% of children (Table 1). Fever was the most prevalent symptom (99%), followed by fatigue/weakness (80%), anorexia (79%), vomiting (59%), and diarrhea (44%); unexplained bleeding was rare (1%). Median reported duration of symptoms before presentation was 3 days (IQR 2–4 days). Of 193 children for whom exposure data were available, 151 (78%) had documented prior contact with a person with EVD, and 42 (22%) had documentation of no prior exposure to EVD.

Additional clinical information was available for a subset of 88 patients for the duration of EHU/ETC admission. In addition to 3 children with spontaneous bleeding at presentation, 7 (8%) had bleeding after admission. In order of frequency, manifestations were bleeding gums, epistaxis, hematemesis, melena, and vaginal bleeding. Only 1 of these 10 children survived to discharge. Seizures were recorded during admission in 6 (7%) children: 3 died, 2 were discharged in a comatose state, and 1 recovered with no reported sequelae.

Laboratory Features

Additional blood test results were available for 36 children from 3 health facilities (Table 2). These children were of similar age (median 6 years, IQR 3–7 years) to children in the overall cohort, but the CFR was lower (11/36 [31%; 95% CI 16%–48%]). Children who died were younger than children who survived (data not shown).

Tests were taken a median of 3 days (range 0–18 days) after admission at an EHU. Leukocyte count (predominantly granulocytes) and C-reactive protein were elevated, and there was considerable renal and liver function derangement, particularly among children who died. Hyponatremia occurred among children who survived and who died, but potassium levels tended to be within normal limits. Hypoglycemia (blood glucose <4.0 mmol/L [reference 4–6.9 mmol/L) was common among children who died (55% [95% CI 23%–83%]) and who survived (30% [95% CI 13%–53%]).

Cycle threshold (C_t) represented the point at which a quantitative PCR was interpreted as positive: the lower the

Table 2. Blood test results for children attending Ebola holding units and Ebola treatment units for whom blood test results were availat Western Area, Sierra Leone, August 2014–March 2015*

Laboratory value (reference)	Median value among patients who died	Median value among patients who survived	p value†
Leukocyte count (4–11), × 10^9/L	33, n = 7	9.2, n = 22	0.067
Lymphocyte count (1–3.2), × 10^9/L	6.1, n = 7	2.9, n = 21	0.007
Granulocyte count (2.5–7.5), × 10^9/L	19.9, n = 7	4.9, n = 22	0.009
Hemoglobin (135–175), g/L	123, n = 7	110, n = 22	0.097
Platelets (150–430), × 10/L	376, n = 7	179, m = 22	0.17
Sodium (128–145), mmol/L	127, n = 10	131, n = 24	0.11
Potassium (3.6–5.1), mmol/L	3.9, n = 7	4.1, n = 23	0.9
Urea (2.5–7.9), mmol/L	16.2, n = 11	4.2, n = 24	<0.001
Creatinine (53–106), mmol/L	120, n = 10	49, n = 24	0.003
Albumin (33–50), g/L	26, n = 11	31, n = 20	0.25
Aspartate transaminase (11–35), U/L	2,000, n = 8	159, n = 17	0.001
Alanine transaminase (10–48), U/L	667, n = 11	131, n = 18	<0.001
Creatine kinase (39–380), U/L	2,544, n = 8	623, n = 18	0.13
C-reactive Protein (0–7.5), mg/L	96, n = 10	8, n = 17	0.007
Viral cycle threshold, range‡	14.3–35.8, n = 40	17–38.2, n = 42	NA

*n values indicate number of patients in category for whom value was available. NA, not applicable
†Wilcoxon rank-sum test used because of small sample size.
‡Different reference ranges used.

C_t, the higher the initial viral load. Results were available from 90 children (42 who survived; 40 who died; 8 unknown; median age [IQR] 8.5 years [4–11 years], 5.0 years [3–10 years], and 6.0 years [2.9–10.5 years], respectively). The range of C_t was similar for children who died and who survived (14.3–35.8 vs. 17.0–38.2) (Table 2).

Health Systems and Clinical Management Factors

Median duration of admission at EHU before transfer or death was 2 days (IQR 1–3 days). Outcomes were available for 193 children who survived to transfer to an ETC. Twenty-five (13%) were transferred directly onto an onsite treatment ward, but most (168 [87%]) traveled 5–380 km (median 25 km [IQR 19.5–45.0 km]). For 201 children for whom information was available about caregiver accompaniment (written documentation in 119 [59%], information from staff interviews alone in 84 [41%]), 74 (37%) were documented as unaccompanied admissions.

Treatment was primarily supportive but ranged from aggressive intravenous or intraosseous fluid resuscitation with laboratory monitoring (more common in ETCs) to ORS and oral medications. Medications received at either EHUs or ETCs were recorded for 178 (58%) children: 99% received antimicrobial drugs and ORS, 85% antimalarial drugs, and 19% intravenous fluids.

Risk Factors for Death

In univariable analysis, younger age (odds ratio [OR] per year of life 0.91 [95% CI 0.85–0.97]) and diarrhea at presentation (OR 1.94 [95% CI 1.11–3.39]) were significantly associated with death. CFR was highest for infants (70%) (Figure 3, panel C).

In multivariable analysis, age was the strongest predictor of death (adjusted OR 0.92 [95% CI 0.86–0.98] per 1-year increment in age) (Table 1; Figure 3, panel C). Diarrhea at presentation was associated with death (OR 1.91 [95% CI 1.08–3.39]). None of the following were associated with death: time from symptom onset to EHU admission, receipt of specific medications or intravenous fluids, attendance at any particular EHU, or being accompanied by a caregiver (Table 1; online Technical Appendix Table 2). We found minimal difference between estimates obtained by analyzing only complete records compared with accounting for missing data using multiple imputation (online Technical Appendix Tables 3, 4).

For 193 children transferred to ETCs for whom outcomes were known (online Technical Appendix Tables 5, 6), neither longer duration of EHU stay before transfer nor longer transfer distances appeared detrimental. Outcomes between ETCs varied considerably, but CIs were wide. Receipt of medications or intravenous fluids at the ETC also were not associated with survival.

Health Status of Survivors

Of 122 surviving children, data from survivor clinics or telephone interviews were available for 42 (34%). Twenty-five (60%) reported no problems; 6 (14%) were referred for ophthalmologic review with possible uveitis, 1 with monocular blindness; 3 (7%) had hearing problems; 2 (5%) had alopecia; 2 (5%) had joint pains; and 1 (2%) depressed affect. Of 2 children discharged without fully recovering consciousness, 1 was recovering and being cared for at home; the second had made little improvement in hospital.

Discussion

We aimed to identify modifiable operational and clinical management factors that could affect the outcome of EVD in children. The relative completeness of outcome data (91%) was possible only through a high level of collaboration between government and nongovernment organizations,

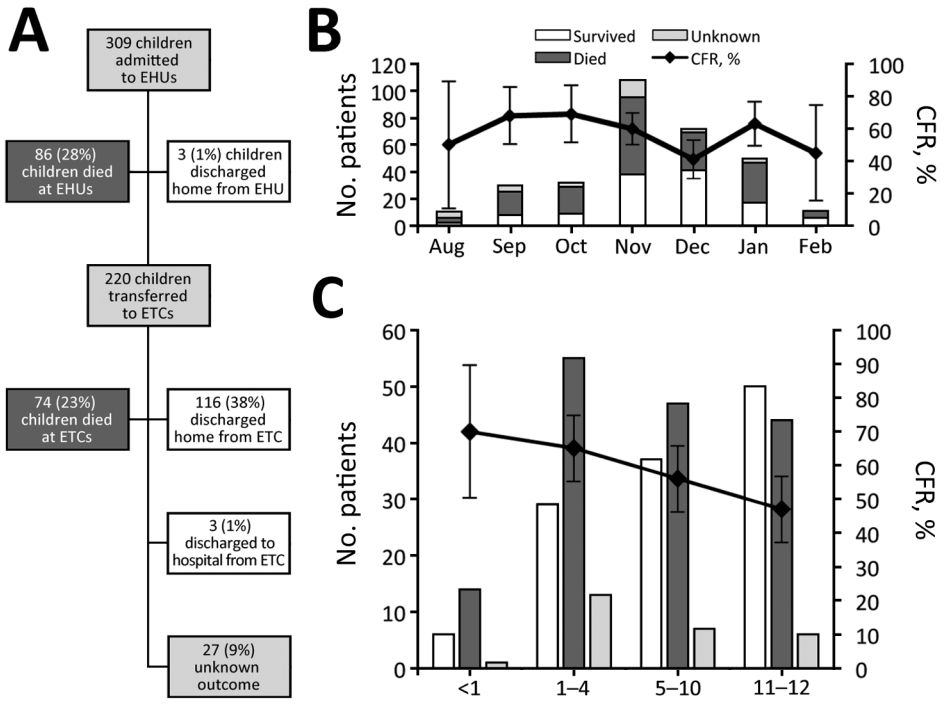

Figure 3. Outcome characteristics of children <13 years of age with Ebola virus disease, Western Area, Sierra Leone, August 2014–March 2015. A) Patient outcome flowchart; B) patient count and case-fatality rate (CFR) by month; C) Patient count and CFR by age. Error bars indicate 95% CIs.

triangulating available data sources. This collaboration enabled crucial matching of clinical management data between EHUs and ETCs, which had not been undertaken previously. That 55% of deaths occurred in EHUs highlights the importance of data pooling. Data from ETCs alone need to be interpreted in the context of substantial survivorship bias (*11*,*12*,*18*). Patients spent a median of 2 days at an EHU before ETC transfer, a critical period given the rapid progression to death.

Cohorts of children reported from previous outbreaks have been smaller, 1 comprising 20 laboratory-confirmed cases and another comprising 55 patients <22 years of age (*4*,*19*). The largest study of children from this outbreak was an international cohort documenting epidemiologic findings from across the 3 most affected countries based on data reported to regional control centers (*3*). Although the sample size included was much larger (2,991 confirmed or probable cases in patients ≤16 years of age), outcomes were available for 42%–59% (varying with age), versus 91% in our study. Furthermore, data were not available about clinical management or caregiver accompaniment.

Our study concurs with previous analyses reporting that young age, particularly infancy, is a risk factor for death from EVD (*2*,*3*,*20*–*23*). Progression to death was swift (median time 3 days from admission [IQR 1–5 days]), more rapid than reported in mixed age cohorts, and the overall death rate was high (*2*,*9*). Compared with the international cohort data from this outbreak, the children in this cohort progressed more rapidly to death. The shortest mean duration from admission to death in the international cohort was 3.7 days for children 1–4 years of age and in other age groups was longer (*3*). Gastrointestinal symptoms predominated here as in mixed age cohorts, although diarrhea was less common in our study (45%) than in the international cohort study (60%) (*5*,*13*,*24*). Diarrhea at presentation, most likely a proxy for more severe disease, appeared to nearly double the risk for death. Few other clinical features at presentation appeared to be associated with death, possibly because of missing or unreliable data; in particular because so many children were unaccompanied, contact history, history of symptoms before attendance, and symptom duration must be cautiously interpreted. Contact history was denied in 42 (22%) children, implying a potential incentive for concealment, which further highlights the need for caution in interpreting these data. This study was powered to detect effects with an OR of ≈2, and the wide CIs for most risk factors mean that real associations not detected by this study cannot be ruled out.

Hypoglycemia in children with EVD has been assumed but not previously demonstrated (*15*). In this cohort, hypoglycemia was frequent and severe (40% of children tested had blood sugar <4.0 mmol/L). Hypoglycemia should be actively sought and treated as a priority, and dextrose should be included in maintenance intravenous fluids to minimize risk for hypoglycemia if monitoring is unavailable. The blood tests in this study were taken at the point of cannulation with no prior intravenous fluids received. As reported in a previous mixed-age cohort (*12*),

other laboratory features include a dramatically raised leukocyte count (predominantly neutrophils) and derangement of renal and liver function. These tests were conducted a median of 3 days after EHU admission and thus are likely to represent a less severe phenotype of disease than in those who died more rapidly. Data from 12 children <6 years of age in the Guinea JIKI trial also demonstrated raised serum creatinine, creatine kinase, and liver function enzymes, although less frequently than in our study (*25*). In contrast, a study from Gulu, Uganda, found no relation between serum chemistry results and death, although an association was seen with markers of immune activation (*19*). A high viral load (low C_t on PCR) has been demonstrated to be associated with death, but because our study was multicenter, the tests were conducted in different laboratories using different assays, making comparisons inappropriate (*25*–*28*).

In terms of clinical management factors, we found no evidence that intravenous fluids had a protective effect on survival, although our study lacked power for this analysis. A case series of adults and children in Liberia also found that intravenous fluids were not protective (*29*). This finding may reflect sicker patients being prioritized for fluid resuscitation. However, aggressive fluid resuscitation in the context of limited monitoring (as was the case in most EHUs) may also have entailed risks. Further research into the safest methods of fluid resuscitation and effective fluid balance monitoring in the Red Zone are crucial, alongside accurate documentation of caregiving.

Nearly 40% of children, even infants, were admitted unaccompanied to EHUs. Unaccompanied children are vulnerable to inadequate oral fluid resuscitation and the hazards of high-concentration chlorine on tap or in basins before even considering the emotional trauma of separation (*30*,*31*). Furthermore, such children could contribute to nosocomial EBOV transmission because they are difficult to keep in their bed space. The heat of personal protective equipment meant that healthcare workers could not spend long within the Red Zone supervising children (*30*,*31*). Many units had started to have survivors work in Red Zones because they are understood to be at vanishingly low risk for re-infection (*32*,*33*). This option could be explored in future outbreaks to ensure adequate care for children.

The overwhelming majority (87%) of children who survived long enough to be moved from an EHU were transferred at least 5 km. Even unaccompanied children and young infants faced transfers up to 380 km to the nearest available ETC bed in very basic ambulances. All of the children in this study who had unknown outcomes were transferred out of EHUs and might have been lost to their families in addition to epidemiologic follow up. Prioritizing transferring children to locations close to their homes could minimize the chance of this loss. Although in our study, neither caregiver presence nor transfer distance was significantly associated with outcome, in the interests of the child both issues could be planned for in future outbreaks.

Although our study has outcomes for 91% of children, missing data and data reliability are a limitation. Transferring information, such as patient observations and medications given in the Red Zone to the low-risk Green Zone where notes were kept, was challenging. Solutions varied from scanners or radios to shouting over the fence, but none were ideal. Stethoscopes were banned, and even simple equipment, such as weighing scales and clocks, had a limited lifespan because of the high concentrations of chlorine used for cleaning. Some analyses lacked power, with wide CIs for some risk factors. Pooling data in this study with other similar cohorts could help identify additional risk factors for survival.

Children have previously been relatively spared by EVD (*20*,*34*,*35*). As the West Africa epidemic progressed, children constituted an increasing proportion of EVD cases (*3*,*36*,*37*). It is possible that earlier in the outbreak, more children were dying unreported at home, given that before the EVD epidemic, Sierra Leone had one of the highest infant mortality rates in the world (*38*). Thus, data presented here also might be subject to survivorship bias. A change in health-seeking behavior, namely bringing very sick children to hospital, might have contributed to the second peak in CFR later in the outbreak (Figure 3, panel B). Documented duration of symptoms before attendance did not change over time (data not shown) but might have been unreliably recorded.

The overarching messages of our study are 3-fold. First, death rates in children were high, and children died even more rapidly than previously documented (*3*). Second is the apparent lack of association between death and potentially modifiable factors that could alter outcome after infection. This lack of association calls for urgent prioritization of interventions targeted to prevent EVD in children. Children have been neglected thus far in EVD vaccine development, and this knowledge gap should be addressed (*39*,*40*). Furthermore, children should be included as a priority in future clinical trials of supportive care methods and of antiviral drugs. Third, the paucity and quality of data and the lack of studies pooling clinical management data from different sites need to be tackled. Many records were already lost when this study was conducted; any available data need to be urgently salvaged and shared. Plans for safe, rapid, and accurate data collection must be prioritized in outbreak planning to identify simple interventions that might improve outcome. We should act or miss a vital opportunity to learn how better to combat EVD in future epidemics.

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Dr. Fitzgerald is a pediatrician specializing in infectious diseases and currently holds an MRC clinical research training fellowship at the UCL Great Ormond Street Institute of Child Health. Her research interests include Ebola virus disease and HIV in children.

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Address for correspondence: Felicity Fitzgerald, Infection, Immunity, Inflammation and Physiological Medicine, UCL Institute of Child Health, 30 Guilford St, London WC1N 1EH, UK; email: felicity.fitzgerald@ucl.ac.uk

Systematic Review and Meta-Analysis of Doxycycline Efficacy for Rectal Lymphogranuloma Venereum in Men Who Have Sex with Men

Charussri Leeyaphan, Jason J. Ong, Eric P.F. Chow, Fabian Y.S. Kong, Jane S. Hocking, Melanie Bissessor, Christopher K. Fairley, Marcus Chen

Rectal lymphogranuloma venereum (LGV) has reemerged as a sexually transmitted infection among men who have sex with men (MSM), particularly those who are HIV-positive. We undertook a systematic review and meta-analysis to determine the efficacy of doxycycline (100 mg 2×/d for 21 days) for rectal LGV in MSM. Nine studies were included: 4 prospective, 4 retrospective, and 1 combined retrospective and prospective. In total, 282 MSM with rectal LGV were included in the studies. All studies reported using nucleic acid amplification tests to assess microbial cure. Most patients (>80%) had symptomatic rectal infection. The fixed-effects pooled efficacy for doxycycline was 98.5% (95% CI 96.3%–100%, I^2 = 0%; p = 0.993). Doxycycline at 100 mg twice daily for 21 days demonstrated a high microbial cure rate. These data support doxycycline at this dosage and duration as first-line therapy for rectal LGV in MSM.

Lymphogranuloma venereum (LGV) has reemerged since the early 2000s as a cause of proctitis in men who have sex with men (MSM). Rectal LGV infections in MSM have been associated with high-risk sexual behaviors, increased rates of concurrent sexually transmitted infections (STIs), and hepatitis C, and the infections have been overrepresented among HIV-positive MSM (*1*). During 2003–2012, a total of 2,138 LGV cases were diagnosed in the United Kingdom: 98% were in MSM, of whom 80% were HIV-positive and 20% had hepatitis C infection (*2*). Surveys from Spain and Germany have shown that rectal LGV accounts for 8%–16% of rectal chlamydia infections in MSM (*3*,*4*).

Author affiliations: Mahidol University, Bangkok, Thailand (C. Leeyaphan); Alfred Health, Melbourne, Victoria, Australia (C. Leeyaphan, J.J. Ong, E.P.F. Chow, M. Bissessor, C.K. Fairley, M. Chen); Monash University, Melbourne (J.J. Ong, E.P.F. Chow, C.K. Fairley, M. Chen); University of Melbourne, Melbourne (F.Y.S. Kong, J.S. Hocking)

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LGV is caused by *Chlamydia trachomatis* serovars L1–L3 which, following mucosal inoculation, disseminate via underlying tissue to regional lymph nodes. This infection process contrasts with that of chlamydia infections caused by *C. trachomatis* serovars A–K, which are limited to the mucosa (*5*). Prior to the 21st century, LGV was endemic to Southeast Asia, the Caribbean, Latin America, and Africa, where infections mainly involved genital inoculation and ulceration with lymphatic spread, resulting in bubo formation (*5*,*6*). More recently, LGV infections among MSM have been largely attributable to the L2b variant of *C. trachomatis* and have predominantly presented as rectal infections following inoculation of the rectal mucosa (*3*). Compared with MSM who have rectal infections caused by chlamydia strains unrelated to LGV, MSM who have rectal LGV are more likely to have symptoms of proctitis and more likely to be HIV-positive (*7*). Symptomatic proctitis is a syndrome commonly seen among MSM attending STI clinics; cases caused by LGV and other sexually acquired pathogens are often clinically indistinguishable (*8*,*9*). Exudative proctitis has frequently been observed, by proctoscopy, in patients with rectal LGV (*10*,*11*). Asymptomatic rectal LGV also occurs and has accounted for different proportions of LGV cases in various studies (*7*,*12*,*13*).

A comparative study published in 1957 provided early evidence for the efficacy of tetracyclines for reducing bubo duration when used for 14 days (*14*). Several national and regional guidelines currently recommend doxycycline (100 mg 2×/d for 21 d) as first-line therapy for rectal LGV (*5*,*6*,*15*,*16*). The deep-seated nature of LGV infection is one rationale for this 3-week duration of treatment (*12*). In a prospective study using repeated testing to verify response to therapy, rectal chlamydia RNA was detectable for up to 16 days following commencement of doxycycline treatment, adding weight to the need for a longer course of doxycycline for LGV (*17*). However, several case reports

have described doxycycline failing to cure LGV in MSM despite 21 days of therapy, including cases of LGV buboes and rectal LGV (*18*–*21*). To provide an evidence base for the use of doxycycline as treatment for rectal LGV infections in MSM, we conducted a systematic review and meta-analysis of studies reporting microbial cure among MSM with rectal LGV treated with 100 mg doxycycline twice daily for 21 days.

Methods

Protocol and Registration

This systematic review and meta-analysis was conducted and reported according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) Statement (http://prisma-statement.org/). The study protocol was registered with PROSPRERO (International Prospective Register of Systematic Reviews, http://www.crd.york.ac.uk/PROSPERO/; registration no. CRD42016036038).

Search Strategy

Six electronic bibliographic databases (Medline, Embase, PubMed, ClinicalTrials.gov, Cochrane Central Register of Controlled Trials, and the Australian New Zealand Clinical Trials Registry) were searched for studies from 1940 to February 2016. In addition, we hand-searched conference abstracts that were available from the International Society for Sexually Transmitted Diseases Research for 2003–2013 and from the International Union against Sexually Transmitted Infections for 2009–2015; we also searched the reference lists of identified papers. Only abstracts published in English were included in the review. No attempt was made to identify unpublished studies.

We used the following search terms: "lymphogranuloma venereum" or "LGV" or "lymphogranuloma venereum" and "treatment" or "LGV and treatment." Medical subject headings (MeSH) were used where possible. To capture all relevant articles, we did not restrict the search strategy specifically to doxycycline or rectal LGV.

Inclusion and Exclusion Criteria

We searched for any published studies providing data on microbial cure of rectal LGV in MSM. Patients were considered cured if a repeat anal swab sample was negative for *C. trachomatis* by nucleic acid amplification testing (NAAT) after treatment with 100 mg doxycycline twice daily for 21 days. So that study cure rates could be included in the meta-analysis, we required the following data for study inclusion: 1) the number of MSM with rectal LGV treated with 100 mg doxycycline twice daily for 21 days; 2) the number of these men who had repeat testing for rectal chlamydia infection following this treatment; and 3) the results of repeat testing for rectal chlamydia infection. Studies with bisexual men were included as studies with MSM. For studies in which the 3 data above were not clear from the published papers or abstracts, we contacted authors to directly request the information. If the information was obtained, the studies and their data were included in the meta-analysis. Studies were excluded if 1) cure rates for rectal infection specifically, as distinct from inguinal buboes, could not be obtained; 2) the total sample size of the study was <10; 3) infections were in heterosexual men only; or 4) if different drugs or dosing regimens were used. Conference abstracts were also included if they fulfilled the inclusion criteria.

Data Extraction Process

We extracted the following data from each study: study design, treatment administered, sample size, proportion of rectal LGV infections that were symptomatic, the diagnostic method for assessing microbial cure, attrition rate of study subjects, and microbial cure after treatment. One author (C.L.) undertook selection of studies, and another (J.J.O.) checked the selection. Disagreements were resolved by discussion and consultation with a third author (M.C.) until a consensus was reached.

Outcome

Treatment efficacy for doxycycline, as determined by microbial cure, was calculated by using the number of treated men with a negative repeat test result by NAAT as the numerator and the number of treated men who underwent repeat testing as the denominator. A single *C. trachomatis*–negative anal swab sample after treatment was considered confirmation of microbial cure. Likewise, a repeat anal swab test positive for a chlamydia strain genotype not associated with LGV was also considered confirmation of cure but was associated with chlamydia reinfection.

Analysis

Meta-analysis was applied to calculate the pooled estimates of doxycycline efficacy. We used the I^2 test to estimate the approximate proportion of variability in point estimates attributed to heterogeneity other than that due to chance (*22*). Random-effects model results were shown if I^2 was >25%, and fixed-effects model results were shown if I^2 was ≤25%.

Assessment of Bias and Quality

Publication bias was not assessed using a funnel plot because <10 studies met the inclusion criteria (*23*). Two authors (C.L. and J.J.O.) independently assessed within-study bias using evaluation criteria reported elsewhere (*24*). Any discrepancies were resolved by recourse to a third author (M.C.). Meta-analysis was conducted using STATA version 13 (StataCorp LP, College Station, TX, USA).

Results

Study Selection and Characteristics

We reviewed 93 of 2,037 identified studies; 9 met our inclusion criteria (Figure 1; Table 1). Five studies were presented in published articles (*3*,*17*,*25*–*27*), and 4 were presented as conference abstracts (*28*–*31*). For 6 of these 9 studies, we obtained additional data on rectal microbial cure rates through personal communications so that the studies could be included in our analysis (Table 1) (*3*,*26*,*28*–*31*). In 1 study, 100 mg doxycycline twice daily for 21 days was used as the comparator group; we used the microbial cure in the doxycycline group in our meta-analysis (*31*). Of the 9 studies, 4 were prospective (*3*,*17*,*27*,*31*), 4 were retrospective (*26*,*28*–*30*), and 1 was combined prospective and retrospective (*25*). All studies used NAAT for retesting after treatment. From these studies, data for a total of 282 MSM with rectal LGV who were retested after treatment with doxycycline were available and included in our meta-analysis. Eight studies reported that >80% of men had rectal symptoms when they sought medical care (*3*,*25*–*31*). All but 2 studies (*3*,*25*) reported the average time between treatment and repeat testing. A study by de Vries et al. (*17*) was the only study that undertook multiple repeat testing over time after doxycycline treatment; for the purposes of our analysis, we used the results from week 3 of the study because some men were given additional courses of doxycycline beyond 21 days. Five studies that reported attrition rates for repeat testing and the percentage of men who had repeat testing ranged from 0 to 65% (*17*,*26*,*27*,*30*,*31*).

Treatment Efficacy

The microbial cure rates for each of the 9 studies ranged from 95% (95% CI 76%–99%) (*29*) to 100% (95% CI 88%–100%) (*31*). Based on these 9 studies, the fixed-effects pooled efficacy was 98.5% (95% CI 96.3%–100%; $I^2 = 0\%$; p = 0.993) (Figure 2).

Study Bias

We assessed the risk of biases within each of the 9 included studies (Table 2; online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/10/16-0986-Techapp1.pdf). Of the 9 studies, 7 recruited patients from STI clinics (*3*,*25*–*29*,*31*); 8 recruited MSM regardless of whether they had rectal symptoms (*3*,*17*,*25*–*28*,*30*,*31*); and 1 recruited only patients with symptomatic proctitis (*29*). All studies described in-house methods for LGV identification: 2 used a real-time PCR targeting the polymorphic membrane protein H gene (*17*,*26*); 1 used nested PCR and restriction fragment length polymorphism (RFLP) analysis targeting the major outer membrane protein gene (*25*); 1 used real-time multiplex PCR including LGV (*31*); 1 used

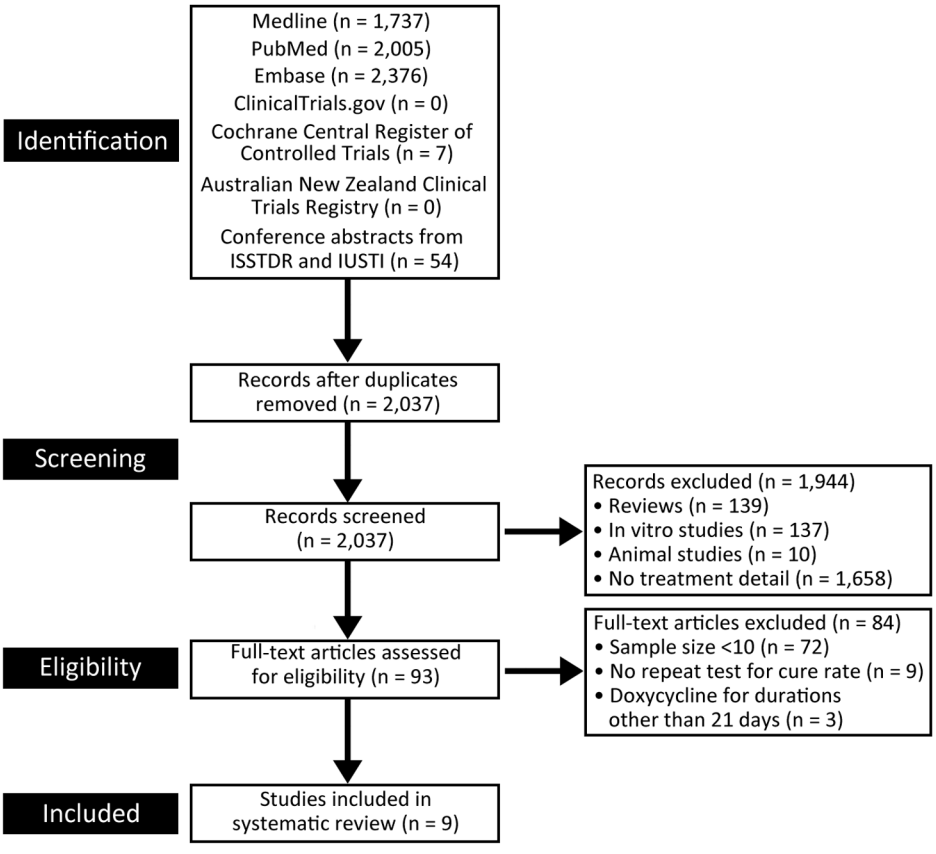

Figure 1. Studies reporting microbial cure after doxycycline treatment (100 mg 2×/d for 21 d) of rectal lymphogranuloma venereum in men who have sex with men. ISSTDR, International Society for Sexually Transmitted Diseases Research; IUSTI, International Union against Sexually Transmitted Infections.

Table 1. Studies from 2006 to 2015 reporting the efficacy of 100 mg doxycycline twice daily for 21 days for the treatment of rectal LGV in men who have sex with men*

Ref.	Study type	Serovar	No. men tested positive and treated for LGV/no. retested after treatment	No. symptomatic/ no. total (%)	No. HIV-positive/no. total (%)	Method for retesting	Time from treatment to retesting	No. negative repeat test results/no. repeat tests (% negative; 95% CI)
(*25*)	RS/PS	L2	21/21	18/21 (86)	13/21 (62)	Cobas Amplicor Analyzer†	NS	21/21 (100; 85–100)
(*17*)	PS	L	20/17	NS	NS	Cobas Amplicor Analyzer†	3 wk	17/17 (100; 82–100)
(*26*)	RS	L	55/19	59/63 (94)	52/63 (82)	BD ProbeTec ET System‡	3 mo	19/19 (100; 83–100)§
(*28*)	RS	L	70/70¶	80/99 (81)	78/99 (79)	BD ProbeTec ET System‡	≤6 mo	68/69 (99; 92–100)¶
(*29*)	RS	L2b	20/20#	20/20 (100)	18/25 (72)	BD ProbeTec ET System‡	3 mo	19/20 (95; 76–99)#
(*30*)	RS	L	80/43	71/83 (85)	69/83 (83)	APTIMA Combo 2 assay**	6 wk	42/43 (97; 88–100)††
(*27*)	PS	L2	13/13	13/13 (100)	9/13 (69)	Versant CT/GC DNA 1.0 assay‡‡	3 mo	13/13 (100; 77–100)
(*3*)	PS	L2, L2b	53/53§§	73/82 (89)	66/82 (80)	Abbott RealTime CT/NG assay¶¶, BD ProbeTec ET System‡	NS	51/53 (96; 87–99)§§
(*31*)	PS	L	28/27	28/28 (100)	27/28 (96)	Real-time multiplex PCR	3 wk	27/27 (100; 88–100)##

*LGV, lymphogranuloma venereum; NS, not specified; PS, prospective; ref., reference; RS, retrospective; RS/PS, retrospective and prospective.
†Roche, Basel, Switzerland.
‡Becton Dickinson Microbiology Systems, Sparks, MD, USA.
§S.C. Hill, St. Mary's Hospital, London, UK, pers. comm., 2016 Apr 28.
¶Seventy men treated for rectal LGV underwent repeat testing; 1 had an equivocal result and was excluded from further analyses (S. Pallawela, Royal Berkshire Hospital, Reading, UK, pers. comm., 2016 Apr 28).
#M. Bissessor, Melbourne Sexual Health Centre, Carlton, Victoria, Australia, pers. comm., 2016 Apr 1.
**Gen-Probe Inc., San Diego, CA, USA.
††A. Garner, Stockport NHS Foundation Trust, Stockport, UK, pers. comm., 2016 May 26.
‡‡Siemens Healthcare Diagnostics, Terrytown, NY, USA,
§§J.C. Galan, Hospital Ramon y Cajal, Madrid, Spain, pers. comm., 2016 May 16.
¶¶Abbott Laboratories, Des Plaines, IL, USA
##J.L. Blanco, University of Barcelona, Barcelona, Spain, pers. comm., 2016 May 10.

PCR amplification of the outer membrane protein 1 (*omp*1) gene followed by RFLP analysis (*27*); 1 used *omp*1 gene sequencing (*29*); 1 used real-time polymorphic membrane protein H gene PCR and *omp*1 gene sequencing (*3*); 1 used LGV-specific molecular serovar typing (*28*); and 1 used an in-house assay (*30*).

Studies differed in the manner in which they dealt with the possibility of LGV reinfection or non-LGV chlamydia as a cause of a positive test result after treatment. Three studies with participants whose repeat test results were positive reported that reinfection could have contributed to these results (*28–30*). Six of the studies conducted genotyping of positive repeat chlamydia specimens (*17*,*26*,*27*,*29–31*). Two of these studies reported genotyping results: Foschi et al. (*27*) reported that 1 patient had a non-LGV serovar on repeat testing, indicating chlamydia reinfection, and Bissessor et al. (*29*) demonstrated an LGV-associated serovar in a sample from a man who had unprotected sexual intercourse after treatment, making it impossible to determine whether the infection was due to treatment failure or reinfection. None of the studies reported the application of stringent criteria or algorithms, such as an algorithm based on a combination of sexual reexposure and chlamydia genotyping (*32*), to distinguish treatment failure from reinfection. All but 1 study based microbial cure on a single repeat test.

None of the studies discussed compliance with the full course of doxycycline or the concurrent use of other antimicrobial drugs, both of which could influence treatment outcomes. Three studies reported the authors' conflicts of interest and funding sources (*3*,*17*,*27*). Through the review process, no randomized controlled trials comparing doxycycline with other treatments were identified.

Discussion

Our meta-analysis found a pooled treatment efficacy of 98.5% (96.3%–100%; I^2 = 0%) for 100 mg doxycycline twice daily for 21 days for the treatment of rectal LGV infections in MSM. This result supports the recommendation that doxycycline be used at this dosage and duration for the treatment of rectal LGV in MSM, including MSM who are HIV-positive. Several guidelines, including those from Europe, the United Kingdom, and the United States, recommend doxycycline at this dosage and duration as

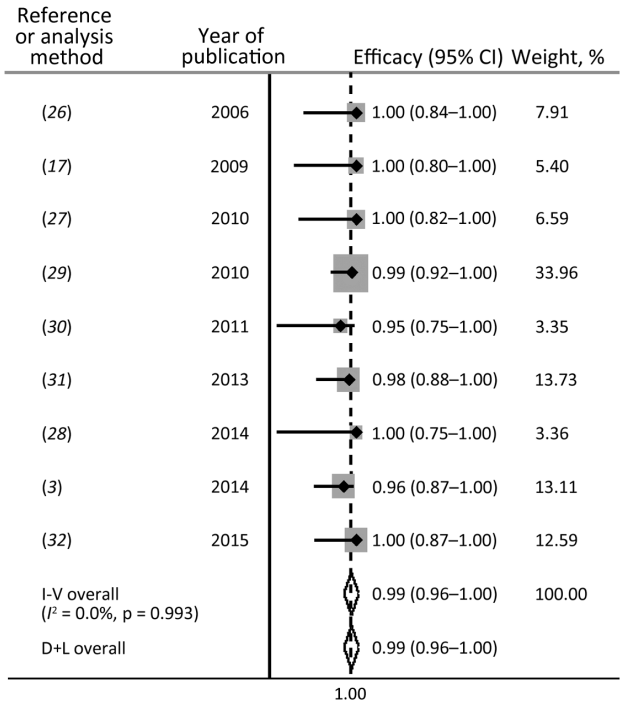

Figure 2. Efficacy of doxycycline (100 mg 2×/d for 21 d) for treatment of rectal lymphogranuloma venereum infection in men who have sex with men. I-V, inverse-variance (fixed) method; D+L, DerSimonian and Laird (random-effects) method; I^2, test for heterogeneity.

first-line therapy for the treatment of LGV (*5*,*6*,*15*). The results of our meta-analysis provide a high degree of precision to support the continuation of this recommendation.

Although rectal infections with LGV-associated variants of *C. trachomatis* have been concentrated among MSM, rectal chlamydia infections in MSM are still, overall, more likely to be caused by other chlamydia serovars. Infections with these other serovars have generally been treated with 100 mg doxycycline twice daily for 7 days, which has been shown from meta-analysis (*33*) to have a pooled efficacy of 99.6%. The efficacy of azithromycin (1 g) for rectal chlamydia infection has been questioned, and randomized trials comparing this with doxycycline for rectal chlamydia are needed. In a prospective study of rectal LGV treatment, 100 mg doxycycline twice daily for 7 days achieved a negative repeat chlamydia test result for only 15 (88%) of 17 MSM (*17*). Persistent rectal LGV infection despite 10 days of doxycycline treatment has also been reported (*18*). These reports underscore the value of genotyping positive rectal chlamydia specimens from MSM because the identification of LGV-associated variants will indicate the need for a longer course of doxycycline to ensure cure.

Although our meta-analysis showed that most rectal LGV infections were cured with a 21-day course of doxycycline, clinical failures have been reported with this regimen. In a case from France, an HIV-negative man with LGV proctitis and inguinal lymphadenopathy from the L2 serovar experienced clinical antimicrobial drug treatment failure even though doxycycline treatment was extended beyond 3 weeks. He subsequently achieved clinical resolution with moxifloxacin (*18*). In a report from Portugal, 2 HIV-positive MSM with rectal LGV from the L2b variant (1 with inguinal lymphadenopathy and the other with fever) did not clinically respond after 3 weeks' treatment with doxycycline (*19*). In a case from Spain, a man with L2 serovar–associated LGV proctitis and inguinal lymphadenopathy received 200 mg doxycycline daily for 21 days, resulting in improved rectal symptoms, but progression of the lymphadenopathy required azithromycin followed by moxifloxacin to achieve clinical cure (*20*). There have also been reports of LGV buboes in MSM that have not resolved clinically after treatment with doxycycline for 21 days. In some cases, abscess formation and rupture with sinus formation have occurred despite this course of treatment (*21*,*34*). These reports suggest that in some cases of more clinically severe or extensive rectal LGV infection, 3 weeks' treatment with doxycycline may not be sufficient for clinical and microbial cure. When abscesses are present, treatment failure might reflect poor penetration of antimicrobial drugs. Several national guidelines recommend that LGV infections be clinically observed until completely resolved and that routine test of cure is not necessary if a

Table 2. Summary of risk of bias in studies from 2006 to 2015 included in a systematic review and meta-analysis of the efficacy of doxycycline for rectal lymphogranuloma venereum in men who have sex with men*

Ref.	Method for selection of participants	Methods for measuring exposure and outcome variables	Design-specific sources of bias, excluding confounding	Method to control confounding	Statistical methods	Conflict of interest
(*25*)	+	+	NR	NR	+	NR
(*17*)	+	+	+	NR	+	+
(*26*)	+	+	++	NR	+	NR
(*28*)	+	+	++	NR	+	NR
(*29*)	+	+	++	NR	+	NR
(*30*)	+	+	++	NR	+	NR
(*27*)	+	+	++	NR	+	+
(*3*)	+	+	NR	NR	+	+
(*31*)	+	+	++	NR	+	NR

*NR, not reported; ref., reference; +, low risk of bias; ++, moderate risk of bias; +++, high risk of bias.

21-day course of doxycycline has been completed (*5*,*6*,*15*). Our study findings support these recommendations.

Cases of rectal LGV among MSM have mainly been attributable to *C. trachomatis* serovars L2b and L2. Co-circulation of these 2 serovars among MSM in Spain has been shown with distinct clinical manifestations: LGV cases with rectal bleeding and pain have been associated with serovar L2b more than with L2 (*3*). Previous studies have, however, reported failure of doxycycline treatment in patients infected with the L2 serovar (*18*,*20*). Recently, a new LGV strain, L2c, a recombination of L2 and D strains, has been identified and may be associated with more severe infection (*35*). However, *C. trachomatis* serovar L, compared with serovars D–K, has no additional genes that determine disease outcome (*36*,*37*). Further research is required to define the determinants of LGV invasiveness and pathogenesis (*37*).

Several points should be considered when interpreting the results of our study. We identified only 9 studies that specifically reported microbial cure rates for MSM with rectal LGV, 4 of which were conference abstracts rather than published papers. The overall pooled efficacy of doxycycline was based on results for a total of 282 men. Although this combined number of cases is limited, the pooling together of otherwise small, individual studies provides a more precise estimate of doxycycline efficacy with tighter CIs than was previously available for each of the separate component studies (Table 1). Only 4 studies were prospective, and several were retrospective studies. We did not identify any randomized controlled trials. We identified considerable limitations in the quality of studies. The studies showed considerable variation in the timing of repeat testing after doxycycline, and only 1 study undertook repeat testing on more than 1 occasion (*17*). A single negative repeat test could, in theory, miss ongoing infection if organism shedding is intermittent and thus could lead to an overestimation of treatment efficacy (*38*). Conversely, a positive repeat test could indicate reinfection with LGV or another chlamydia strain rather than treatment failure, leading to an underestimation of treatment efficacy. None of the studies reported criteria or algorithms, as described previously (*32*), to distinguish treatment failure from reinfection based on sexual reexposure and chlamydia genotyping. Future randomized trials aimed at determining the efficacy of alternative antimicrobial drugs for the treatment of LGV are warranted and should include carefully considered algorithms that distinguish treatment failure from reinfection.

Despite these caveats, our study demonstrated a high microbial cure rate for 100 mg doxycycline twice daily for 21 days for the treatment of rectal LGV in MSM. The data support doxycycline at this dosage and duration as first-line therapy for rectal LGV.

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Dr. Leeyaphan, an instructor in the Department of Dermatology, Faculty of Medicine Siriraj Hospital, Mahidol University, in Bangkok, Thailand, undertook research for this study during her position as Research Fellow at the Melbourne Sexual Health Centre, Alfred Health, in Melbourne, Australia. Her primary research interests are the epidemiology and dermatologic manifestations of sexually transmissible infections.

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Address for correspondence: Marcus Chen, Central Clinical School Monash University, Melbourne Sexual Health Centre, 580 Swanston St, Carlton, VIC 3053, Australia; email: mchen@mshc.org.au

Increase in Meningococcal Serogroup W Disease, Victoria, Australia, 2013–2015

Kylie S. Carville,[1] Kerrie Stevens, Asma Sohail, Lucinda J. Franklin, Katherine A. Bond, Aicha Brahmi, Finn Romanes, Katherine S. Ong

In Victoria, Australia, invasive meningococcal disease caused by *Neisseria meningitidis* serogroup W increased from 4% of all cases in 2013 to 30% in 2015. This increase resulted largely from strains similar to those in the serogroup W sequence type 11 clonal complex, previously described in the United Kingdom and South America.

Neisseria meningitidis serogroup W (MenW) disease was believed to have little epidemic potential before the first international outbreak in 2000, which was related to the Hajj, and has subsequently been described in the African meningitis belt, South Africa, and Saudi Arabia (*1*). This serogroup emerged in the South Cone of South America in the mid-2000s and in the United Kingdom in 2009–2010. Strains belonging to sequence type (ST) 11 are the most prevalent; those from the South Cone of South America are part of ST11 but have diversified (*1,2*).

Since 2009–2010, the United Kingdom (England and Wales) has seen an increase in disease caused by 1 endemic hypervirulent strain of MenW that began in older adults and subsequently spread to persons in all age groups (*3,4*). Historically, MenW accounted for <5% of laboratory-confirmed cases of invasive meningococcal disease (IMD) in England, but in 2014–2015, this serogroup was responsible for 25% of all cases (*3*). The ST11 strain was shown by whole-genome sequencing (WGS) to be similar to strains in the South Cone nations of South America (*2,4,5*). In response, vaccination programs against MenW have been initiated in the United Kingdom and Chile (*1,3*).

The Department of Health and Human Services (Melbourne, Victoria, Australia) collects information from doctors and laboratories about diagnoses of certain health-related conditions in Victoria under the Public Health and Wellbeing Act, 2008. The incidence of IMD in Victoria decreased from 2.5 cases/100,000 population (125 cases) in 2003, when the conjugate meningococcal C vaccine was added to the National Immunization Program, to 0.6 cases/100,000 population in 2014 (*6*).

Serogroup B is currently the most common cause of IMD in Victoria. However, the department has observed a significant increase in reports of MenW; 17 (30% of IMD cases) cases were reported in 2015, compared with 4 (12%) cases in 2014 and 1 (4%) case in 2013 ($p<0.01$ by test of proportions for 2013–2015) (Figure 1). In 2015, a total of 55 confirmed cases of IMD were reported to the department: 29 were caused by serogroup B, 17 serogroup W, 9 serogroup Y, and 1 probable case without laboratory confirmation. We analyzed the status of meningococcal serogroup W disease in Victoria during 2013–2015.

The Study

The 22 MenW cases reported during January 1, 2013–December 31, 2015, were in older persons than is typically seen for IMD (9 cases were in persons >70 years of age). Median age of these 22 case-patients was 56 years; median age was 19 years for patients with serogroup B infections. Eleven (50%) MenW cases were in women.

Bacteremia was the predominant manifestation among MenW case-patients (55%), followed by septic arthritis (with or without bacteremia) (18%) (Table). Epiglottitis and pneumonia were also observed. Seven (32%) MenW case-patients were admitted to intensive care units. The case-fatality rate was low (5%); 1 death occurred in a young man who had no previous illnesses. In comparison, the most common manifestation in patients with serogroup B disease during the same period was meningitis (45%), followed by bacteremia (37%), and meningitis with bacteremia (17%). The case-fatality rate for patients with serogroup B disease was also low (1.3%).

Variable region typing (finetyping) for *PorA* and *FetA* genes was conducted for 22 specimens from MenW case-patients (21 isolates and 1 cerebrospinal fluid sample). WGS was conducted for 21 isolates, and multilocus sequence typing was conducted in silico (*7*). One case, in the person who died, was diagnosed by PCR of a cerebrospinal fluid sample (WGS was not possible for this patient).

Seventeen specimens were identified as strain type W:P1.5,2:F1–1; sixteen were ST11 (Table). This strain type has been observed only once in Victoria for an isolate in 2006 (Microbiological Diagnostic Unit Public Health Laboratory, unpub. data). This pattern is similar to that for the predominant strain type that emerged in the United

Author affiliations: Department of Health and Human Services, Melbourne, Victoria, Australia (K.S. Carville, A. Sohail, L.J. Franklin, A. Brahmi, F. Romanes, K.S. Ong); Microbiological Diagnostic Unit Public Health Laboratory, Melbourne (K. Stevens, K.A. Bond); Austin Health, Heidelberg, Victoria, Australia (K.A. Bond)

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[1]Current affiliation: Victorian Infectious Diseases Reference Laboratory, Melbourne, Victoria, Australia.

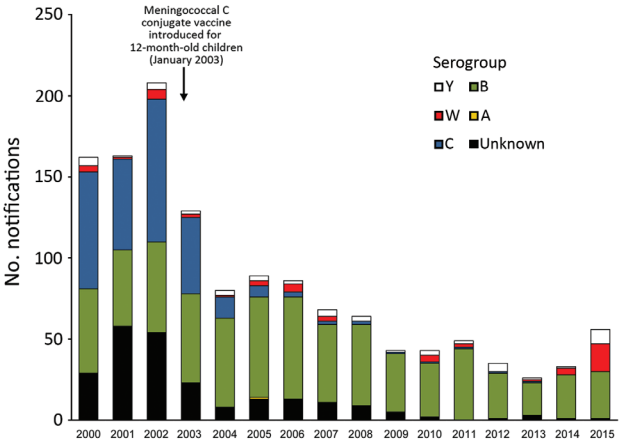

Figure 1. Invasive meningococcal disease notifications, by serogroup and year, Victoria, Australia, January 1, 2000–December 31, 2015.

Kingdom (*4*). The remaining 5 isolates were not ST11. Two isolates had the same strain type (P1.18-1,3:F4-1:ST184), and the remaining strain types were observed only once each (Figure 2). Phylogenetic analysis showed that isolates from Victoria clustered with isolates from the United Kingdom and South America but not with isolates from South Africa or Hajj strains (*8*).

Among the 22 case-patients, 3 did not have any close contacts (i.e., persons who lived in the same household or with whom they had intimate physical contact). Risk factors were not common. Five smokers were identified, and 1 person was immunosuppressed (early myeloma). No case-patients had traveled during the 1–2 weeks before onset of illness. Additional travel history was obtained for 15 of 16 case-patients infected with ST11. Travel among close contacts was reported in the 12 months preceding onset of illness in 4 instances to countries in Southeast Asia, southeastern Europe, and the United Kingdom.

Conclusions

Routine surveillance detected an increase in invasive MenW disease in Victoria, Australia. Although most disease manifestations were mild, there was 1 death in an otherwise healthy young adult. Milder manifestations might be related to older ages of many case-patients or be influenced by serogroup. Overrepresentation of MenW (and serotype Y) in patients with septic arthritis, pericarditis, and pneumonia has been described, often in older persons, but meningococcal pneumonia might be more common among younger persons infected with these serogroups (*9–11*). Other states and territories in Australia have not shown a similar increase in MenW disease.

Absence of travel for most case-patients indicates that transmission of W:P1.5,2: F1-1:ST11 might be endemic in Victoria, although investigations remain ongoing. There is substantial population movement between Australia and the United Kingdom; the United Kingdom is the primary source of permanent migrants (*12*) and a major source and destination for short-term visits (*13*). This population movement and genetic similarity suggest that the strain was originally introduced from the United Kingdom.

The atypical nature of disease manifestations observed could result in underdetection of cases. Absence of close contacts for some case-patients raises questions about disease transmission, including potential for transmission from persons not typically defined as being close contacts (e.g., a carrier to which exposure is short). ST11 strains have been described as being highly transmissible and persistent (including carriage for several months postacquisition) (*14,15*).

In response to the increase in MenW cases, the Department of Health and Human Services advised health professionals in Victoria to consider MenW disease in the differential diagnosis of atypical infections in older patients.

Table. Characteristics of 22 patients with invasive meningococcal serogroup W disease, Victoria, Australia, January 1, 2013–December 31, 2015*

Characteristic	All serogroup W	W:P1.5,2:F1-1:ST11†	Other ST†
Median age (range), IQR, y	56 y (<1 mo–89 y), 23–72 y	56 y (<1 mo–85 y), 25–72 y	45 y (<1 mo–89 y), 1–84 y
Indigenous status			
Aboriginal or Torres Strait Islander	0	0	0
Non-Aboriginal	21 (95)	16 (100)	5 (100)
Unknown	1 (5)	0	0
Manifestation with petechial rash	2 (10)	1 (6)	0
Manifestation			
Bacteremia	12 (55)	8 (50)	3 (60)
Septic arthritis	4 (18)	3 (19)	1 (20)
Meningitis	1 (5)	0	1 (20)
Meningitis and bacteremia	2 (9)	2 (12)	0
Pneumonia	2 (9)	2 (12)	0
Epiglotittis	1 (5)	1 (6)	0
Admission to intensive care unit	7 (32)	6 (38)	0
Death	1 (5)	0	0
Total	22	16	5

*Values are no. (%) unless otherwise indicated. IQR, interquartile range; ST, sequence type.
†Excludes 1 patient for whom ST was not determined.

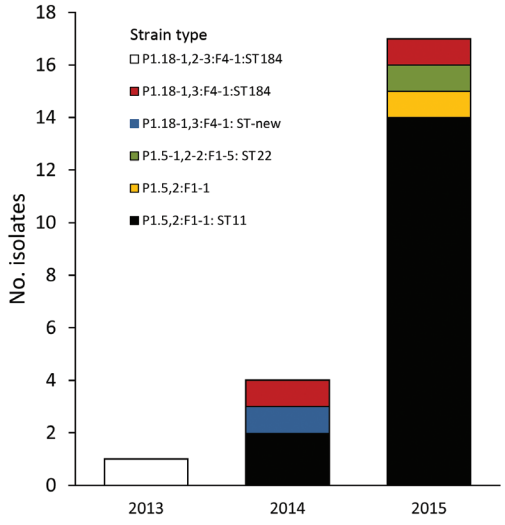

Figure 2. Finetypes of 17 invasive meningococcal serogroup W isolates, Victoria, Australia, January 1, 2000–December 31, 2015.

Enhanced surveillance has been initiated to capture more detail on travel history and contacts to better understand the current epidemiology of MenW. WGS, which was not used previously for detection of IMD, is being used in conjunction with standard typing methods for higher resolution of genetic relatedness of isolates. Although no changes have been made in vaccination recommendations for Victoria, additional data will provide useful information for future discussions regarding merits of vaccination programs.

The response in Victoria benefited from documentation of rapid endemic expansion of similar strains of MenW in the United Kingdom and South America. Public health officials in Victoria were able to detect and monitor the situation early, and the public health response was aided by actions in these 2 other regions. Given that MenW ST11 was established in older populations in the United Kingdom before spreading to younger populations and has been described as being hypervirulent (*3*), disease progression in Victoria is being closely monitored for a similar transition. The situation is evolving, epidemiologic and molecular investigations are ongoing, and other jurisdictions are encouraged to monitor emergence of this strain.

Ms. Carville is an infectious diseases epidemiologist at the Victorian Infectious Diseases Reference Laboratory, Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia. Her research interests are disease surveillance and response, vaccine-preventable diseases, and Indigenous health.

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Address for correspondence: Kylie Carville, Victorian Infectious Diseases Reference Laboratory, The Peter Doherty Institute for Infection and Immunity, 792 Elizabeth St, Melbourne 3000, VIC, Australia; email: kylie.carville@vidrl.org.au

Distinct Zika Virus Lineage in Salvador, Bahia, Brazil

Samia N. Naccache,[1] Julien Thézé,[1] Silvia I. Sardi, Sneha Somasekar, Alexander L. Greninger, Antonio C. Bandeira, Gubio S. Campos, Laura B. Tauro, Nuno R. Faria, Oliver G. Pybus, Charles Y. Chiu

Sequencing of isolates from patients in Bahia, Brazil, where most Zika virus cases in Brazil have been reported, resulted in 11 whole and partial Zika virus genomes. Phylogenetic analyses revealed a well-supported Bahia-specific Zika virus lineage, which indicates sustained Zika virus circulation in Salvador, Bahia's capital city, since mid-2014.

Zika virus is an arthropodborne RNA virus primarily transmitted by mosquitoes of the species *Aedes* (*1*). The virus has 2 genotypes: African, found only in the continent of Africa; and Asian, associated with outbreaks in Southeast Asia, several Pacific islands, and, recently, the Americas (*2*). In May 2015, Brazil reported its first autochthonous cases of Zika virus infection, which occurred in northeast Brazil (*3*,*4*). As of June 30, 2016, all 27 federal states in Brazil had confirmed Zika virus transmission (http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=35262&lang=en).

The rapid geographic expansion of Zika virus transmission and the virus's association with microcephaly and congenital abnormalities (*5*) demand a rapid increase in molecular surveillance in areas that are most affected. Molecular surveillance is particularly relevant for regions where other mosquitoborne viruses, particularly dengue and chikungunya viruses, co-circulate with Zika virus (*2*); surveillance on the basis of clinical symptoms alone is highly inaccurate. Genetic characterization of circulating Zika virus strains can help determine the origin and potential spread of infection in travelers returning from Zika virus–endemic countries. Previous analyses have suggested that Zika virus was introduced in the Americas at least 1 year before the virus's initial detection in Brazil (*1*). The state of Bahia, Brazil, reported most (93%) suspected Zika virus infections in Brazil during 2015 (*2*), including cases of Zika virus–associated fetal microcephaly (*6*); however, except for 1 complete genome, no genetic information from the region has been available (*2*,*7*). We report molecular epidemiologic findings resulting from 11 new complete and partial Zika virus genomes recovered from serum samples from patients at the Hospital Aliança in the city of Salvador in Bahia, Brazil.

The Study

Symptomatic patients with suspected Zika virus infection were enrolled in a research study approved by the Brazil Ministry of Health (Certificado de Apresentação para Apreciação Ética 45483115.0.0000.0046, no. 1159.184, Brazil). During April 2015–January 2016, acute Zika virus infection was diagnosed for 15 patients whose serum samples tested positive by a qualitative reverse transcription PCR (RT-PCR) by using primers targeting the nonstructural 5 gene (*8*). Clinical samples were retested for Zika virus positivity by using a separate quantitative RT-PCR (QuantiTect SYBR Green PCR kit; QIAGEN, Valencia, CA, USA) and primers targeting the envelope gene (*9*). Metagenomic next-generation sequencing libraries were constructed from serum RNA extracts, as described (*10*,*11*; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/16-0663-Techapp1.pdf). Pathogen identification from metagenomic next-generation sequencing data was performed by using the Sequence-based Ultra-Rapid Pathogen Identification bioinformatics pipeline (*12*; http://chiulab.ucsf.edu/surpi/). Results of the metagenomic analyses and identification of co-infections with chikungunya virus are reported elsewhere (*13*).

For Zika virus genome sequencing, 2 isolates (Bahia07 and Bahia09; Table) with Zika virus titers >10^4 copies/mL generated sufficient viral metagenomic data for complete genome assembly. For the remaining samples with lower titers, metagenomic next-generation sequencing libraries were enriched for Zika virus sequencing by using xGen biotinylated lockdown capture probes (Integrated DNA

Author affiliations: University of California San Francisco, San Francisco, California, USA (S.N. Naccache, S. Somasekar, C.Y. Chiu); University of California San Francisco–Abbott Viral Diagnostics and Discovery Center, San Francisco (S.N. Naccache, S. Somasekar, C.Y. Chiu); University of Oxford, Oxford, UK (J. Thézé, N.R. Faria, O.G. Pybus); Federal University of Bahia, Salvador, Brazil (S.I. Sardi, G.S. Campos); University of Washington, Seattle, Washington, USA (A.L. Greninger); Hospital Alianca, Salvador (A.C. Bandeira); Gonçalo Moniz Research Center–Oswaldo Cruz Foundation, Salvador (L.B. Tauro); Evandro Chagas Institute, Ananindeua, Brazil (N.R. Faria)

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

Table. Clinical information for isolates from serum samples of patients with acute symptomatic Zika virus infection*

Isolate	Patient age, y/sex	Collection date†	Genbank accession no.	Zika virus RT-PCR	Zika virus qRT-PCR C_t	Viral load, copies/mL	160-nt single-end metagenomic reads		250-nt paired-end Zika virus–specific enrichment	
							Genome recovery, %‡	Mean fold coverage	Genome recovery, %‡	Mean fold coverage
Bahia01	72/F	2015 May 16	KX101066	Pos	34.6	1,042	23.1	0.4	65.3	16,288.2
Bahia02	37/M	2015 May 5	KX101060	Pos	32.5	4,086	26.0	0.4	73.4	20,045.8
Bahia03	35/M	2015 May 5	KX101061	Pos	32.8	3,272	1.1	0.0	77.7	220.0
Bahia04	40/M	2015 Jun 1	KX101062	Pos	34.1	1,464	5.1	0.1	42.0	4,659.5
Bahia05	U/M	2015 Dec 10	KX101063	Pos	33.7	1,901	5.0	0.1	42.8	8,547.5
Bahia07	37/F	2015 Aug 29	KU940228	Pos	13.7	9.1×10^8	100	3,603.5	ND	ND
Bahia08	U/M	2015 Jul 15	KU940227	Pos	33.3	2,470	75.1	9.2	84.9	23,805.1
Bahia09	40/F	2015 Apr 25	KU940224	Pos	29.9	23,121	99.98	41.5	ND	ND
Bahia11	40/F	2015 Apr 27	KX101064	Pos	Neg (no C_t)	NA	27.8	0.9	64.0	28,704.1
Bahia12	36/M	2015 May 7	KX101067	Pos	34.2	1,327	11.2	0.2	50.4	10,461.8
Bahia15	U/M	2016 Jan 25	KX101065	Pos	Neg (no C_t)	NA	4.6	0.2	45.4	3,706.8

*C_t, cycle threshold; NA, not applicable; ND, not done; Neg, negative; Pos, positive; qRT-PCR, quantitative reverse transcription PCR; RT-PCR, reverse transcription PCR; U, unknown.
†Samples were collected from Salvador in Bahia, Brazil, except for Bahia05, which was collected in Camaçari, Bahia, Brazil.
‡Assumes a genome size of 10,676 nt, the size of the prototype Brazilian Zika virus strain SPH2015 (KU321639).

Technologies, Redwood, CA, USA) designed to tile across all sequenced Zika virus genomes >10,000 nt in GenBank (http://www.ncbi.nlm.nih.gov/genbank) as of March 1, 2016. Capture probes were curated for redundancy at a 99% nt similarity cutoff. Enrichment was performed on the metagenomic libraries in pools of 8 libraries (including Zika virus–negative serum sample controls) by using the xGen lockdown probe protocol and the SeqCap EZ Hybridization and Wash Kit (Roche, Indianapolis, IN, USA). Eleven Zika virus genomes with >40% genome recovery (mean 69.4% ± 2.0%) were assembled (Table). Distribution of single nucleotide variants across the 11 recovered genomes exhibited distinct patterns (online Technical Appendix Figure 1), indicating that the assembled genomes were unlikely to result from cross-contamination by a single high-titer Zika virus sample.

Multiple sequence alignment was performed by using MAFFT version 7 (http://mafft.cbrc.jp/alignment/software/); maximum-likelihood (ML) and Bayesian phylogenetic inferences were determined by using PhyML version 3.0 (http://www.atgc-montpellier.fr/phyml/) and BEAST version 1.8.2 (http://beast.bio.ed.ac.uk/), respectively. The best-fit model was calculated by using jModelTest2 (https://github.com/ddarriba/jmodeltest2; details in online Technical Appendix). Coding regions corresponding to the 11 complete or partial genomes from Bahia were aligned with all published and available near-complete Zika virus genomes and longer subgenomic regions (>1,500 nt) of the Asian genotype as of April 2016 (mean sequence size 8,402 nt with 1,652 distinct nucleotide site patterns). The ML phylogeny was reconstructed by using the best-fit general time-reversible nucleotide substitution model with a proportion of invariant sites (GTR+I). Statistical support for phylogenetic nodes was assessed by using a bootstrap approach with 1,000 bootstrap replicates. A Bayesian molecular clock phylogeny was estimated by using the best-fitting evolutionary model (*2*); specifically, a GTR+I substitution model with 3 components: a strict molecular clock, a Bayesian skyline coalescent prior, and a noninformative continuous time Markov chain reference prior for the molecular clock rate.

The isolates from patients in Salvador clustered together within 1 strongly supported clade (posterior probability 1.00, bootstrap support 100%, Bahia clade C) (Figure; online Technical Appendix Figure 2). This support is notable; most Zika virus genomes in this clade are incomplete, and uncertainty is accounted for in phylogenetic inference. The tree topology accords with previous findings (*2*,*4*,*5*), and time to most recent common ancestor (TMRCA) of the epidemic in the Americas is similar to that previously estimated (*2*) (American epidemic clade A; Figure). The overall ML and molecular clock phylogenies exhibited many well-supported internal nodes with bootstrap support >60% and posterior probability >0.80 (Figure; online Technical Appendix Figure 2), although several nodes near the ancestor of clade A were less well supported.

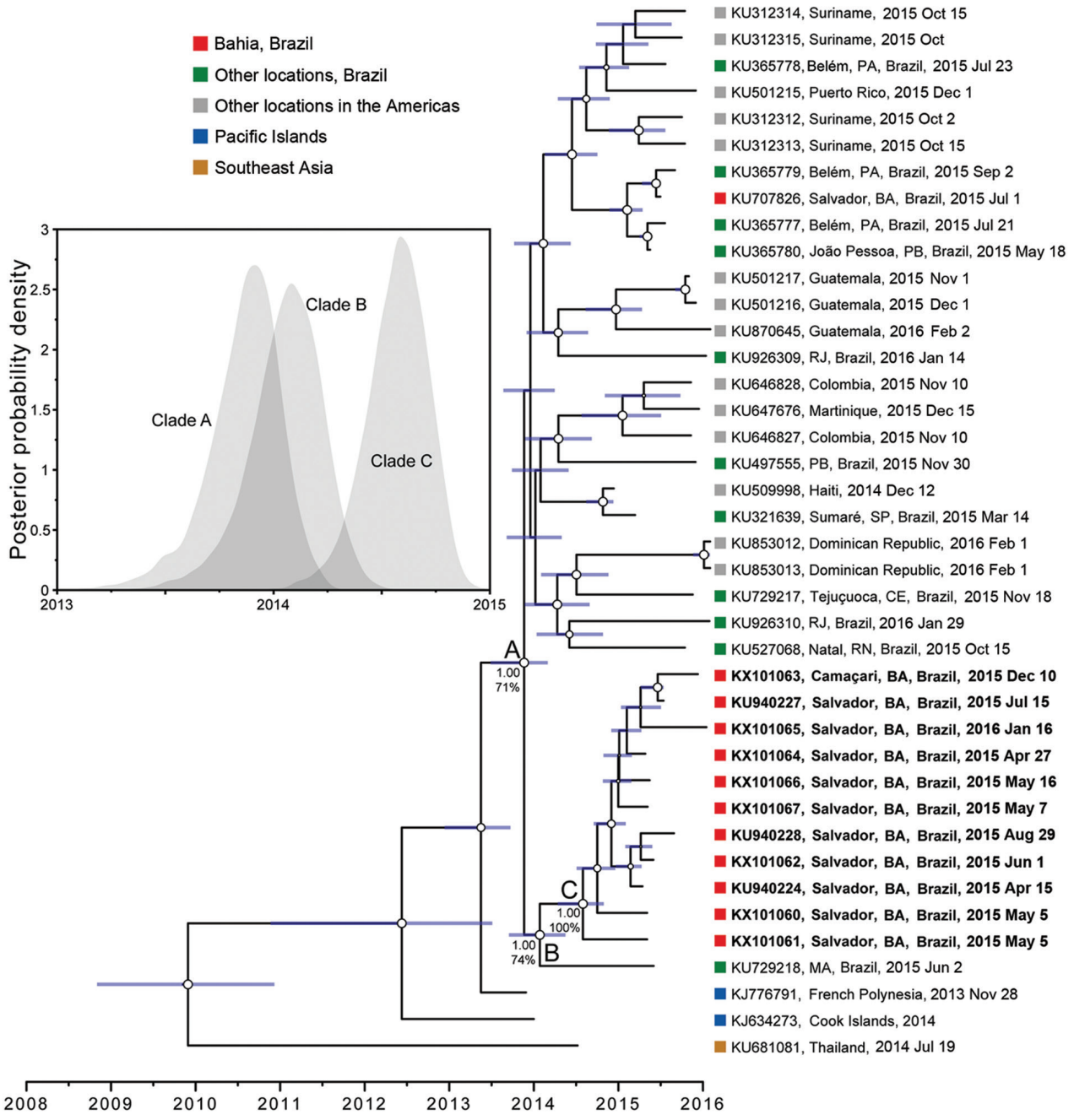

Figure. Timeframe of Zika virus outbreaks in the Americas. A molecular clock phylogeny is shown with the Zika virus outbreak lineage estimated from complete and partial (>1,500 nt) coding region sequences. For visual clarity, 5 basal Southeast Asia sequences (GenBank accession nos. HQ23499 [Malaysia, 1966]; EU545988 [Micronesia, 2007]; KU681082 [Philippines, 2012]; JN860885 [Cambodia, 2010]; and KU681081 [Thailand, 2013]) are not displayed. Blue horizontal bars represent 95% Bayesian credible intervals for divergence dates. A, B, and C denote the current American epidemic, the northeastern Brazil (Maranhão sequence and Bahia), and the Bahia clades, respectively; numbers next to the clade denote posterior probabilities and bootstrap scores in percentages. Circle sizes at each node represent the posterior probability support of that node. Taxa are labeled with the Genbank accession numbers, sampling location, and sampling date. Names of sequences generated in this study are in bold. The inset graph on the left shows the posterior probability distributions of the estimated ages (time to most recent common ancestor) for clades A, B, and C. The posterior probability density is plotted on the vertical axis as a function of time on the horizontal axis (tick marks designate 3-month intervals). Estimated ages were determined with BEAST version 1.8.2 (http://beast.bio.ed.ac.uk/) by using the best-fitting evolutionary model. The posterior probability distributions were visualized by using Tracer version 1.6 (http://tree.bio.ed.ac.uk/software/tracer/). Brazil states: BA, Bahia; CE, Ceará; MA, Maranhão; PA, Pará; PB, Paraíba; RN, Rio Grande do Norte; RJ, Rio de Janeiro; SP, São Paulo. .

The updated phylogenetic analyses, including the newly identified clade C, suggest that Zika virus was introduced in Bahia during March–September 2014. An isolate from Maranhão in northeastern Brazil (≈1,000 km from Bahia) is ancestral to the Bahia clade (posterior probability 1.00, bootstrap support 74%, northeastern Brazil clade B) (Figure; online Technical Appendix Figure 2). The TMRCA of clade B (comprising the Bahia clade and the Maranhão sequence) is estimated to be September 2013–April 2014, an early stage of the epidemic. This TMRCA is consistent with the hypothesis that Zika virus in the Americas originated in Brazil (*2*). A previously reported sequence from Bahia (*6*) clustered with an isolate from Belém in the state of Pará in northern Brazil, ≈3,000 km from Bahia (posterior probability 0.99, bootstrap support 81%) (Figure; online Technical Appendix Figure 2). The patient denied history of travel, suggesting that multiple Zika virus lineages may circulate in Bahia.

Conclusions

Our results suggest an early introduction and presence (mid-2014) of Zika virus in the Salvador region in Bahia, Brazil. Given the size of the cluster and statistical support for it, this lineage likely represents a large and sustained chain of transmission within Bahia state. Most cases of this Zika virus lineage clustered closely to a sequence from Maranhão, and we found evidence for an additional potential introduction to Bahia from Pará state. Consequently, Zika virus in Salvador during mid-2014 was likely introduced from other regions in Brazil rather than from outside the country. Current findings of Zika virus emergence in Bahia state during mid-2014 are consistent with first-trimester viral infection in pregnant women corresponding to the initial reported cases of fetal microcephaly, which began in January 2015 (*5*) and peaked in November 2015.

Broader sampling across Bahia is needed to determine whether the Salvador lineage (clade C) identified in this article comprises most Zika virus cases in the state. Brazil currently faces a major public health challenge from cocirculation of Zika, dengue, and chikungunya viruses (*2*–*4*,*14*,*15*). Additional molecular surveillance in the Americas and beyond is urgently needed to trace and predict transmission of Zika virus.

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Dr. Naccache is a researcher at the University of California, San Francisco; her interests are genomic assay development for clinical infectious disease diagnosis and sequencing-based characterization of emerging viruses. Dr. Thézé is a postdoctoral researcher in computational biology at the University of Oxford, UK; he is interested in viral evolution, especially the spatiotemporal dynamics of pathogen spread.

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Address for correspondence: Charles Y. Chiu, University of California, San Francisco, 185 Berry St, Box # 0134, UCSF China Basin, San Francisco, CA 94107, USA; email: charles.chiu@ucsf.edu

Streptococcus suis Serotype 2 Capsule In Vivo

Jean-Philippe Auger,[1] Nattakan Meekhanon,[1] Masatoshi Okura,[1] Makoto Osaki, Marcelo Gottschalk, Tsutomu Sekizaki, Daisuke Takamatsu

Many *Streptococcus suis* isolates from porcine endocarditis in slaughterhouses have lost their capsule and are considered avirulent. However, we retrieved capsule- and virulence-recovered *S. suis* after in vivo passages of a nonencapsulated strain in mice, suggesting that nonencapsulated *S. suis* are still potentially hazardous for persons in the swine industry.

Streptococcus suis is a gram-negative bacterium that infects pigs and causes severe economic losses to the swine industry. Moreover, it causes severe disease in persons in close contact with diseased pigs or their products (*1*). In Japan, *S. suis* has been frequently isolated from pigs with endocarditis in slaughterhouses; most of the isolates were expected to be sequence types (STs) that are potentially hazardous to humans (*2*). Many isolates from porcine endocarditis lost their capsule, and all the nonencapsulated isolates analyzed had mutations in the capsular polysaccharide synthesis (*cps*) genes (*3*,*4*). The capsule of *S. suis* is a major virulence factor (*1*). Although loss of the capsule gives *S. suis* some benefit in causing endocarditis by enhancing the ability of bacterial cells to adhere to porcine and human platelets, a major virulence determinant for infective endocarditis (*3*), nonencapsulated *S. suis* are generally considered avirulent (*5*). However, whether nonencapsulated *S. suis* lurking in porcine endocarditis poses a threat to persons working in the swine industry is unknown. To investigate whether nonencapsulated *S. suis* can restore the ability to express the capsule and become virulent again, we repeated in vitro or in vivo passages of nonencapsulated *S. suis* and attempted to retrieve capsule-recovered strains.

The Study

For the in vitro passages, we used 29 *S. suis* strains isolated from pigs with endocarditis. These isolates had the *cps* gene cluster of serotype 2 but had lost their capsule because of mutations in the *cps* genes (Table). We subcultured them twice in liquid media and separated the cells according to the buoyant density by Percoll density gradient centrifugation (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/15-1640-Techapp1.pdf). Because encapsulated cells show lower density than nonencapsulated cells (*6*,*7*), we investigated capsular expression of *S. suis* cells with low density by coagglutination tests using serotype 2 antiserum (online Technical Appendix). The retrieved *S. suis* was also used for the next subcultures. We repeated 4 cycles of this experiment (in total 8 subcultures) but obtained no encapsulated *S. suis* from any of the strains tested.

Although these results suggested that mutations in *cps* genes are not repaired easily, the conditions faced by *S. suis* in vivo could influence capsular expression. To investigate this possibility, we selected strain NL119 as a representative. NL119 is an ST1 strain, one of the types hazardous to humans, but one that has lost the capsule because of a point mutation that occurred at nt 490 (T490C, Cys164Arg) of a glycosyltransferase gene (*cps2F*) (Table; Figure 1, panel A) (*4*). We inoculated groups of 5 mice with 5×10^8 CFU of NL119 (online Technical Appendix). Bacteria persistent in mice were retrieved 36 h after infection from the blood, in which capsular expression works favorably for survival. We investigated capsular expression of the retrieved NL119 by coagglutination tests and used the colony giving the strongest reaction within 30 s for the subsequent in vivo passage.

As expected, the coagglutination test of the parental strain NL119 showed a negative result. Similarly, NL119 after the first and second passages (NL119 P1 and P2, respectively) reacted weakly, comparable to those of the parental strain, suggesting poor encapsulation. Meanwhile, NL119 after the third and fourth passages (NL119 P3 and P4, respectively) reacted strongly, suggesting recovery of the capsule. To confirm this finding, we further analyzed formalin-killed bacteria by dot-ELISA using monoclonal antibody Z3, which reacts with the sialic acid moiety of the serotype 2 capsule (*8*), and an anti–*S. suis* serotype 2 serum adsorbed with parental strain NL119 to select the capsule-specific antibodies (online Technical Appendix). In accordance with the coagglutination test, NL119 P1 and P2 gave weak reactions similar to those of NL119, whereas strong signals were detected in NL119 P3 and P4 with both the monoclonal antibody and serum (Figure 1, panels B, C). Because NL119 P1–P4 were also ST1 as determined by multilocus sequence

Author affiliations: Université de Montréal, St-Hyacinthe, Quebec, Canada (J.-P. Auger, M. Gottschalk); The University of Tokyo, Tokyo, Japan (N. Meekhanon, T. Sekizaki); Kasetsart University, Bangkok, Thailand (N. Meekhanon); National Agriculture and Food Research Organization, Tsukuba, Japan (M. Okura, M. Osaki, D. Takamatsu); Gifu University, Gifu, Japan (D. Takamatsu)

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[1]These authors contributed equally to this article.

Table. Nonencapsulated *Streptococcus suis* strains isolated from pigs with endocarditis and used for in vitro passages to investigate possible capsule recovery

Strain	Affected gene(s)	Types of mutations	Affected nucleotide(s) (affected amino acid)	Reference
NL100	*cps2F*	Nonsense	T696G (Tyr232TERM)	(*4*)
NL119	*cps2F*	Missense	T490C (Cys164Arg)	(*4*)
NL122	*cps2F*	Missense	G52A (Gly18Ser)	(*4*)
NL126	*cps2F*	Frameshift by insertion	TCCG	(*4*)
NL132	*cps2E*	Missense	G1199A (Arg400Lys)	(*4*)
	cps2H	Frameshift by deletion	TA	(*4*)
NL143	*cps2F*	Missense	G493T (Asp165Tyr)	(*4*)
	cps2K	Insertion	AATCATTGG	(*4*)
	cps2R	Missense	G496A (Gly166Arg)	(*4*)
NL146	*cps2F*	Nonsense	T482A (Leu162TERM)	(*4*)
NL171	*cps2E*	Insertion	IS element: 1,619 bp	(*3*)
NL174	*cps2H*	Frameshift by deletion	A	(*4*)
NL175	*cps2H*	Frameshift by deletion	A	(*4*)
NL184	*cps2E*	Insertion	IS element: 1,115 bp	(*3*)
NL194	*cps2E*	Insertion	IS element: 1,416 bp	(*3*)
NL208	*cps2E*	Frameshift by deletion	TAAG	(*4*)
NL219	*cps2E*	Frameshift by deletion	TAAG	(*4*)
NL225	*cps2F*	Frameshift by insertion	CCAAA	(*4*)
NL230	*cps2F*	Frameshift by insertion	A	(*4*)
NL240	*cps2E*	Nonsense	C1189T (Gln397TERM)	(*4*)
NL245	*cps2E*	Frameshift by insertion	T	(*4*)
NL249	*cps2E*	Frameshift by insertion	AGCA	(*4*)
NL255	*cps2E*	Insertion	IS element: 1,619 bp	(*3*)
NL257	*cps2E*	Frameshift by insertion	ATCT	(*4*)
NL266	*cps2E*	Frameshift by deletion	A	(*4*)
NL278	*cps2F*	Missense	T259C (Ser87Pro)	(*4*)
NL295	*cps2F*	Missense	T492G (Cys164Trp)	(*4*)
NL303	*cps2F*	Deletion	81 bp	(*4*)
NL322	*cps2B*	Missense	G469A (Asp157Asn)	(*4*)
	cps2G	Deletion	50 bp	(*4*)
NL328	*cps2F*	Frameshift by deletion	AG	(*4*)
NL342	*cps2E*	Frameshift by deletion	TAAG	(*4*)
NL345	*cps2H*	Deletion	23 bp	(*4*)
	cps2N	Missense	C706T (Pro236Ser)	(*4*)

typing, these results suggested that NL119 had recovered the capsule during passages in animals.

To find mutations that had contributed to the capsule recovery, we sequenced the *cps2F* gene of NL119 P1–P4. Although the cytosine residue at nt 490 was not changed in comparison with the parental strain, we found a further missense mutation at nt 491 (G491C, Arg164Pro) of the *cps2F* gene in NL119 P3 and P4 (Figure 1, panel A). To investigate whether this mutation was involved in the capsule recovery, we cloned *cps2F* of NL119 P4 into a gene expression vector pMX1 (*9*) and introduced it into the parental strain NL119 (online Technical Appendix). A coagglutination test using serotype 2 antiserum showed positive reactions in all transformants tested, demonstrating that the further missense mutation restored the function of *cps2F*, resulting in capsule recovery of NL119 P3 and P4, although how the Cps2F function was recovered by the amino acid substitution is unknown. Isolation of the capsule-recovered strains in vivo could have been the consequence of selection of encapsulated cells, which were already present as a subpopulation in the original nonencapsulated NL119 population, by resisting host immunity including phagocytosis. However, because NL119 was a nonencapsulated strain originally recovered from a single colony and well-isolated by repeated passages in vitro, and no encapsulated subpopulation was ever retrieved in vitro by the selection using Percoll density gradient centrifugation, the most plausible hypothesis would be that the capsule-recovered *S. suis* was generated in vivo.

To evaluate whether the capsule-recovered *S. suis* isolate also recovered its virulence, we infected mice with either NL119 or NL119 P4 (online Technical Appendix). Rates of death differed significantly ($p<0.05$): 50% death in the NL119 P4-infected mice 14 days after infection, compared with 0% for the nonencapsulated NL119 (Figure 2, panel A). Recovery of the capsule also significantly increased its survival in blood 24 h after infection ($p<0.05$). All but 1 surviving NL119 P4-infected mice had significant blood bacterial titers ($\geq 5 \times 10^3$ CFU/mL; geometric mean 10^4 CFU/mL). In contrast, except for 1 mouse, all mice infected with NL119 had blood bacterial titers $<10^4$ CFU/mL (geometric mean 10^2 CFU/mL) (Figure 2, panel B).

Conclusions

Although capsule loss might contribute to *S. suis* infection by enhancing bacterial adherence to host cells and

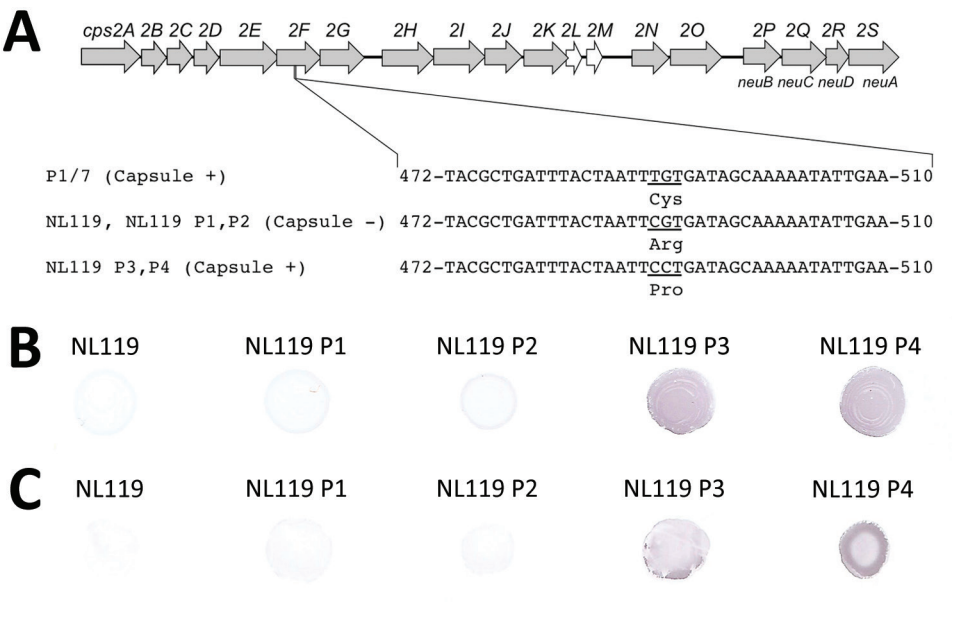

Figure 1. Capsule recovery of *Streptococcus suis* strain NL119 in vivo. A) The genetic organization of the *S. suis* serotype 2 capsular polysaccharide synthesis (*cps*) gene cluster and mutations observed in isolate NL119 and strains retrieved from NL119-infected mice after each in vivo passage (NL119 P1–P4; DDBJ/EMBL/GenBank accession nos. LC147077, LC147078, LC147079, LC147080, and LC077855, respectively). Gray arrows indicate genes putatively involved in capsule synthesis; open arrows indicate genes with unknown functions; numbers indicate nucleotide positions in *cps2F*. NL119 lost the ability to synthesize the capsule because of a missense mutation at nt 490 (T490C, Cys164Arg) of *cps2F* (*4*). B, C) NL119 P1 and P2 retrieved from mice after the first and second in vivo passages, remained nonencapsulated, and their *cps2F* sequences were identical to that of NL119. In NL119 P3 and P4 retrieved after the third and fourth passages a further missense mutation at nt 491 (G491C, Arg164Pro) of *cps2F* restored the function of the gene, resulting in capsule recovery of the strains. Dot-ELISA of NL119 and strains retrieved from NL119-infected mice after each in vivo passage (NL119 P1–P4) using monoclonal antibody Z3 (B) and polyclonal anti–*S. suis* serotype 2 serum adsorbed with NL119 (C). Monoclonal antibody Z3 specifically recognizes the sialic acid moiety of the *S. suis* serotype 2 capsule.

biofilm formation (*3*,*10*–*12*), capsule loss makes *S. suis* cells susceptible to phagocytosis; therefore, the virulence of nonencapsulated mutants was attenuated when evaluated in animal models (*5*). In accordance with previous studies, nonencapsulated NL119 was avirulent. However, NL119 P4, which recovered its capsule in vivo, also recovered virulence. Because various mutations in *cps* genes, including large deletions and insertions, cause capsule loss in *S. suis* (*3*,*4*), not all mutations will be repaired like NL119. However, our results demonstrated the presence of a nonencapsulated mutant, which can recover the capsule and virulence in vivo. Hence, nonencapsulated *S. suis* strains can cause severe diseases to the next hosts by recovering the capsule, which indicates that some nonencapsulated *S. suis* lurking in pigs with endocarditis are still potentially hazardous to

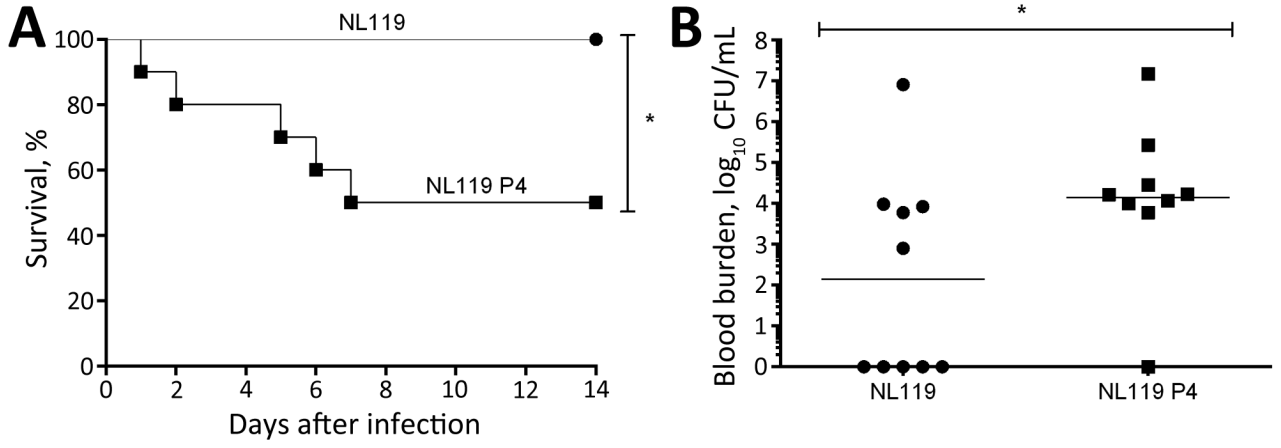

Figure 2. Virulence of nonencapsulated *Streptococcus suis* strain NL119 and capsule-recovered NL119 P4 in mice. A) Survival of C57BL/6 mice (n = 10 mice per strain; until 14 days after infection) inoculated intraperitoneally with 5×10^7 CFU of either NL119 or NL119 P4. B) Blood bacterial burden at 24 h after infection. Data of individual mice are presented as $\log_{10}$ CFU/mL with the geometric mean. Asterisks indicate a significant difference between NL119 and NL119 P4 ($p<0.05$).

persons handling such pigs and their products. Further investigations using a variety of naturally occurring and laboratory-derived mutants are needed for a comprehensive understanding of the biological significance and mechanisms of this phenomenon.

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Mr. Auger is a doctoral student at the Research Group on Infectious Diseases of Swine at the Université de Montréal in St-Hyacinthe, Quebec, Canada. His primary research interest is the host response to pathogens.

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Address for correspondence: Daisuke Takamatsu, Division of Bacterial and Parasitic Diseases, National Institute of Animal Health, National Agriculture and Food Research Organization, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan; email: p1013dt@affrc.go.jp

Estimation of Severe Middle East Respiratory Syndrome Cases in the Middle East, 2012–2016

Justin J. O'Hagan, Cristina Carias, Jessica M. Rudd, Huong T. Pham, Yonat Haber, Nicki Pesik, Martin S. Cetron, Manoj Gambhir, Susan I. Gerber, David L. Swerdlow

Middle East respiratory syndrome has been reported among travelers returning from the Middle East, where most cases have been recorded. Using data from travelers, we estimated 3,250 (1,300–6,600) severe cases occurred in the Middle East during September 2012–January 2016. This estimate is 2.3-fold higher than the total laboratory-confirmed cases recorded in these countries.

Middle East respiratory syndrome (MERS), caused by MERS coronavirus (MERS-CoV), was first recognized in September 2012 (*1*). From that time until January 2016, >1,600 cases were laboratory-confirmed, and ≈600 deaths have been attributed to the virus (*2*). Cases have been detected among persons who traveled from the Middle East to 16 countries, and a MERS-CoV outbreak in South Korea introduced by a traveler caused >100 cases (*3*).

Estimates of the epidemic size in the Middle East are required to understand the level of MERS-CoV circulation and the likelihood of MERS-CoV exportations. However, these estimates have not been calculated for >2 years, during which time the number of recorded cases has increased by >15 times (*2*,*4*). We used data from travelers to this region to update estimates of severe MERS cases in the Middle East.

The Study

We estimated the cumulative number of severe MERS cases in source countries from which laboratory-confirmed MERS-CoV infections were reported by the World Health Organization for nonresident travelers during September 2012–January 2016. The source countries were Saudi Arabia, United Arab Emirates, Jordan, and Qatar (*1*); only the emirates of Abu Dhabi and Dubai were included in the United Arab Emirates calculations due to a lack of traveler data on the other 5 emirates, but no MERS cases have been reported from these other emirates.

Author affiliations: IHRC Inc., Atlanta, Georgia, USA (J.J. O'Hagan. C. Carias); Centers for Disease Control and Prevention, Atlanta (J.J. O'Hagan, C. Carias, J.M. Rudd, H.T. Pham, Y. Haber, N. Pesik, M.S. Cetron, M. Gambhir, S.I. Gerber, D.L. Swerdlow); Imperial College London, London, UK (M. Gambhir); Monash University, Melbourne, Victoria, Australia (M. Gambhir)

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All MERS traveler case-patients whose data were used in the analysis were hospitalized because of respiratory symptoms and therefore likely experienced severe disease. However, 30% of reported MERS case-patients were listed as mildly symptomatic or asymptomatic and were likely missed by the passive surveillance systems that detected severe travel-associated cases (*1*). Consequently, we used the term "severe" to indicate that our estimates of case numbers are for those with more serious disease.

To estimate the cumulative number of severe cases, we used data for 1) the number of travelers to the 4 source countries, 2) average trip lengths, 3) number of confirmed MERS-CoV infections among travelers to the source countries, and 4) population sizes of source countries. Our estimates are for the period September 2012, when MERS-CoV was first identified, through January 2016.

We estimated the number of severe cases in each source country using methods used previously by Cauchemez et al., which assume that travelers and local residents have similar per-day risk of infection (*4*). The infection rate among travelers to source countries was multiplied by the total person-time at risk for the population of that country using the following formula:

$$\text{cumulative number of severe MERS-CoV cases in Country X} = \text{severe case rate among travelers to source countries} \times \text{country X person-time} =$$

$$\frac{\text{No. severe cases reported among travelers returning from source countries}}{\text{Annual no. travelers to source countries} \times \text{average trip length} \times \text{epidemic period}} \times \text{country X population size} \times 365 \times \text{epidemic period}$$

where the epidemic period = 3.33 years (September 2012–January 2016). We estimated CIs using profile-likelihoods (4). This approach can estimate the cumulative incidence of disease regardless of seasonality in infection rates (5).

We analyzed 11 travel-associated MERS cases, including 6 case-patients from high-income countries (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/15-1121-Techapp1.pdf). It has been suggested that MERS-CoV surveillance may be better in high-income countries than in lower-income countries (*6*). We tested this hypothesis by comparing the frequency of case detection among travelers returning to high-income

Table 1. Estimated cumulative incidence of severe Middle East respiratory syndrome cases in Middle Eastern source countries calculated on the basis of illnesses among travelers, September 2012–January 2016*

Traveler origins	Estimated no. cases (95% CI)				
	All countries	Saudi Arabia	Jordan	Qatar	UAE†
Visitors from high-income OECD countries	3,263 (1,297–6,613)	2,269 (902–4,599)	483 (192–979)	163 (65–330)	347 (138–704)
All non–Middle Eastern visitors	1,431 (743–2,452)	995 (517–1,705)	212 (110–363)	72 (37–123)	152 (79–261)

*OECD, Organization for Economic Cooperation and Development; UAE, United Arab Emirates.
†Only the emirates of Abu Dhabi and Dubai were included in calculations due to a lack of traveler data on the other 5 emirates; no cases have been reported from these other emirates.

countries, as defined by the Organization for Economic Cooperation and Development (http://www/oecd.org/about/membershipandpartners/), versus lower-income countries (all other non–Middle Eastern countries worldwide). We found that significantly more cases have been identified in high-income settings ($p<0.001$ by Fisher exact test; online Technical Appendix). Consequently, we produced 2 sets of calculations, 1 using data only from high-income countries and 1 that combined data from all non–Middle Eastern countries. The high-income country analysis does not assume that no cases occurred in lower-income countries but shows different case detection rates across travelers' home countries.

Using data for 32 high-income countries, we estimated ≈3,263 severe cases (95% CI 1,297–6,613; Table 1) for all source countries during September 2012–January 2016. We calculated that Saudi Arabia had the largest number of cases (2,269, 95% CI 902–4,599). We estimated ≈1,431 severe cases (95% CI 743–2,452) when data from high-income and lower-income countries were combined.

We conducted sensitivity analyses in which we included 1) laboratory-confirmed cases among travelers for whom it was unclear in which country they had been infected or if they had been infected by another travel-associated case-patient and 2) probable but non–laboratory-confirmed MERS-CoV cases reported in travelers. These analyses indicated there could have been up to 4,895 severe cases across source countries (95% CI 2,352–8,824). We also conducted sensitivity analyses to assess the effect of uncertainty of travelers' average length of stay in source countries (Table 2; online Technical Appendix Tables 2, 3). Increases in travelers' assumed lengths of stay produced lower cumulative incidence estimates related to lower estimated infection rates, and decreases in travelers' assumed lengths of stay produced higher case estimates. For example, using data for travelers from high-income nations, a 2-day increase in average length of stay produced estimates of 2,326 severe cases across source countries (95% CI 924–4,714; online Technical Appendix Table 2), and a 2-day decrease in lengths of stay produced estimates of 5,463 severe cases (95% CI 2,171–11,071).

Conclusions

We used data on the incidence of MERS among travelers returning from the Middle East to better estimate the occurrence of severe disease in the most affected countries. We estimated that there were ≈3,300 cases of severe disease in the 4 source countries during September 2012 January 2016. This estimate was 2.3-fold higher than the total number of laboratory-confirmed cases across source countries from September 2012–January 2016.

Using data up to August 2013, Cauchemez et al. estimated the total case count to be 11-fold higher than the number of laboratory-confirmed cases reported across source countries (*4*). The closer agreement between observed and estimated cases in our analysis is consistent with improvements in surveillance practices across source countries during 2014 (*6–8*). Our results are also complementary to a serologic study from Saudi Arabia that reported antibodies to MERS-CoV were found in 0.15% of the population (*10*). Our study adds information by focusing on severe infections (which are of greatest clinical concern) and providing more up-to-date information by including data from the 2-year period after the serologic samples were collected.

Our estimates were based on a small sample size (11 travel-associated cases) and assumed that travelers and residents

Table 2. Estimated cumulative incidence of severe Middle East respiratory syndrome cases in Middle Eastern source countries calculated on the basis of illnesses among travelers and traveler LOS, September 2012–January 2016*

Traveler data	Estimated no. cases (95% CI)				
	–2 Days LOS	–1 Day LOS	Average LOS†	+1 Day LOS	+2 Days LOS
Visitors from high-income OECD countries	5,463 (2,171–11,071)	4,086 (1,623–8,280)	3,263 (1,297–6,613)	2,716 (1,079–5,504)	2,326 (924–4,714)
All non–Middle Eastern visitors	2,043 (1,061–3,499)	1,683 (874–2,883)	1,431 (743–2,452)	1,245 (647–2,132)	1,102 (572 – 1,887)

*LOS, length of stay; OECD, Organization for Economic Cooperation and Development.
†The average length of stay of travelers from OECD countries in the 4 source countries (Saudi Arabia, Jordan, Qatar, and United Arab Emirates) was estimated to be 5.0 d, and the average length of stay of travelers from all non–Middle Eastern countries in the 4 source countries was estimated to be 6.7 d (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/15-1121-Techapp1.pdf). Only the emirates of Abu Dhabi and Dubai were included in United Arab Emirates calculations due to a lack of traveler data on the other 5 emirates; no cases have been reported from these other emirates.

of the Middle East had similar infection risks. Our sensitivity analyses demonstrated that results are sensitive to travelers' estimated lengths of stay and also showed that estimates of the epidemic size that incorporated data from lower-income countries were 60% lower than estimates obtained by using data from high-income countries alone. This finding implies different levels of case detection across travelers' home countries or different MERS-CoV exposure between visitors of different nationalities. Additional data (e.g., larger sample size, travel volume, and lengths of stay, stratified by age and immigration status, frequencies of testing, and contact with camels) could provide further estimates.

Public health officials are concerned about MERS-CoV, both in the source countries and from exported cases in persons who can seed outbreaks elsewhere (*9*,*11*). By better estimating the epidemic size in the Middle East, our results can help guide public health preparedness efforts in source countries and contribute to projections of the number of cases that could occur among travelers (*9*,*11*–*13*).

Dr. O'Hagan is an epidemiologist and transmission modeler at Centers for Disease Control and Prevention. His interests include the combined use of mathematical modeling and epidemiologic studies to better understand the effect of infectious disease risk factors and interventions.

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Address for correspondence: Justin J. O'Hagan, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop A16, Atlanta, GA 30329-4027, USA; email: johagan@cdc.gov

Hypervirulent Clone of Group B *Streptococcus* Serotype III Sequence Type 283, Hong Kong, 1993–2012

Margaret Ip, Irene Ang, Kitty Fung, Veranja Liyanapathirana, Ming Jing Luo, Raymond Lai

We describe a hypervirulent clone of group B *Streptococcus* serotype III, subtype 4, sequence type 283, that caused invasive disease with a predilection for meningitis in Hong Kong during 1993–2012. The organism is associated with high mortality and increased summer prevalence and is linked to diseased fish from freshwater fish farms.

Group B S*treptococcus* (GBS) has been implicated in neonatal sepsis and infections in pregnant women. Worldwide, the predominant clone belongs to serotype III, subtype 2 (III-2), sequence type (ST) 17 (*1*), which is less genetically diverse and heterogeneous than strains from the other serotypes (Ia, Ib, II, IV–IX). In July 2015, the Singapore Ministry of Health reported an outbreak of GBS invasive disease in adults and suspected a link to raw fish consumption (*2*). The Ministry subsequently reported that this GBS belonged to ST283 (*3*), and its whole genome has been recently sequenced (*4*). GBS strains of ST283 were linked to a series of adult meningitis cases in Singapore and Hong Kong dating from 1998 (*5*). In addition, GBS III-4/ST283 is a hypervirulent lineage that has been associated with invasive GBS disease in nonpregnant adults (*6*). We investigated the epidemiology of GBS infections caused by GBS III-4/ST283 during 1993–2012 in Hong Kong and examined possible associations with seasons, temperature, and humidity. We also compared the invasive potential of different GBS serotypes among neonates, pregnant women, and nonpregnant adults.

The Study

We investigated GBS serotypes found among 1,645 isolates from patients admitted to a university hospital in Hong Kong during 1993–2012. Of the isolates, 437 were invasive GBS (i.e., isolated from sterile blood, cerebrospinal fluid, and body fluid specimens), and 1,208 were noninvasive (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/10/15-1436-Techapp1.pdf). We stratified these patients into 3 groups: 249 neonates, 704 pregnant women, and 692 nonpregnant adults (women and adults were ≥16 years of age). We used PCR, pulsed-field gel electrophoresis, and multilocus sequence typing for serotyping and subtyping of the isolates, as described (*1,6*). We reviewed demographic and medical records for the 1,645 patients and performed statistical analysis by using Fisher exact and χ^2 tests. SPSS version 19 (IBM, Armonk, New York, USA) was used for Spearman correlation analysis. We obtained ethical approval from the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee (CRE-2012.054).

Using an empirical odds ratio (OR) (*7*), we compared the invasive potential of serotype III-4 isolates with that of all other serotypes (Table 1). Serotypes III-2 (n = 76; OR 1.5, 95% CI 1.1–2.1; p<0.01) and III-4 (n = 50; OR 19.4, 95% CI 9.1–41.2; p<0.001) had significantly higher (OR >1.0) invasive potential than other serotypes. Serotypes III-2 was the predominant cause of invasive disease in neonates (OR 5.3, 95% CI 2.7–10.3; p<0.001). Serotype III-4 was highly invasive in nonpregnant adults (OR 18.4, 95% CI 7.7–43.9; p<0.001) and in neonates (OR 6.3, 95% CI 0.7–54.3; p<0.01).

By using pulsed-field gel electrophoresis, all 48 serotype III-4 strains showed indistinguishable patterns and were confirmed as GBS III-4/ST283 (Figure 1, http://wwwnc.cdc.gov/EID/article/22/10/15-1436-F1.htm). Medical records for these 48 patients (44 nonpregnant adults, 3 neonates, and 1 pregnant woman) revealed that invasive disease has been identified in nonpregnant adults since 1995 and invasive illness in neonates and pregnant women since 2009.

Mean age of the 44 nonpregnant adults with GBS III-4/ST283 infection was 63 (range 23–96) years; half were ≥65 years of age. Twenty-eight (63.6%) of the 44 patients had underlying diseases (e.g., diabetes, hypertension, malignancy, chronic rheumatic heart, and gout). Serious complications developed in 35 (80%) of the 44 nonpregnant adult patients: 17 (38.6%) had sepsis, 10 (22.7%) had septic arthritis, 7 (15.9%) had meningitis, and 2 (4.5%) had infective endocarditis. Twelve (27.3%) patients died. For patients ≥65 years of age, presence of underlying diseases or serious complications was not statistically associated with fatal outcome (p>0.05).

Of the 44 nonpregnant adults with GBS III-4/ST283, 41 (93.2%) had isolates from invasive sites. Sex of patients had no effect on whether the GBS disease was invasive: 20 of these patients were women and 21 were men.

Author affiliations: Chinese University of Hong Kong, Hong Kong, China; Prince of Wales Hospital, Hong Kong

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Table 1. Invasive potential of individual group B *Streptococcus* serotypes among neonates, nonpregnant adults, and pregnant women, Hong Kong, 1993–2012*

Serotype	Odds ratio (95% CI), p value			
	All patients, n = 1,645	Neonates, n = 249	Nonpregnant adults, n = 692	Pregnant women, n = 704
Ia	**0.6 (0.4–0.8), <0.001**	0.7 (0.4–1.4), 0.37	**0.4 (0.2–0.6), <0.001**	1.0 (0.6–1.6), 0.94
Ib	1.0 (0.7–1.3), 0.92	1.1 (0.5–2.5, 0.74	1.2 (0.8–1.8), 0.30	0.7 (0.4–1.3), 0.30
II	0.9 (0.6–1.3), 0.49	**0.2 (0.1–0.5), <0.01**	0.9 (0.5–1.6), 0.76	**2.0 (1.0–3.7), <0.04**
III-1	1.1 (0.8–1.5), 0.69	1.2 (0.6–2.3), 0.65	1.3 (0.8–2.1), 0.33	0.8 (0.4–1.6), 0.58
III-2	**1.5 (1.1–2.1), <0.01**	**5.3 (2.7–10.3), <0.001**	**0.5 (0.3–1.0), <0.05**	1.4 (0.8–2.5), 0.22
III-3	1.1 (0.4–2.9), 0.83	8.6 (0.4–169.2), 0.16	0.8 (0.2–3.3), 0.79	0.4 (0.0–6.3), 0.49
III-4	**19.4 (9.1–41.2), <0.001**	**6.3 (0.7–54.3), <0.01**	**18.4 (7.7–43.9), <0.001**	6.3 (0.4–101.8), 0.19
IV	0.1 (0.0–2.2), 0.16	0.4 (0.0–9.9), 0.57	0.2 (0.0–4.2), 0.32	0.6 (0.0–10.2), 0.70
V	**0.6 (0.5–0.9), <0.02**	**0.1 (0.0–0.4), 0.001**	0.9 (0.5–1.4), 0.60	0.8 (0.4–1.6), 0.56
VI	0.8 (0.4–1.5), 0.51	0.2 (0.0–2.0), 0.19	1.0 (0.5–2.2), 0.95	0.7 (0.4–2.8), 0.57
VII	2.3 (0.7–7.6), 0.17	0.4 (0.0–9.9), 0.57	14.5 (0.7–282.2), 0.08	2.5 (0.5–13.2), 0.27
VIII	0.1 (0.0–2.2), 0.16	0.4 (0.0–9.9), 0.57	0.2 (0.0–4.2), 0.32	0.6 (0.0–10.2), 0.70
IX	0.9 (0.0–22.7), 0.96	1.2 (0.0–61.1), 0.93	2.0 (0.0–103.5), 0.72	2.1 (0.1–51.3), 0.66
NT	1.4 (0.1–15.3), 0.79	1.2 (0.0–61.1), 0.93	2.1 (0.1–33.0), 0.61	2.1 (0.1–51.3), 0.66

*Bold indicates statistical significance; p values determined by Fisher exact or χ^2 test. NT, nontypeable.

Among the total 227 nonpregnant adults with invasive GBS infection, 12 (5.5%) had meningitis (Table 2). Of these patients, 7 (58.3%) were infected with serotype III-4 alone. Compared with other GBS serotypes, GBS III-4 had significantly higher potential ($p<0.001$ by χ^2 test) to cause meningitis in nonpregnant adults.

Among the 3 neonates with invasive disease caused by GBS III-4/ST283, one infant had early-onset sepsis, and 2 had late-onset sepsis (1 with meningitis). Thirty neonates had meningitis, 16 (53.3%) caused by serotype III-2 and 1 (3.3%) caused by serotype III-4.

To assess contributing factors, we examined possible climatic associations with isolation of serotypes causing the 437 cases of invasive GBS disease in Hong Kong during our study period. We reviewed Hong Kong's average monthly temperature and humidity records for 1997–2012. Serotype III-4 was the only serotype with a prevalence significantly associated with monthly temperature (Spearman correlation $r = 0.622$; $p = 0.031$). Isolation of serotype III-4 peaked during the summer months (June–September), when mean temperature was ≥28°C) (Figure 2, panel A). Humidity was not significantly associated with serotype III-4 prevalence ($p>0.05$) (Figure 2, panel B).

GBS infects many host species, has become an important pathogen in the aquaculture industry, and has resulted in significant economic loss (*8*). Serotype Ia/ST7 has been associated with large outbreaks of streptococcosis in cultured tilapia in China (*9*). The pathogenicity of human ST7 isolates in fish has been well established (*10*). Although no epidemiologic evidence confirms GBS as a zoonosis in human infections (*11*), comparative genome analysis revealed that cultured tilapia GBS ST7 was closely related to human ST7 strain A909. These fish and human strains share highly similar clustered regularly interspaced short palindromic repeats, prophages, virulence-associated genes, and extremely short evolutionary relationships in phylogenetic analysis (*12*).

ST283 and its single-locus variant ST491 have been detected in diseased tilapia with high death rates in Southeast Asia, where fish isolates shared the same mobile genetic elements and surface proteins as the human isolates

Table 2. Distribution of GBS serotypes in patients with group B *Streptococcus* invasive disease, Hong Kong, 1993–2012*

Serotype	Nonpregnant adults, n = 267			Neonates, n = 113		
	No. (%)			No. (%)		
	Meningitis†	Nonmeningitis‡	p value	Meningitis†	Nonmeningitis‡	p value
Ia	1 (3.0)	32 (97.0)	0.53	4 (21.1)	15 (78.9)	0.55
Ib	1 (2.1)	46 (97.9)	0.28	2 (14.3)	12 (85.7)	0.27
II	0	17 (100.0)	0.31	0	4 (100.0)	0.22
III-1	1 (3.6)	27 (96.4)	0.66	5 (26.3)	14 (73.7)	0.56
III-2	0	13 (100.0)	0.38	16 (35.6)	29 (64.4)	0.08
III-3	0	3 (100.0)	0.68	2 (66.7)	1 (33.3)	0.11
III-4	7 (15.9)	37 (84.1)	**<0.001**	1 (20.0)	4 (80.0)	0.73
V	2 (7.1)	26 (92.9)	0.64	0	3 (100.0)	0.29
VI	0	10 (100.0)	0.44	0	1 (100.0)	0.55
VII	0	3 (100.0)	0.68	0	0	NA
VIII	0	0	NA	0	0	NA
IX	0	0	NA	0	0	NA
NT	0	1 (100.0)	0.81	0	0	NA
Total	12 (5.3)	215 (94.7)	NA	30 (26.5)	83 (74.3)	NA

*GBS, group B *Streptococcus*; NA, nonapplicable; NT, nontypeable. Bold indicates statistical significance; p values determined by Fisher exact or χ^2 test.
†Meningitis was confirmed by cerebrospinal fluid culture of GBS.
‡Nonmeningitis was confirmed by GBS culture from sterile body site or from cerebrospinal fluid.

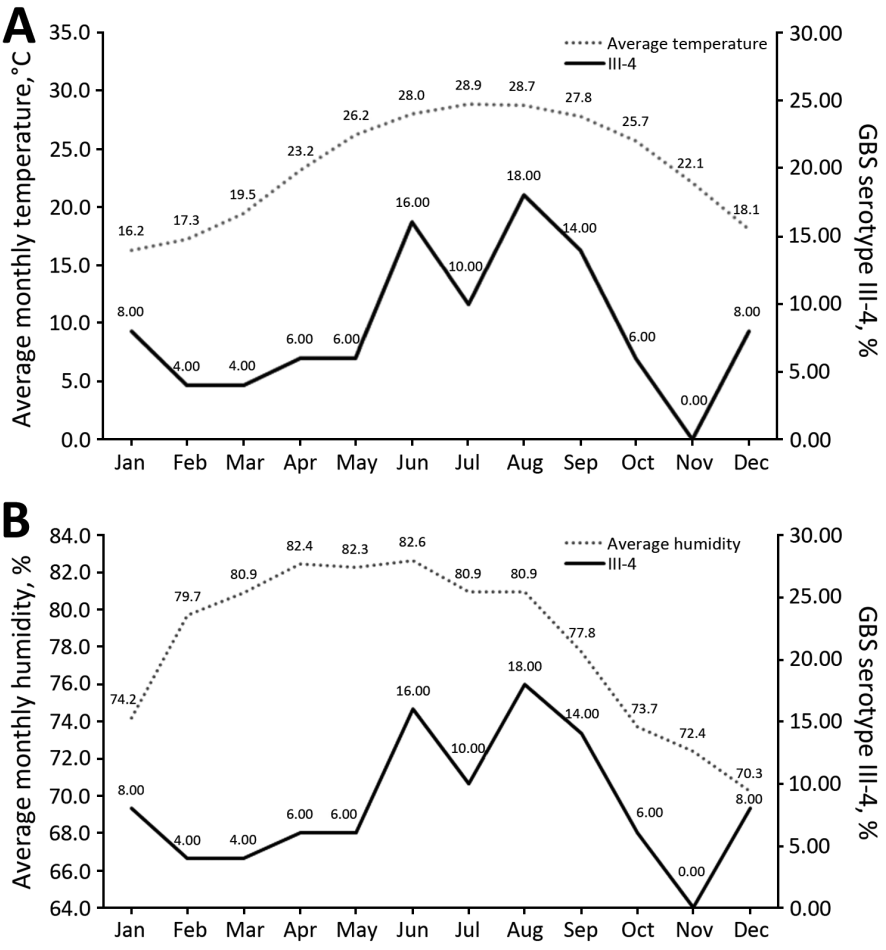

Figure 2. Association of temperature and humidity with distribution of isolates by month of collection from patients infected with invasive Group B *Streptococcus* (GBS) serotype III, subtype 4 (III-4), Hong Kong, 1993–2012. A) Average annual monthly temperature and distribution of invasive GBS III-4 isolates. B) Average annual monthly humidity and distribution of invasive GBS III-4 isolates. Numbers along data lines indicate monthly values.

described in this article (*8,13*). This similarity provides molecular evidence for the linkage of ST283 strains in humans and fish. The outbreak in Singapore showed a rise in GBS sepsis and septic arthritis, which were linked to a probable source of raw fish consumption; subsequently, health authorities advised discontinuing sales of the raw fish dish as a precaution (*2*). A previous study showed that fish consumption caused a 7.3-fold increased risk for acquiring GBS serotype Ia and Ib colonization in humans (*14*). GBS serotypes Ia, III, and V of clonal complex (CC) 19, CC23, and CC103 from a human and a cow have been shown to infect tilapia experimentally (*15*); these cross-infections support GBS pathogenicity across different species. Epidemiologic investigations and comparative genomics on a wider scale of animal and human strains may further reveal evolutionary relationships between these GBS lineages.

Conclusions

Our finding of an association of the GBS III-4/ST283 lineage with summer months coincides with the timing of the outbreak in Singapore. Because GBS grows rapidly in warm temperatures, a higher infective dose may occur in warmer months than at other times. Warm temperatures might also cause increased pathogenicity of this organism, a postulation supported by the association of higher temperatures with tilapia deaths caused by GBS III-4 in freshwater fish farms (*13*). The pathogenicity of this strain in fish has been shown in vivo and further highlights the pathogenic potential of this lineage.

Reports dating from the 1990s indicate that GBS ST283 isolated from humans came exclusively from invasive sites in nonpregnant adults. Our data suggest that these isolates might have undergone adaptive evolution in recent years to colonize and invade neonates with early- and late-onset GBS sepsis. Studies addressing the changing epidemiology of this hypervirulent lineage and its relationship to humans and fish are warranted to reduce potential transmission between the 2 hosts.

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Dr. Ip is a clinical microbiologist at the Chinese University of Hong Kong and works in bacteriology in the Department of

Microbiology. Her research interests include clinical and molecular epidemiology of gram-positive cocci (e.g., methicillin-resistant *Staphylococcus aureus*, Group B *Streptococcus,* and *Streptococcus pneumoniae*) and antimicrobial resistance determinants of *Streptococcus pneumoniae* and other pathogenic bacteria.

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Address for correspondence: Margaret Ip, Department of Microbiology, Chinese University of Hong Kong, The Prince of Wales Hospital, 30-32 Ngan Shing St, Sha Tin, New Territories, Hong Kong; email: margaretip@cuhk.edu.hk

Chikungunya Virus in Febrile Humans and *Aedes aegypti* Mosquitoes, Yucatan, Mexico

Nohemi Cigarroa-Toledo, Bradley J. Blitvich, Rosa C. Cetina-Trejo, Lourdes G. Talavera-Aguilar, Carlos M. Baak-Baak, Oswaldo M. Torres-Chablé, Md-Nafiz Hamid, Iddo Friedberg, Pedro González-Martinez, Gabriela Alonzo-Salomon, Elsy P. Rosado-Paredes, Nubia Rivero-Cárdenas, Guadalupe C. Reyes-Solis, Jose A. Farfan-Ale, Julian E. Garcia-Rejon, Carlos Machain-Williams

Chikungunya virus (CHIKV) was isolated from 12 febrile humans in Yucatan, Mexico, in 2015. One patient was co-infected with dengue virus type 1. Two additional CHIKV isolates were obtained from *Aedes aegypti* mosquitoes collected in the homes of patients. Phylogenetic analysis showed that the CHIKV isolates belong to the Asian lineage.

Chikungunya virus (CHIKV; family *Togaviridae*, genus *Alphavirus*) is transmitted to humans by *Aedes* spp. mosquitoes (*1*,*2*). The virus is the etiologic agent of chikungunya, an acute febrile illness that is often accompanied by debilitating arthralgia. Historically, CHIKV has been restricted to the Eastern Hemisphere, but in 2013, the virus was reported in the Western Hemisphere during a large outbreak in the Caribbean region. CHIKV spread rapidly to South America, Central America, Mexico, and the United States. The Pan American Health Organization estimated that >1.7 million suspected and laboratory-confirmed cases of chikungunya have occurred in the Western Hemisphere (http://www.paho. org/hq/index.php?option=com_topics&view=readall&cid=5927&Itemid=40931&lang=en).

CHIKV was isolated in Mexico from a patient from Jalisco in whom symptoms developed in May 2014 shortly after the patient returned from the Caribbean region (*3*). The first autochthonous case was reported in October 2014 after CHIKV was isolated from a patient in southeastern state of Chiapas (*4*). CHIKV-infected *Aedes aegypti* mosquitoes and additional chikungunya cases were identified in Chiapas later in 2014 (*5*,*6*). To our knowledge, no reports of CHIKV in any other states in Mexico have been published. In this study, we tested febrile patients in the state of Yucatan and mosquitoes temporally and spatially associated with these patients for CHIKV infection.

Author affiliations: Universidad Autonoma de Yucatan, Merida, Mexico (N. Cigarroa-Toledo, R.C. Cetina-Trejo, L.G. Talavera-Aguilar, C.M. Baak-Baak, O.M. Torres-Chablé, P. González-Martinez, G. Alonzo-Salomon, E.P. Rosado-Paredes, N. Rivero-Cárdenas, G.C. Reyes-Solis, J.A. Farfan-Ale, J.E. Garcia-Rejon, C. Machain-Williams); Iowa State University, Ames, Iowa, USA (B.J. Blitvich, M. Hamid, I. Friedberg)

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The Study

We obtained written informed consent from all patients who participated in the study or their legal guardians. The study population was composed of patients who came to hospitals or clinics in Yucatan during August–October 2015 with chikungunya-like illness. These patients were referred to the hematology laboratory at the Hideyo Noguchi Research Center (Merida, Yucatan, Mexico). A patient was considered to have chikungunya-like illness if he or she had fever and arthralgia. Travel history of each study participant was recorded, and any patient who had traveled outside Yucatan in the past 30 days before disease onset was excluded from the study.

Blood was collected from the cephalic vein of each patient, dispensed into a vacutainer tube (BD Diagnostics, Franklin Lakes, NJ, USA), and centrifuged. Serum was collected and stored at −80°C. Resting mosquitoes were collected from the homes of each study participant by using Centers for Disease Control and Prevention (Atlanta, GA, USA) backpack-mounted aspirators. Each house was examined once, and collections were made between 9:00 AM and noon. All rooms were inspected, particularly dark areas (i.e., underneath furniture, in closets, and in curtains). Backyards were also searched, particularly shaded areas (i.e., pet homes, tool sheds, and underneath vegetation).

Mosquitoes were transported alive to the laboratory and identified on chill tables by using morphologic characteristics (*7*). Female mosquitoes were sorted into pools of ≤10 and homogenized in phosphate-buffered saline (pH 7.2) by using a mortar and pestle. Male mosquitoes were discarded.

An aliquot of each serum sample and mosquito homogenate was filtered and inoculated onto subconfluent monolayers of *Ae. albopictus* (C6/36) cells in 25-cm^2 flasks. Cells were incubated for 7 days at 28°C. Second and third blind passages were performed in C6/36 and African green monkey kidney (Vero) cells, respectively. Vero cells were incubated for 3–7 days at 37°C in an atmosphere of 5% CO_2. Cells were scraped from flasks after each passage and centrifuged at 10,000 × *g* for 10

min at 4°C. Supernatants were collected and stored at −80°C. Cell pellets were resuspended in Trizol (Invitrogen, Carlsbad, CA, USA), and total RNA was extracted following the manufacturer's instructions.

We analyzed total RNA by using reverse transcription PCR (RT-PCR) and CHIKV-specific primers for a 107-nt region of the nonstructural protein 1 gene (primer sequences available upon request from the authors) and dengue virus (DENV)–specific primers for a 511-nt region of the capsid–membrane genes of all 4 serotypes (*8*). If DENV RNA was detected, we performed a semi-nested RT-PCR with serotype-specific primers. If CHIKV RNA was detected, we amplified a 3,744-nt region that spans the structural protein genes (capsid-E3-E2-6K-E1) (E, envelope; 6K, membrane-associated peptide) as 2 overlapping fragments (primer sequences available upon request from the authors).

Complementary DNAs were generated by using Superscript III reverse transcriptase (Invitrogen), and PCRs were performed by using *Taq* polymerase (Invitrogen). RT-PCR products were purified by using the Purelink Gel Extraction Kit (Invitrogen) and sequenced by using a 3730x1 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

We isolated CHIKV from 12 (23.5%) of 51 study participants. DENV type 1 was also isolated from 1 CHIKV-positive patient. DENV was readily detected in cultured cells after the first blind passage, but its ability to replicate decreased after subsequent passages, presumably because CHIKV outcompeted this slower-replicating flavivirus.

The most common symptoms in patients infected with only CHIKV, in addition to fever, during the first 3 days of disease onset were arthralgia (100%), myalgia (100%), asthenia (90.9%), and rash (45.5%) (Table). Symptoms of the co-infected patient (a 31-year-old woman) included headache, myalgia, and rash. Age range of patients infected with only CHIKV was 9–59 years (mean age 31 years).

Table. Signs and symptoms of 12 patients infected with CHIKV during the first 3 days of disease onset, Yucatan, Mexico*

	No. (%) patients	
Sign/symptom	CHIKV infected, n = 11	Co-infected with DENV 1, n = 1
Arthralgia	11 (100.0)	1
Ankles	4 (36.4)	1
Knees	11 (100.0)	1
Shoulders	0 (0)	0
Wrists	11 (100.0)	1
Asthenia	10 (90.9)	0
Fever	11 (100.0)	1
Headache	0 (0)	1
Myalgia	11 (100.0)	1
Rash	5 (45.5)	1
Vomiting	1 (9.1)	0

*CHIKV, chikungunya virus; DENV 1, dengue virus type 1.

We collected a total of 237 female mosquitoes, and all were identified as *Ae. aegypti* mosquitoes. CHIKV was isolated from 2 pools. One pool contained mosquitoes collected in the living room of a 53-year-old patient who had a confirmed CHIKV infection. The other pool contained mosquitoes collected in bedroom of the co-infected patient. DENV was not isolated from any mosquitoes.

The capsid-E3-E2-6K-E1 region of each CHIKV isolate was sequenced and submitted to GenBank under accession nos. KU295117–KU295130. Pairwise alignments of the nucleotide and deduced amino acid sequences were performed by using Clustal Omega (http://www.ebi.ac.uk/Tools/ msa/clustalo/). Analysis showed that nucleotide sequences had 99.41%–99.97% identity and amino acid sequences 99.44%–100% identity with each other. The nucleotide sequence of 1 isolate (GenBank accession no. KU295121) was aligned with all other CHIKV sequences in GenBank and shown to have highest identity (99.52%) with the corresponding gene region of CHIKV isolates from Panama and El Salvador, followed by an identity of 99.49% with isolates from Chiapas, Mexico; Guatemala; Puerto Rico; Guyana; and elsewhere in the Western Hemisphere. Analysis of deduced amino acid sequences showed that mutations associated with increased infectivity of *Ae. albopictus* mosquitoes (E1-A226V and E2-L210Q) (*9*,*10*) were not present in genomes of any isolates.

Complete structural gene sequences of 60 CHIKV isolates, including the 14 isolates from Yucatan, were aligned by using MUSCLE (*11*), and phylogenetic trees were constructed by using the neighbor-joining algorithm as implemented in PHYLIP (*12*) (Figure). We observed 4 lineages, Asian, East/Central/South African, Indian Ocean, and West African lineages, which was consistent with results of previous studies (*1*,*5*). CHIKV isolates from Yucatan belonged to the Asian lineage and shared a close phylogenetic relationship with other isolates from the Western Hemisphere (Figure). Our isolates formed a nested clade within the Asian lineage. However, bootstrap support (0.61) for this topologic arrangement was not strong.

Conclusions

We isolated CHIKV from febrile patients and *Ae. aegypti* mosquitoes in Yucatan, Mexico, which provided additional evidence that this virus is spreading throughout the Americas at an alarming rate. Concurrent isolation of CHIKV and DENV from a patient in this study and patients in previous studies (*14,15*) underscores the need for differential diagnosis in areas where these viruses co-circulate.

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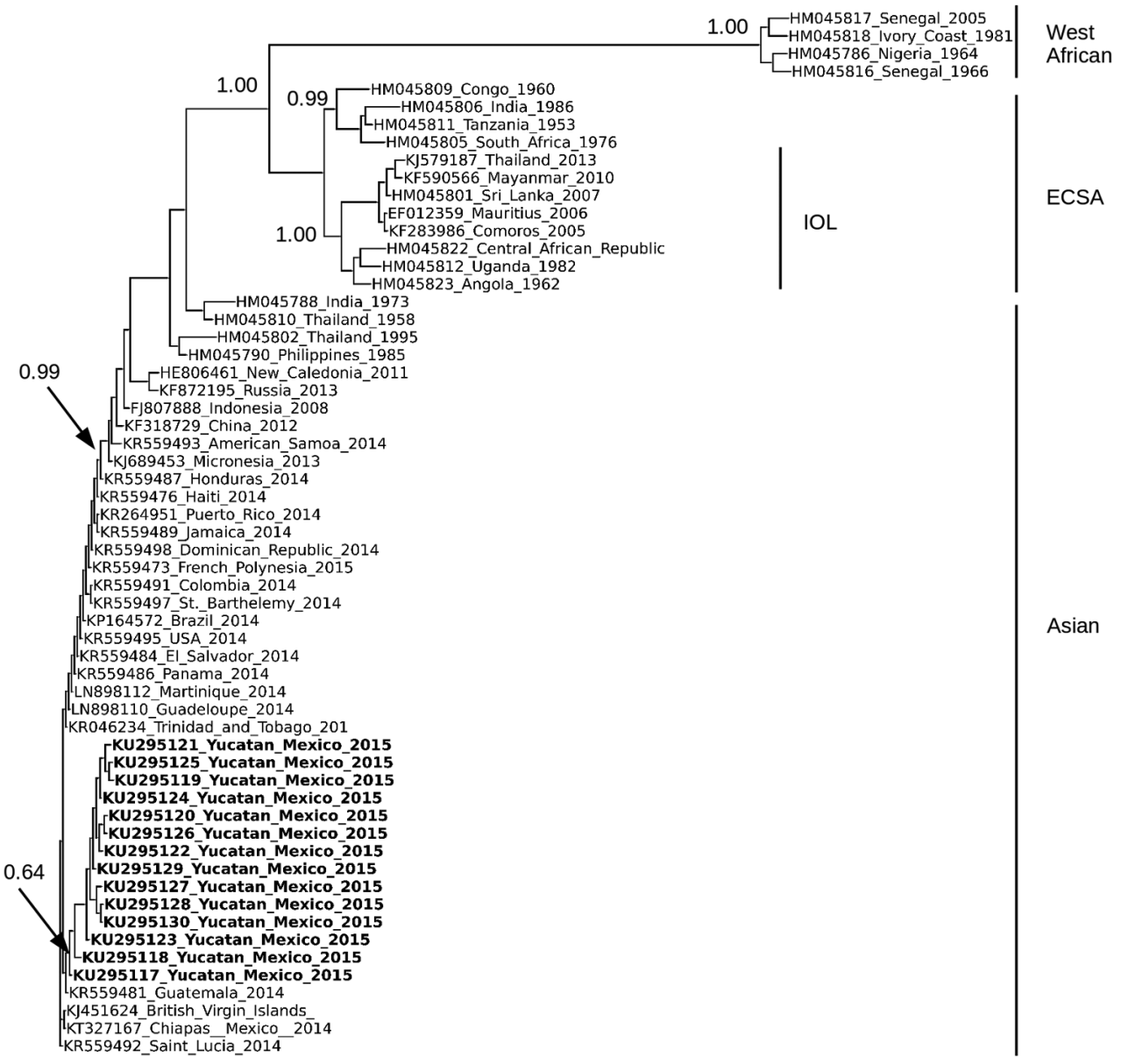

Figure. Phylogenetic analysis of chikungunya virus (CHIKV) isolates from Yucatan, Mexico. Analysis was based on a 3,744-nt structural gene region (capsid-E3-E2-6K-E1) of 63 CHIKV isolates, including the 14 isolates from Yucatan. Sequences were aligned by using MUSCLE (*11*), and the tree was constructed by using the neighbor-joining algorithm as implemented in PHYLIP (*12*) and using ETE3 (Environment for Tree Exploration 3) (*13*). Isolates are identified by GenBank accession number, country, and year isolated. CHIKV isolates from the Yucatan are shown in bold. Bootstrap values were generated by using 1,000 repetitions and normalized on a scale of 0–1. Bootstrap values for select branches are shown. 6K, membrane-associated peptide; E, envelope; ECSA, East/Central/South African lineage; IOL, Indian Ocean lineage.

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Ms. Cigarroa-Toledo is a graduate student in the Arbovirus Laboratory at the Universidad Autonoma de Yucatan, Merida, Mexico. Her research interests include arbovirus epidemiology, evolution, and surveillance.

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Address for correspondence: Carlos Machain-Williams, Laboratorio de Arbovirologia, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autonoma de Yucatan, Merida, Yucatan, Mexico; email: carlos.machain@correo.uady.mx

etymologia

Aedes aegypti [a-e'dēz a-jip'tē]

In 1757, Fredrik Hasselqvist (a protégé of Carl Linnaeus) first described a mosquito collected in Egypt as *Culex* (Latin for "gnat") *aegypti*, noting as the most salient feature the "glistening white" rings on the legs. *Aedes* (Greek for "unpleasant") *aegypti* is the principal vector of several human diseases, including chikungunya, dengue, yellow fever, and Zika. Yellow fever virus was among the first human viral pathogens to be discovered, and the US Army Yellow Fever Commission's work showing that *Ae. aegypti* (also known as the "yellow fever mosquito") was the principal vector remains one of the cornerstones of medical virology and tropical medicine.

Ae. aegypti arrived in the New World shortly after Europeans, transported on ships, where conditions selected for the anthropophilic *Ae. aegypti* subsp. *aegypti*. (Forest-breeding zoophagous *Ae. aegypti* subsp. *formosus* are still found in sub-Saharan Africa.) From the New World, *Ae. aegypti* spread across the Pacific to Asia and Australia.

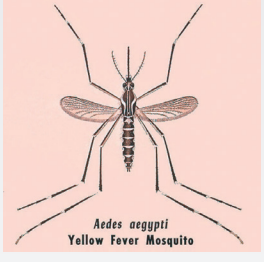

Illustration of *Aedes aegypti* adult mosquito. CDC / James M. Stewart

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Address for correspondence: Ronnie Henry, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E03, Atlanta, GA 30329-4027, USA; email: boq3@cdc.gov

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Daily Reportable Disease Spatiotemporal Cluster Detection, New York City, New York, USA, 2014–2015

Sharon K. Greene, Eric R. Peterson, Deborah Kapell, Annie D. Fine, Martin Kulldorff

Each day, the New York City Department of Health and Mental Hygiene uses the free SaTScan software to apply prospective space–time permutation scan statistics to strengthen early outbreak detection for 35 reportable diseases. This method prompted early detection of outbreaks of community-acquired legionellosis and shigellosis.

The Bureau of Communicable Disease (BCD) at the New York City Department of Health and Mental Hygiene (DOHMH) monitors and investigates >70 reportable diseases among the city's 8.49 million residents. Each day, healthcare providers and laboratories submit ≈1,000 communicable disease reports to BCD. Clusters (significant increases in observed vs. expected cases) and outbreaks (clusters believed to be associated with a common infection source) are detected through several methods, including notification by astute healthcare providers and by applying the modified historical limits method to detect increases in disease counts during the previous 4 weeks (*1*). This temporal analysis is applied weekly citywide and for each of 5 boroughs and 42 neighborhoods.

Cluster detection methods have been applied to syndromic data sources (e.g., emergency department visits) since the early 2000s (*2*,*3*). Less extensively described is cluster detection using reportable disease data, which reflect specific laboratory-confirmed diagnoses, contain patient home addresses, and may include illness onset dates and work addresses collected during patient interviews and medical record reviews. Other public health practitioners have applied purely temporal prospective cluster detection methods to reportable disease data (*4*,*5*) or conducted proof-of-concept spatiotemporal prospective analyses (*6*,*7*). However, published descriptions of actual prospective application of spatiotemporal methods to reportable diseases are rare (*8,9*), suggesting lack of widespread adoption among public health officials. We describe BCD's experience with automated daily reportable disease spatiotemporal cluster detection using prospective space–time permutation scan statistics (*3*) in SaTScan (*10*) during February 2014–September 2015, highlighting instances in which findings guided public health action.

Author affiliations: New York City Department of Health and Mental Hygiene, Queens, New York, USA (S.K. Greene, E.R. Peterson, D. Kapell, A.D. Fine); Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA (M. Kulldorff)

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The Study

For 35 reportable communicable diseases for which cluster detection could inform programmatic activities (*1*), we analyzed disease counts for patients of all ages combined. For amebiasis, cryptosporidiosis, and giardiasis, for which outbreaks among young children are of particular interest, additional analyses were restricted to disease counts among patients <5 years of age, for 38 total daily analyses.

In BCD's application, the space–time permutation scan statistic detects disease clusters in space–time cylinders centered on every census tract centroid; the circular base represents space (maximum geographic cluster size of 50% of all reported cases), and the height represents time (maximum temporal window length of 30 days, for most diseases). For each cylinder, a likelihood ratio–based test statistic is calculated. The test statistic is considered elevated if the observed disease count during the time window in census tracts with centroids inside the cylinder's circular base exceeds the expected number of cases, which is a function of 1) the case count in the circle during a baseline period (which accounts for any purely geographic variations in disease occurrence, diagnosis, and reporting) and 2) the total case count citywide during the time window (which accounts for citywide purely temporal patterns, such as seasonality or secular trends) (*3*). The cylinder with the maximum test statistic is the cluster least likely to be due to chance under the null hypothesis that the same process generated disease counts inside and outside the cylinder.

To create a simulated dataset, cases' dates are randomly shuffled and assigned to the original census tracts. The maximum statistic for each simulated dataset is calculated in the same way as for the observed dataset. For each disease, this process is repeated daily 999 times. The maximum value for the observed dataset is ranked among the 999 trial maxima. A p value (range 0.001–1) is derived from this ranking; p = 0.001 represents the highest significance relative to the permutation trials. The Monte Carlo approach to deriving significance by using repeated trials,

each permuting observed data attributes, is designed to control for multiple testing.

A recurrence interval (RI) is calculated as the reciprocal of the p value and represents the number of days of daily surveillance required for the expected number of clusters at least as unusual as the observed cluster to be equal to 1 by chance (*11*). We defined a signal as any cluster with an RI ≥100 days; that is, during any 100-day daily analysis period, the expected number of clusters at least as unlikely as the current cluster is 1.

We developed a SAS program (SAS Institute, Inc., Cary, NC, USA) to generate case and parameter files (Table 1), read in a coordinate file of census tract centroids, invoke SaTScan in batch mode, read analysis results back into SAS for further processing, and output files to secured folders. For any signals, the program also generated emails notifying BCD leadership and staff responsible for follow-up (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/10/16-0097-Techapp1.pdf).

This automated analysis detected the second largest US outbreak of community-acquired legionellosis (*12*), identifying a cluster of 8 cases centered in the South Bronx on Friday, July 17, 2015 (RI = 500 days) (Figure), before any human public health monitor noticed it. On Monday, July 20, an increase in cases was independently noticed by BCD staff members routinely investigating individual cases, and on July 21, an infection-control nurse working in the outbreak area called BCD to report an increase. The DOHMH and state and federal partners conducted an extensive epidemiologic, environmental, and laboratory investigation to identify and remediate the outbreak source, a cooling tower.

A shigellosis outbreak among the observant Jewish community in Brooklyn (*13*) began in late October 2014 and was detected with 9 cases on November 14, 2014 (RI = 333 days). BCD does not routinely investigate individual shigellosis reports, so automated analysis alone prompted early outbreak identification. Shigellosis outbreaks within this community occur cyclically and have been linked to daycare and preschool attendance (*14*). Starting in mid-November, BCD staff visited community schools, daycare centers, and health fairs to promote appropriate handwashing. The outbreak subsided by mid-March 2015. Other clusters prompting investigations

Table 1. Case file specifications for routine reportable disease analyses in New York City, New York, using the prospective space–time permutation scan statistic

Feature	Selection	Notes
Geographic aggregation	Census tract (defined using US Census 2000 boundaries) of residential address at time of report*	The less data are spatially aggregated, the more precisely areas with elevated rates can be identified. New York City has 2,216 census tracts in an area of 305 square miles.
Date of interest for analysis	Event date, defined using hierarchy of onset date → diagnosis date (collection date of first specimen testing positive) → report date → date event created in surveillance database	Defining reportable disease clusters according to when case-patients became ill is preferred. However, onset date is missing for most case-patients who have not yet been interviewed, and each case needs a date to be included in analysis. Thus, the best available proxy for onset date is used. Because we use daily data (rather than weekly, monthly, or yearly data), the time precision is specified as day on the SaTScan (http://www.satscan.org/) input tab. The time precision parameter indicates the temporal resolution of the data in the case file.
Study period	1 y for most diseases, ending the day before analysis†	One year is a reasonable choice, balancing the need for a period long enough to establish a stable local baseline for each spatial unit, yet short enough to avoid variable secular trends (e.g., geographically different increases in the underlying population of a spatial unit). Analyses are run each morning using data with event dates through the previous day.
Case inclusion criteria	Include all reported cases, regardless of current status (e.g., confirmed, probable, suspected, pending, noncase)†	Depending on the disease, cases initially might be assigned a transient pending status and, upon investigation, be reclassified as a case (confirmed, probable, or suspected) or a noncase. Timeliness is preserved by analyzing all reported cases, including noncases and pending cases, regardless of whether they ultimately will be confirmed. By analyzing all reported cases, case inclusion criteria are consistent across the study period. If instead the case file were restricted to confirmed and pending cases, then analyses would be biased toward false signaling, as some cases with an initial pending status will be ultimately reclassified after investigation as a noncase. This reclassification process is complete for the baseline but ongoing for the current period of interest (*1*), and the speed of reclassification might vary geographically.
Day-of-week variable	Include a variable that indicates the day of the week (1–7)	The analysis automatically adjusts for day-of-week effects but not for space by day-of-week interaction. Including this variable in the SaTScan case file accounts for how the daily pattern of health-seeking behavior and diagnoses might vary geographically.

*Exception to residential address at time of report: if the residential address is not geocodable (e.g., because the case-patient is not a resident of the city or because a post office box is reported instead of a street address), then the geocoded work address, if available, is substituted.
†For exceptions, see online Technical Appendix (http://wwwnc.cdc.gov/EID/article/22/10/16-0097-Techapp1.pdf).

Figure. Automated output from spatiotemporal analysis on July 17, 2015, indicating a cluster (dark gray) of 8 legionellosis cases over 8 days centered in the South Bronx, New York City, New York, USA. In subsequent days, this cluster expanded in space and time into the second largest US outbreak of community-acquired legionellosis.

included legionellosis (Queens, April–May 2015) and campylobacteriosis (Brooklyn, October 2014). During a 1-year period, 28 unique signals were observed across 15 diseases (Table 2), which staff perceived as a reasonable number for investigation.

Not all detected clusters were actionable. No public health response was conducted for an amebiasis cluster (Manhattan, April 2015; RI = 143 days) consisting of 6 men (34–49 years of age) diagnosed within a 12-day period and residing within a 0.35-mile radius because no case-patients were identified as food handlers or daycare workers. A public health response also was not conducted for a giardiasis cluster (Bronx, April 2015; RI = 1,000 days) that consisted of 6 household members who acquired the infection during international travel. Investigators were interested in being notified of and following such clusters over time, even if they ultimately were not actionable or verified as true outbreaks.

Conclusions

Several outbreaks in New York City, New York, were detected by daily automated spatiotemporal analyses. Early cluster detection facilitated prioritization of individual case investigations, outbreak recognition and investigation, provider and community outreach, and timely intervention to limit sickness and death. This method has proven particularly useful for identifying and monitoring outbreaks of shigellosis (*6*,*8*,*9*) and legionellosis and might be useful for monitoring additional diseases with outbreak potential, including pertussis, syphilis, and tuberculosis.

Key to the system's success is a strong informatics infrastructure, especially electronic laboratory reporting and near real-time geocoding of surveillance data. Other facilitators include a powerful statistical disease surveillance methodology, knowledgeable epidemiologists to interpret signals, and adequate outbreak investigation resources.

These methods could be useful to other health departments receiving more reports than can be rapidly reviewed manually. State health departments could consider conducting similar analyses to detect clusters spanning multiple jurisdictions.

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Table 2. Signaling rates at 3 recurrence interval thresholds for 35 reportable diseases under surveillance in New York City, New York, USA, 2014–2015*

Disease	Annual no. cases‡	No. signals during 365 d of prospective surveillance†		
		Recurrence interval ≥365 d§	Recurrence interval ≥100 d	Recurrence interval ≥30 d
Amebiasis	476	0	1.2	4.3
Babesiosis	57	0	0	0
Campylobacteriosis	1,663	0.6	0.6	4.9
Chikungunya	171	0.6	1.8	3.1
Cholera	0	0	0	0
Cryptosporidiosis	135	0	0	0.6
Cyclosporiasis	51	0	0	1.2
Dengue	57	0	0	1.8
Encephalitis	2	0	0	0
Giardiasis	871	1.2	1.8	5.5
Hemolytic uremic syndrome	4	0	0	0
Hepatitis A	78	1.9	1.9	5.8
Acute hepatitis B	51	0.6	1.2	3.7
Hepatitis D	0	0	0	0
Hepatitis E	0	0	0.6	0.6
Human granulocytic anaplasmosis	51	0.6	0.6	0.6
Human monocytic ehrlichiosis	8	0	0.6	0.6
Invasive group A *Streptococcus* disease	263	0	0	1.8
Invasive group B *Streptococcus* disease	33	0.6	1.2	2.4
Invasive *Haemophilus influenzae* disease	97	0	0	1.8
Invasive *Streptococcus pneumoniae* disease	647	0	1.2	1.8
Legionellosis	434	9.1	9.1	11.4
Listeriosis	34	0	0	0.6
Malaria	187	0.6	1.8	4.3
Meningococcal disease	8	0	0	0.6
Noncholera *Vibrio* spp. infection	18	0	0	0
Paratyphoid fever	11	0	0	0
Rickettisalpox	9	0	0	0
Rocky Mountain spotted fever	6	0	0	2.4
Shiga toxin–producing *Escherichia coli*	96	0	0	0
Shigellosis	806	1.8	1.8	6.1
Typhoid fever	31	0	1.9	3.8
Vancomycin-intermediate *Staphylococcus aureus* infection	28	0	0	0
West Nile virus disease	19	0	0	0
Yersiniosis	25	0	0	0
Total signals across all diseases under surveillance	NA	17.8	27.6	69.8

*Signals were detected by using the prospective space–time permutation scan statistic. NA, not applicable.
†A signal for a particular disease was defined as unique if the first most likely cluster on a particular day did not encompass any of the same census tracts as the first most likely cluster on the prior day. The signaling rate for most diseases was based on 598 d of surveillance (February 10, 2014–September 30, 2015). For 5 diseases, the signaling rate was based on a shorter surveillance period to reflect analytic adjustments: hepatitis A, paratyphoid fever, and typhoid fever (190 d under surveillance after extending to a 60-d maximum temporal cluster size); legionellosis (160 d under surveillance after excluding unresolved cases); and Shiga toxin–producing *E. coli* (21 d under surveillance after excluding cases with only a positive multiplex PCR gastrointestinal panel test).
‡Confirmed, probable, and suspected cases among residents with event dates October 1, 2014–September 30, 2015.
§The signal was detected at the lower ≥100-d threshold on the same day for 50% of the signals, 1 d earlier for 19% of signals, 2 d earlier for 19% of signals, 3 d earlier for 6% of signals, and 7 d earlier for 6% of signals.

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Dr. Greene is director of the Data Analysis Unit at the Bureau of Communicable Disease of the New York City Department of Health and Mental Hygiene, Queens, New York. Her research interests include infectious disease epidemiology and applied surveillance methods for outbreak detection.

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Address for correspondence: Sharon K. Greene, New York City Department of Health and Mental Hygiene, 42-09 28th St, CN 22A, WS 06-154, Queens, NY 11101, USA; email: sgreene4@health.nyc.gov

Viral RNA in Blood as Indicator of Severe Outcome in Middle East Respiratory Syndrome Coronavirus Infection

So Yeon Kim, Sun jae Park, Sook Young Cho, Ran-hui Cha, Hyeon-Gun Jee, Gayeon Kim, Hyoung-Shik Shin, Yeonjae Kim, Yu Mi Jung, Jeong-Sun Yang, Sung Soon Kim, Sung Im Cho, Man Jin Kim, Jee-Soo Lee, Seung Jun Lee, Soo Hyun Seo, Sung Sup Park, Moon-Woo Seong

We evaluated the diagnostic and clinical usefulness of blood specimens to detect Middle East respiratory syndrome coronavirus infection in 21 patients from the 2015 outbreak in South Korea. Viral RNA was detected in blood from 33% of patients at initial diagnosis, and the detection preceded a worse clinical course.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a zoonotic, betacoronavirus lineage C RNA virus that was first identified in Saudi Arabia in 2012 (*1*). MERS-CoV causes respiratory and renal illness in humans, and infection often progresses to severe pneumonia, acute respiratory distress syndrome, renal failure, or death in a subset of patients (*2*). Risk factors, including patient age, preexisting health conditions, and high viral load in upper respiratory specimens, have been suggested to be related to disease severity and death (*3*,*4*). However, pathogenesis and clinical characteristics promoting recovery from infection or progression to serious organ failure have not been well elucidated.

Respiratory specimens are preferred for viral RNA detection and confirmatory diagnosis of MERS-CoV infection in humans (*5*). MERS-CoV has broad tissue tropism, including the kidney, intestinal tract, liver, histiocytes, macrophages, and T lymphocytes, but viral RNA has been found inconsistently in blood, urine, and fecal specimens (*6*–*10*). Reports have described small numbers of cases with extrapulmonary virus; therefore, it remains unclear whether extrapulmonary specimens have any diagnostic usefulness in determining infection or whether extrapulmonary viral detection has clinical implications in disease management.

Author affiliations: National Medical Center, Seoul, South Korea (S.Y. Kim, S.j. Park, S.Y. Cho, R.-h. Cha, H.-G. Jee, G. Kim, H.-S. Shin, Y. Kim, Y.M. Jung); Korea Centers for Disease Control and Prevention, Cheongju-si, South Korea (J.-S. Yang, S.S. Kim); Seoul National University College of Medicine, Seoul (S.I. Cho, M.J. Kim, J.-S. Lee, S.J. Lee, S.H. Seo, S.S. Park, M.-W. Seong)

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A large MERS-CoV outbreak occurred in 2015 in South Korea. This outbreak comprised the first imported case and subsequent infection of 185 patients (*11*). Our study aimed to evaluate the diagnostic utility of blood specimens for MERS-CoV infection by using large numbers of patients with a single viral origin and to determine the relationship between blood viral detection and clinical characteristics.

The Study

We collected 21 pairs of EDTA whole blood and serum specimens from 21 patients with MERS-CoV after admission to the National Medical Center in Seoul, South Korea. MERS-CoV infection initially was diagnosed by the Korea Centers for Disease Control and Prevention using respiratory specimens (*11*). After admission, each patient was reassessed for epidemiologic information and clinically managed with monitoring.

Specimens were stored at −80°C before analyses. Viral RNA was extracted and eluted with a MagNA Pure LC 2.0 automated nucleic acid extractor and MagNA Pure LC total nucleic acid isolation kit (both from Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions. Specimen volumes were 100 μL EDTA whole blood and 200 μL serum, and elution volumes were 100 μL for EDTA whole blood and 50 μL for serum. One-step, real-time reverse transcription PCR (rRT-PCR) was performed for the 3 MERS-CoV gene regions (upstream envelope [upE], open reading frame [ORF] 1a, and nucleocapsid) with an AgPath-ID One-Step RT-PCR kit (Applied Biosystems, Foster City, CA, USA) and an ABI7500 real-time PCR system (Applied Biosystems). Human ribonuclease (RNase) P was amplified in parallel for sample quality control (*5*,*12*,*13*). In each test, the viral RNA was considered detected when amplification before cutoff was observed from at least 2 different targets in MERS-CoV with pass of sample quality control. The robustness of rRT-PCR was demonstrated for qualitative concordance of positivity or negativity by using different types of specimens (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/10/16-0218-Techapp1.pdf). We calculated the viral copy concentration in the blood using standard curves constructed from the cycle threshold (C_t) of serially diluted 10^5 copies/μL of upE RNA (provided by the University of Bonn Medical Center, Bonn, Germany).

The results of the blood viral RNA analyses did not affect clinical management. We assessed the relationships among clinical and molecular diagnostic factors with the IBM SPSS Statistics program 22.0 (SPSS Inc., Chicago, IL, USA). In each test, $p<0.05$ was considered statistically significant. The institutional review board of the National Medical Center approved this study (H-1508-057-002).

We assessed patient demographics, their clinical features, and disease outcomes (Tables 1, 2; online Technical Appendix Table 2). The time difference was an average of 1.5 days (median 1, range 0–5 days) between when the initial diagnostic respiratory specimens and blood specimens were obtained. At admission, viral RNA was detected in 6 (29%) of 21 EDTA whole blood and 6 (29%) of 21 serum samples from infected patients. Two patients showed viral positivity in either specimen subtype of EDTA whole blood or serum; therefore, the overall detection rate for MERS-CoV was 33% (7/21) in blood. The concordance rate of viral assay was 90% (19/21) between EDTA whole blood and serum specimens. Blood virus concentration was 3,130 copies/mL EDTA whole blood (range 2,080–17,400 copies/mL), equivalent to a median upE C_t of 37.55, and 1,300 copies/mL serum (range 490–10,200 copies/mL, median upE C_t 36.88).

Blood viral RNA positivity at admission was associated with fever ≥37.5°C on the sampling date ($p = 0.007$), requirement for mechanical ventilation during the following clinical course ($p = 0.003$) and extracorporeal membrane oxygenation ($p = 0.025$), and patient death ($p = 0.025$, all by 2-tailed Fisher exact test; Figure). Blood viral RNA positivity was not associated with viral C_t in the initial diagnostic lower respiratory specimens, or requirement of oxygen supplementation during the following clinical course. Between the blood viral RNA-positive and -negative groups, we found no differences in age, duration from symptom onset to diagnosis of MERS-CoV infection, or an invasive procedure before the specimens were obtained (online Technical Appendix Table 3).

Table 1. Demographic characteristics of 21 Middle East respiratory syndrome coronavirus–infected patients, South Korea, 2015

Characteristic	Value
No.	21
Median age, y (range)	64 (23–86)
Sex, no.	
M	9
F	12
Median hospitalization, d (range)	17 (2–138)
Death rate, %	23.8
Median no. days exposed to virus (range)*	3 (1–20)
Median duration between symptom onset and initial diagnosis, d (range)	2 (0–12)†

*Based on contact history.
†Two asymptomatic patients were excluded from analysis.

Viral loads in the lower respiratory specimens at the initial confirmatory diagnosis showed no effect on patient survival (Figure). Patient death was not associated with length of time from symptom onset to diagnosis of MERS-CoV infection (online Technical Appendix Table 3).

Our results showed that the detection rate of blood viral RNA was low in the early phase of infection in patients with a confirmed diagnosis, similar to results from a previous study (*14*). These findings contrasted with those of severe acute respiratory syndrome coronavirus infection (*15*). Therefore, in the case of MERS-CoV infection, blood does not have the highest diagnostic yield for the initial confirmatory diagnosis. The viral load in blood was low, even in detected cases. A proportion of MERS-CoV isolates in the 2015 Korea outbreak harbored a C→T substitution in the third nucleotide of the ORF1a primer binding site (GenBank accession nos. KT374052–374055). This mismatch may partially contribute to the insensitivity of ORF1a assay observed in this study. An alternative sensitive target replacing ORF1a might be useful in studies using blood specimens.

Table 2. Differences in Middle East respiratory syndrome coronavirus detection by rRT-PCR among specimen types, South Korea, 2015*

Characteristic	Respiratory specimen type	Blood specimen type	
		EDTA whole blood	Serum
No. specimens	20 sputum; 1 endotracheal aspirate	21	21
Days from initial confirmatory diagnosis using respiratory specimens to blood sampling	Set as day 0	1.4 (median 1, range 0–5)	1.5 (median 1, range 0–5)
Viral gene region rRT-PCR			
Upstream of E			
Total positive results/total tests (%)	21/21 (100)	6/21 (29)	6/21 (29)
Median C_t (range)	28.48 (19.70–33.46)†	37.55 (35.34–38.07)	36.88 (34.24–38.14)
Open reading frame 1a			
Total positive results/total tests (%)	21/21 (100)	0/21 (0)	0/21 (0)
Median C_t (range)	29.28 (21.00–34.21)†	Not detected	Not detected
Nucleocapsid			
Total positive results/total tests (%)	Not performed	7/21 (33)	7/21 (33)
Median C_t (range)	Not performed	36.37 (35.62–38.04)	34.62 (32.93–38.84)

*C_t, cycle threshold; rRT-PCR, real-time reverse transcription PCR.
†One patient whose C_t was not reported was excluded.

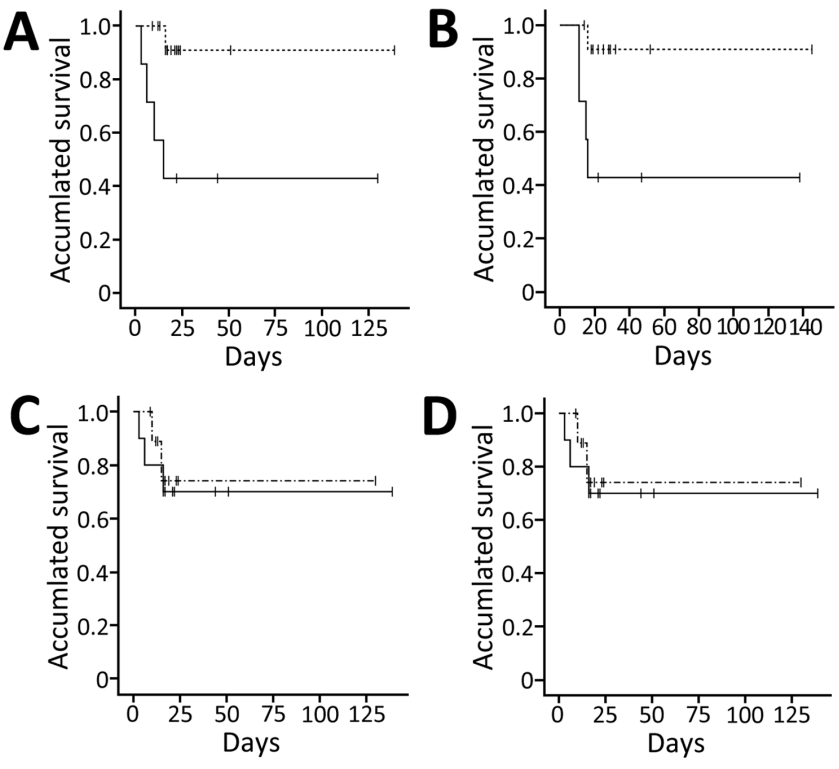

Figure. Differences in survival among Middle East respiratory syndrome coronavirus–infected patients, South Korea, 2015. A, B) Survival difference between the blood viral RNA-positive (solid line) and -negative (broken line) groups. Survival was defined as the time from initial confirmatory diagnosis to death before hospital discharge (A) (Kaplan-Meier survival analysis, log rank p = 0.009; Breslow p = 0.006) and as the time from symptom onset to death (B) (Kaplan-Meier survival analysis, log rank p = 0.017; Breslow p = 0.015). C, D) Survival difference between the high respiratory viral load (solid line) and low respiratory viral load (broken line) groups. Viral loads were classified into 2 groups: patients who harbored viral loads above the median load of patients and patients who harbored below. Survival was defined as time from initial confirmatory diagnosis to death. Cycle threshold (C_t) values were calculated for real-time reverse transcription PCRs targeting the upstream of envelope region (C) and open reading frame 1a region (D) (Kaplan-Meier survival analysis, log rank p = 0.739; Breslow p = 0.630). Tick marks along data lines indicate data-censored time points.

Blood viral RNA has been detected in a few case reports of MERS-CoV fatalities (*8*–*10*). Our data of 42 specimens from cross-sectional time points focusing on early viremia showed that blood viral RNA was present in a subpopulation of patients and that these patients had significantly poorer prognoses, as demonstrated by the need for more frequent mechanical ventilation and the increased risk for death. Further large studies using serial daily specimens that are collected throughout the admission period—, both upper and lower respiratory specimens and paired measurement of viral RNA and antibody in blood—might help overcome the limitation of the current study, which included relatively small numbers of deceased patients (24% [5/21]).

Conclusions

Our data showed a detection rate of 33% for viral RNA in blood at initial diagnosis, which was insufficient for initial confirmatory diagnosis. Blood viral RNA at the early phase was related to a worse clinical course in infected patients and might be a good prognostic indicator of severe outcome. Measuring blood viral RNA at hospital admission might be useful.

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Dr. Kim is a medical doctor at the Department of Laboratory Medicine of National Medical Center in South Korea. Her research interests include molecular diagnostics and infectious diseases.

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Address for correspondence: Moon-Woo Seong, Department of Laboratory Medicine, Seoul National University Hospital, Seoul National University College of Medicine, 101, Daehak-ro, Jongno-gu, Seoul 110-744, South Korea; email: mwseong@snu.ac.kr

Sporotrichosis-Associated Hospitalizations, United States, 2000–2013

Jeremy A.W. Gold, Gordana Derado, Rajal K. Mody, Kaitlin Benedict

To determine frequency and risk for sporotrichosis-associated hospitalizations, we analyzed the US 2000–2013 National (Nationwide) Inpatient Sample. An estimated 1,471 hospitalizations occurred (average annual rate 0.35/1 million persons). Hospitalizations were associated with HIV/AIDS, immune-mediated inflammatory diseases, and chronic obstructive pulmonary disease. Although rare, severe sporotrichosis should be considered for at-risk patients.

Sporotrichosis is a fungal disease caused by the *Sporothrix schenckii* species complex (*1*). In the environment, *S. schenckii* is commonly associated with decaying plant matter, soil, and sphagnum moss (*2*). Infection usually occurs through cutaneous inoculation of the organism and typically is a disease of the skin, subcutaneous tissue, and lymph nodes. Less commonly, disseminated forms of disease can occur if infection spreads from primary to secondary body sites, and pulmonary disease can occur if conidia are inhaled (*3*). Extracutaneous sporotrichosis typically develops in persons with immunosuppression, chronic obstructive pulmonary disease (COPD), diabetes mellitus, or alcoholism (*2*). Infections are usually sporadic, but outbreaks have been associated with traumatic skin injury sustained during outdoor work or with zoonotic spread from infected animals (*4,5*). Considered rare in the United States, sporotrichosis is not a reportable disease, and most information about its epidemiology comes from outbreak investigations.

To develop nationally representative estimates and to assess underlying conditions associated with sporotrichosis-associated hospitalizations, we analyzed data from the Healthcare Cost and Utilization Project (HCUP). This family of databases, sponsored by the Agency for Healthcare Research and Quality, comprises the largest collection of publicly available all-payer healthcare data in the United States.

The Study

The HCUP National (referred to as Nationwide before 2012) Inpatient Sample (NIS) is a database of hospital inpatient stays derived from billing data from ≈1,000 community hospitals (*6*). Data from the 2012 and 2013 NIS represent a 20% stratified sample of discharges from all participating hospitals; before 2012, the NIS contained all discharges from a sample of hospitals. For national estimates, discharges are assigned specific sampling weights based on hospital census region, rural/urban location, teaching status, bed size, and ownership. Weighted, the NIS estimates that >36 million discharges occur yearly.

We identified sporotrichosis-associated hospitalizations in the 2000–2013 NIS by using diagnosis code 117.1 from the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM). We created dichotomous variables to identify patients discharged with selected concurrent conditions by using ICD-9-CM codes for the following: HIV/AIDS (042); solid organ transplant or hematopoietic stem cell transplant (V42 [excluding V42.3–V42.5], 996.8); immune-mediated inflammatory diseases commonly treated with immunosuppressive medications including glucocorticoids and biological agents such as tumor necrosis factor-α (TNF-α) inhibitors: rheumatoid arthritis (714.0, 714.2), inflammatory bowel disease (555.xx, 556.xx), and psoriasis (696.0, 696.1, 696.8); diabetes mellitus (249.xx, 250.xx); alcohol use disorders and associated conditions (291.xx, 303.xx, 305.0, 535.3, 571.2, 571.3); and COPD (490, 491, 492, 494, 496). We categorized sporotrichosis-associated hospitalizations as lymphocutaneous, pulmonary, or arthritic sporotrichosis by using ICD-9-CM codes suggestive of lymphocutaneous disease (289.1, 289.3, 457.2, 681.00, 681.10, 682.0–682.9, 683, 686.9, 709.9), pulmonary disease (484.7, 486, 491.21, 493.22, 518.89, 786.2, 786.39), and infectious arthropathy (711.xx), respectively.

We obtained national estimates of sporotrichosis-associated hospitalizations and 95% CIs by applying the HCUP-supplied discharge weights (*7*) and examined them by age, sex, hospital region, season, presence of certain underlying conditions, in-hospital deaths, length of stay, and hospital charges by using SAS (SAS Institute, Cary, NC, USA) survey procedures. We calculated average annual overall rates and age-, sex-, and region-specific rates per 1 million persons by using population statistics from the US Census Bureau. We compared sporotrichosis-associated and non–sporotrichosis-associated hospitalizations by using the Rao-Scott χ^2 test for categorical variables (*8*) and the Student *t*-test for continuous variables.

During 2000–2013, an estimated 1,471 (95% CI 1,245–1,696) sporotrichosis-associated hospitalizations

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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occurred; the average annual rate was 0.35/1 million persons (95% CI 0.30–0.40) (Table). Yearly rates ranged from 0.19–0.47/1 million persons and showed no apparent trend (Figure). Sporotrichosis was listed as the primary patient diagnosis for 29.5% of all sporotrichosis-associated hospitalizations.

Sporotrichosis-associated hospitalizations occurred almost exclusively among adults, and rates were higher among men (average annual rate 0.40/1 million persons) than women (0.30/1 million). Rates were highest in the western (0.45/1 million) and lowest in the northeastern (0.15/1 million) United States. Nearly half (43.6%) of sporotrichosis-associated hospitalizations were for lymphocutaneous disease, but the clinical category of sporotrichal illness could not be identified for 42.3%. The discharge record diagnosis for sporotrichosis-associated hospitalizations was more likely than that for non–sporotrichosis-associated hospitalizations to mention HIV/AIDS (1.3% vs. 0.4%; p = 0.009), immune-mediated inflammatory disease (8.0% vs. 1.9%; p<0.001), or COPD (16.5% vs. 10.9%; p = 0.006), and the hospital stay was more likely to be longer (6.9 vs. 4.6 days; p<0.001) and costlier (mean charges $36,131 vs. $25,906; p = 0.017).

Conclusions

Our update of the epidemiology of sporotrichosis-associated hospitalizations in the United States demonstrates that the annual incidence remains low, at an estimated 0.35 hospitalizations/1 million persons. To our knowledge, national data on sporotrichosis-associated hospitalizations have not

Table. Characteristics of sporotrichosis-associated hospitalizations versus non–sporotrichosis-associated hospitalizations, United States, 2000–2013*

Characteristic	Sporotrichosis-associated hospitalizations			Non–sporotrichosis-associated hospitalizations, %	p value
	No. hospitalizations (95% CI)	Average annual rate (95% CI)†	% Hospitalizations		
Overall no.	1,471 (1,245–1,696)	0.35 (0.30–0.40)		518,905,960	
Patient age group, y					<0.001
<18‡	–	–	–	16.6	
18–44	329 (240–419)	0.21 (0.15–0.26)	20.9	25.8	
45–64	599 (466–734)	0.57 (0.44–0.70)	40.8	23.0	
≥65	451 (427–655)	1.01 (0.79–1.22)	36.8	34.6	
Mean patient age, y (SE)			58.1 (1.0)	47.9 (0.1)	<0.001
Patient sex					<0.001
M	831 (679–982)	0.40 (0.33–0.48)	56.4	41.4	
F	640 (513–767)	0.30 (0.24–0.36)	43.5	58.6	
Hospital region					<0.001
Northeast	118 (65–171)	0.15 (0.08–0.22)	8.0	19.4	
Midwest	328 (239–418)	0.35 (0.26–0.45)	22.3	22.9	
South	589 (471–706)	0.38 (0.31–0.46)	40.0	38.3	
West	436 (310–561)	0.45 (0.32–0.58)	29.6	19.4	
Season					0.006
Winter	257 (184–330)		17.5	23.2	
Spring	378 (289–467)		25.7	23.5	
Summer	371 (281–461)		25.2	23.2	
Autumn	349 (252–446)		23.7	22.8	
Unknown	116 (68–163)		7.9	7.3	
Underlying conditions					
HIV/AIDS	19 (0–42)		1.3	0.4	0.009
Transplant	19 (0–42)		1.3	0.7	0.289
Immune-mediated inflammatory disease	118 (66–170)		8.0	1.9	<0.001
Diabetes mellitus	285 (204–366)		19.4	17.7	0.294
Alcoholism	49 (16–83)		3.4	2.5	0.262
COPD	242 (149–335)		16.5	10.9	0.006
None of the above	877 (726–1,028)		59.6	69.3	
Patient deceased at discharge	49 (18–79)		3.3	2.0	<0.001
Mean length of hospital stay, d (SE)			6.9 (0.5)	4.6 (0)	<0.001
Mean charges, US$ (SE)			36,131 (4,290)	25,906 (166)	0.017
Disease form§					
Lymphocutaneous	641 (517–766)		43.6		
Pulmonary	160 (98–222)		10.9		
Arthritis	47 (15–80)		3.2		
Unclassified	623		45.5		

*Values are % or no. (%) unless otherwise indicated. COPD, chronic obstructive pulmonary disease.
†Per 1 million persons.
‡Data for this age group not presented because of small sample size.
§Categorized using codes from the International Classification of Diseases, Ninth Revision, Clinical Modification, suggestive of lymphocutaneous disease (289.1, 289.3, 457.2, 681.00, 681.10, 682.0–682.9, 683, 686.9, 709.9); pulmonary disease (484.7, 486, 491.21, 493.22, 518.89, 786.2, 786.39); and infectious arthropathy (711.xx).

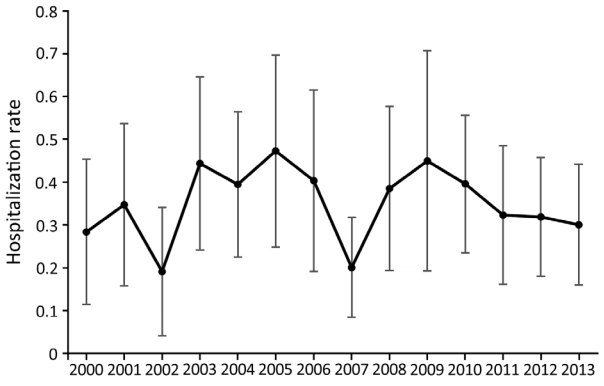

Figure. Annual rates of sporotrichosis-associated hospitalizations (no. hospitalizations/1 million persons), United States, 2000–2013. Error bars represent 95% CIs.

been described since Reingold et al. reported an annual incidence of 2.4/1 million persons during 1980–1982 (*9*). Their estimate was based on a review of hospital discharge data from the Professional Activity Study of the Commission on Professional and Hospital Activities. Our data suggest that the current incidence of sporotrichosis-associated hospitalizations is lower than it was in the 1980s, which may be the result of increased awareness of sporotrichosis prevention methods (such as wearing long-sleeved clothing and protective gloves and avoiding sphagnum moss) among workers in higher risk occupations after several high-profile outbreaks of lymphocutaneous sporotrichosis in the 1980s (*10*). Our estimate is consistent with that obtained from population-based laboratory surveillance during 1992–1993 in the San Francisco Bay area (California, USA), which estimated a yearly sporotrichosis incidence of <1 case/1 million persons (*11*).

We found that slightly more sporotrichosis-associated hospitalizations occurred among men than women and among persons 18–64 years of age. These findings may reflect participation in outdoor activities that could expose persons to *Sporothrix* (*4*). Although most sporotrichosis outbreaks have occurred in midwestern and southern states, sporotrichosis-associated hospitalizations were most common in western states.

Using administrative data has limitations: ICD-9-CM codes may not capture all cases because of possible misclassification and do not distinguish the more common lymphocutaneous form from other, more severe, forms of sporotrichosis. This limitation may be alleviated in future analyses because codes in the International Classification of Diseases, Tenth Revision, do distinguish the different forms of sporotrichosis. Using ICD-9-CM codes indicative of unspecified lymphocutaneous disease, we classified nearly half of all sporotrichosis-associated hospitalizations as the lymphocutaneous form. However, many more cases of lymphocutaneous sporotrichosis not requiring patient hospitalization probably occur; during the largest recorded outbreak of lymphocutaneous sporotrichosis in the United States, only 20% of case-patients were hospitalized (*10*).

Similar to previous reports describing factors that predispose persons to severe sporotrichosis, we found that HIV/AIDS and COPD were more commonly listed on discharge records for sporotrichosis-associated hospitalizations than for non–sporotrichosis-associated hospitalizations. However, factors associated with specific forms of sporotrichosis could not be assessed because of the small sample sizes for these subgroups. Our analysis showed that immune-mediated inflammatory diseases commonly treated with glucocorticoids and TNF-α inhibitors were 4 times more common among hospitalizations for sporotrichosis than hospitalizations for other reasons. The association between invasive mycoses and hypercortisolism has been well documented (*12*). No meaningful increase in sporotrichosis-associated hospitalizations during 2000–2013 was noted; however, as biological agents such as TNF-α inhibitors are being more frequently used to treat immune-mediated inflammatory diseases, and given their association with disseminated mycoses (*13,14*), there remains the potential that sporotrichosis-associated hospitalizations will increase in the future. Although severe forms of sporotrichosis are relatively rare, physicians should continue to be aware of potential for this disease in at-risk patients, particularly those who are receiving immunosuppressive agents and those with HIV/AIDS or COPD.

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Dr. Gold is a first-year resident in the Department of Medicine at Columbia University Medical Center, New York-Presbyterian Hospital, in New York, NY, USA. He participated in the Epidemiology Elective Program at the Centers for Disease Control and Prevention in 2015 while pursuing his medical degree at Albert Einstein College of Medicine in Bronx, NY.

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Address for correspondence: Kaitlin Benedict, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop C09, Atlanta, GA 30329-4027, USA; email: jsy8@cdc.gov

Effect of Geography on the Analysis of Coccidioidomycosis-Associated Deaths, United States

Jason A. Noble, Robert G. Nelson, Gudeta D. Fufaa, Paul Kang, Shira Chani Shafir, John N. Galgiani

Because coccidioidomycosis death rates vary by region, we reanalyzed coccidioidomycosis-associated mortality in the United States by race/ethnicity, then limited analysis to Arizona and California. Coccidioidomycosis-associated deaths were shown to increase among African-Americans but decrease among Native Americans and Hispanics. Separately, in a Native American cohort, diabetes co-varied with coccidioidomycosis-associated death.

In a recent study (*1*), researchers used a publically available database derived from death certificates to identify factors associated with deaths attributed to coccidioidomycosis in the United States. However, because coccidioidomycosis is endemic to only a few states, especially Arizona and California, and the racial/ethnic compositions of these states do not reflect the country as a whole, we refined the original analysis to compare national statistics with those of Arizona and California. Here we report the differences observed after using relevant demographic variables that align with the epidemiology of serious coccidioidal infections.

The Study

We analyzed multiple-cause-of-death data by using established methods (*1*). We then restricted analysis to Arizona and California. Publically available data from the Centers for Disease Control and Prevention National Center for Health Statistics (https://www.cdc.gov/nchs) for 1990–2008 were used to calculate national and state-specific mortality rates. We included in our analysis all coccidioidomycosis-associated deaths (indicated by codes 114.0–114.9 from the International Classification of Diseases (ICD), Ninth Revision, or B38.0–B38.9 from the ICD, Tenth Revision) and calculated mortality rates by using bridged-race population estimates from US census data. We age-adjusted these rates by using weights from the 2000 US census and then calculated incidence rate ratios accordingly. We used a generalized linear model with a log-binomial construct to analyze the effect of sex, age, and race/ethnicity. Interaction terms between race/ethnicity and sex were not statistically significant (United States overall 0.96 [p = 0.15], Arizona 0.92 [p = 0.11], California 0.96 [p = 0.37]).

Our analysis of national coccidioidomycosis-associated deaths confirmed what was previously reported (*1*) (Table). During 1990–2008, a total of 3,088 coccidioidomycosis-related deaths were reported in the United States. Men were disproportionately affected. Although most decedents were non-Hispanic whites, the mortality rate among non-Hispanic whites was the lowest among all racial/ethnic groups reported. For other groups, coccidioidomycosis-related deaths were highest among Hispanics and Native Americans and lowest among African-Americans and Asians.

After we restricted analyses to Arizona and California, where coccidioidomycosis is endemic, coccidioidomycosis-related mortality rates were 2.19 (95% CI 1.38–3.49) and 1.89 (95% CI 1.51–2.37) deaths per 100,000 person-years, respectively. A total of 1,957 coccidioidomycosis-related deaths were reported in Arizona and California during 1990–2008. In both states, mortality rates were highest among men, and compared with non-Hispanic whites, all other racial/ethnic groups had higher mortality rates attributed to coccidioidomycosis. However, in contrast with the national data, African-Americans had the highest and Native Americans and Hispanics the lowest excess mortality compared with non-Hispanic whites. Risk for coccidioidal death increased with age (Figure). Moreover, death rates attributed to coccidioidomycosis were higher across virtually all age groups in the highly endemic states, especially in Arizona, than in the United States as a whole. The only exception to this finding was that there were no deaths among persons <15 years of age in California. A multivariate analysis indicated that older age, male sex, and nonwhite race were independent risk factors for coccidioidomycosis-associated mortality.

Separately, we examined the coccidioidomycosis-related deaths in a Native American population residing near Phoenix, Arizona. We consulted a longitudinal study of diabetes and its complications within the Gila River Indian

Author affiliations: University of Arizona College of Medicine, Phoenix, Arizona, USA (J.A. Noble, P. Kang); National Institutes of Health, Bethesda, Maryland, USA (R.G. Nelson, G.D. Fufaa); University of California, Los Angeles, California, USA (S.C. Shafir); University of Arizona College of Medicine, Tucson, Arizona, USA (J.N. Galgiani)

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Table. Differences in coccidioidomycosis-associated deaths, by geography, sex, and race/ethnicity, Arizona, California, and United States overall, 1990–2008

Characteristic	US mortality rate*	Incidence rate ratio† (95% CI)			Relative risk‡ (95% CI)		
		United States	Arizona	California	United States	Arizona	California
Sex							
F	0.31	Referent	Referent	Referent	Referent	Referent	Referent
M	0.93	3.03 (2.80–3.27)	2.24 (1.95–2.54)	3.49 (2.68–3.69)	2.32 (1.97–2.73)	1.76 (1.38–2.26)	2.79 (2.10–3.71)
Race/ethnicity							
Non-Hispanic white	0.40	Referent	Referent	Referent	Referent	Referent	Referent
Hispanic	1.82	4.45 (4.06–4.88)	1.36 (1.13–1.64)	2.48 (2.09–2.96)	6.74 (5.59–8.14)	2.10 (1.53–2.88)	5.21 (3.87–6.99)
African American	0.69	1.70 (1.52–1.91)	3.41 (2.65–4.38)	5.15 (4.27–6.22)	1.38 (1.16–1.60)	3.42 (2.55–4.60)	3.50 (2.84–4.31)
Asian	1.25	2.84 (2.42–3.35)	2.19 (1.38–3.49)	1.88 (1.51–2.37)	5.48 (4.32–6.79)	5.51 (2.82–10.7)	2.70 (2.01–3.61)
Native American	2.67	6.52 (5.14–8.30)	2.50 (1.91–3.28)	1.39 (0.58–3.38)	8.15 (6.09–10.9)	2.18 (1.52–3.12)	3.01 (1.31–6.89)

*Per 1 million person-years.
†Univariate analysis (sex or race/ethnicity).
‡Multivariate analysis (sex and race/ethnicity).

Community (GRIC) conducted during 1965–2007, a subset of which is published (*2*). The results of that study cover a well-characterized population of persons who are of ≥50% Pima Indian heritage. We included in our analysis all persons ≥15 years of age who resided within the community. We considered all deaths with codes 114.0–114.9 from the ICD, Ninth Revision, listed as the underlying or contributing cause of death to be coccidioidomycosis-associated deaths. We calculated mortality statistics within the GRIC by using population estimates from the 1980 GRIC census and then adjusted these estimates for age and sex.

During 1965–2008, a total of 17 coccidioidomycosis-related deaths were reported in the GRIC. In this cohort, there was no male predominance (9 of the decedents were women). All deaths occurred in persons >45 years of age (range 49–86 years). Death in this group was highly associated with diabetes (15 of the decedents had type 2 diabetes). The overall crude mortality rate was 350 deaths/1 million person-years, and the age-sex adjusted mortality rate was 6.3-fold (95% CI 1.8–11.6-fold) higher among those with diabetes compared with those without.

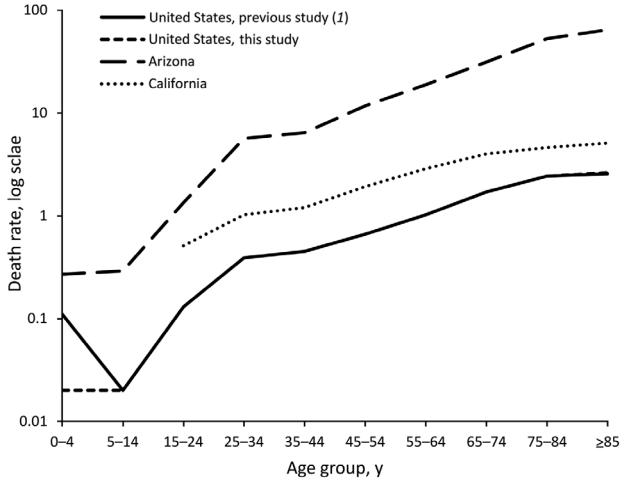

Figure. Coccidioidomycosis-associated mortality rates, by age group, Arizona, California, and United States overall, 1990–2008. The difference in the mortality rate of the 0–4 year age group between previous study (*1*) and this study is attributable to a misprint in the source document.

Conclusions

Although the effect of race/ethnicity on dissemination of coccidioidomycosis has been reported previously (*3*–*6*), little is known about race/ethnicity and coccidioidomycosis-attributed death. By using equivalent methods, we confirmed many of the findings reported by Huang et al. (*1*). We then wondered how a reanalysis, using only the populations at the highest risk for exposure to *Coccidioides* spores, would change the associated mortality incidence rate ratios and relative risks.

In 2000, Arizona had a population of ≈5 million. With ≈80% of the population residing within the highly coccidioidomycosis-endemic area of Maricopa and Pima counties, ≈4.1 million persons were at risk for exposure to *Coccidioides*. In 2000, California had a population of ≈34 million. Only 4% of the population (≈1.5 million) lived in the counties comprising the San Joaquin Valley. These 5.6 million persons, who were most likely to inhale *Coccidioides* spores, make up only ≈2% of the US population (*7*,*8*). With this in mind, we considered how the mortality rate might change if we restricted analysis to the 2 states with the highest endemicity.

Restricted analysis confirmed that the mortality rate increased with age and was associated with male sex. These associations are in agreement with previous reports (*9*,*10*). Increased mortality was observed in nonwhite racial/ethnic groups. However, in contrast to the national data, African-Americans had the highest coccidioidomycosis-associated mortality rate, whereas risk for Hispanics, Native Americans,

and Asians was less elevated compared with non-Hispanic whites. These findings correspond well with what has been reported by Seitz et al. (*6*), that nonwhite race is a risk factor for disseminated coccidioidomycosis-associated hospitalization in Arizona and California.

Examination of the GRIC allowed us to calculate coccidioidomycosis-related mortality rates in a genetically well-defined population. The high coccidioidomycosis-related mortality rate in Pima Indians is probably related at least in part to the high incidence of comorbidities in this population. Further, because none of these deaths occurred in persons <45 years old, the increased mortality rate among Native Americans is probably not solely related to genetic susceptibility; however, persons ≤15 years of age were not included in the GRIC analysis, which might limit this interpretation. Instead, the increased mortality rate is more probably a result of the high rate of debilitating comorbidities in this population. This argument is supported by the longer duration of diabetes associated with increased infection-related mortality in Pima Indians (*11*).

Our study highlights the advantage of calculating rates of endemic diseases within their respective regions. By confining analysis to Arizona and California, our study measures the race/ethnicity-associated risk for death attributed to coccidioidomycosis in a highly disease-endemic region, which differs from the United States overall. Mortality rates are higher for men and all nonwhite racial/ethnic groups, especially for African-Americans. Sex and race/ethnicity affected mortality rates independently. Whether this predilection for nonwhite groups is attributable to genetic susceptibility, socioeconomics, or other factors remains to be determined. Data from a Native American population suggest a relationship between diabetes and coccidioidomycosis-associated death. Whether this phenomenon is applicable to other populations deserves further investigation. We caution against strict interpretation of these data because multiple-cause-of-death data are inherently limited by their use of population estimates and death certificates for statistical analysis (*12*).

Dr. Noble received his doctor of medicine degree in 2016 from the University of Arizona College of Medicine in Phoenix and is beginning his ophthalmology residency. His primary research interests include drug-resistant mechanisms of pathogenic yeast and the epidemiology of serous retinal detachments and coccidioidal mortality.

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Address for correspondence: John N. Galgiani, Valley Fever Center for Excellence, University of Arizona College of Medicine, 1656 East Mabel St, Tucson, AZ 85712, USA; email: spherule@u.arizona.edu

Persistence of Antibodies against Middle East Respiratory Syndrome Coronavirus

Daniel C. Payne, Ibrahim Iblan, Brian Rha, Sultan Alqasrawi, Aktham Haddadin, Mohannad Al Nsour, Tarek Alsanouri, Sami Sheikh Ali, Jennifer Harcourt, Congrong Miao, Azaibi Tamin, Susan I. Gerber, Lia M. Haynes, Mohammad Mousa Al Abdallat

To determine how long antibodies against Middle East respiratory syndrome coronavirus persist, we measured long-term antibody responses among persons serologically positive or indeterminate after a 2012 outbreak in Jordan. Antibodies, including neutralizing antibodies, were detectable in 6 (86%) of 7 persons for at least 34 months after the outbreak.

Middle East respiratory syndrome coronavirus (MERS-CoV) causes acute respiratory illness, which can progress rapidly to respiratory failure and death in ≈40% of persons with laboratory-confirmed cases. The first known cases of MERS-CoV occurred during an outbreak of severe acute respiratory infections in Zarqa, Jordan, during March–April 2012 (*1*). New cases and clusters of MERS-CoV infections continue to occur within the Arabian Peninsula, and the virus has been exported to other countries around the world.

For 2 persons affected by the April 2012 outbreak, the cause of death remained unknown until late 2012, when retained samples produced positive MERS-CoV results according to reverse transcription PCR. In May 2013, we obtained serologic and epidemiologic data from 124 persons: the 2012 outbreak survivors, their exposed contacts, and their household members. In that investigation, we found another 7 persons with positive MERS-CoV results according to ELISA and confirmatory results by immunofluorescence assay (IFA), microneutralization assay, or both (*1*). Results were indeterminate for another 8 exposed persons, whose results were positive by only 1 of these serologic methods; these 8 persons were deemed MERS test–negative overall.

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (D.C. Payne, B. Rha, J. Harcourt, C. Miao, A. Tamin, S.I. Gerber, L.M. Haynes); Jordan Field Epidemiology Training Program, Amman, Jordan (I. Iblan); Jordan Ministry of Health, Amman (S. Alqasraw, A. Haddadin, T. Alsanouri, S. Sheikh Ali, M.M. Al Abdallat); Eastern Mediterranean Public Health Network, Amman (M. Al Nsour, T. Alsanouri)

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For patients with severe acute respiratory syndrome coronavirus (SARS-CoV) infection, antibodies persist for at least 2 years after symptomatic infection (*2*). Recently, antibodies against MERS-CoV were found (by ELISA and IFA) in 9 healthcare workers in Saudi Arabia with symptomatic MERS-CoV infection at least 18 months after infection (*3*). Duration of antibody responses beyond 18 months has not been reported (*4*). Our objective was to evaluate long-term antibody responses among persons with laboratory-confirmed to MERS-CoV infection.

The Study

All surviving members of the 2012 outbreak in Jordan, their exposed contacts, and their household members who were identified serologically as either MERS-CoV positive or indeterminate were asked to consent to further participation. Participants were asked to provide a follow-up serologic specimen so we could compare 34-month results with 13-month results.

Specimens were prepared by the Jordan Central Public Health Laboratory (Amman, Jordan) and tested at the US Centers for Disease Control and Prevention (Atlanta, GA, USA). Antibody titers in serum samples were determined by an anti–MERS-CoV nucleocapsid indirect ELISA and by MERS-CoV (Hu/Jordan-N3/2012 strain) indirect IFA (*1*). The presence of neutralizing antibody titers was determined by microneutralization with live MERS-CoV (Hu/Jordan-N3/2012 strain) in a Biosafety Level 3 laboratory as described previously (*1*). Neutralization titers were defined as the reciprocal of the highest serum dilution completely protecting the Vero cell monolayer from cytopathic effect in at least 1 of 3 parallel wells. Titers ≥1:20 were reported as positive.

Of the 15 surviving persons with ≥1 positive serologic test result, 13 (87%) consented to follow-up testing. All 7 (100%) surviving persons with ≥2 positive serologic test results 13 months after the MERS-CoV outbreak also consented. Each of these 7 persons was considered to be a probable MERS-CoV case-patient according to World Health Organization criteria; each had had a symptomatic acute respiratory infection during the outbreak period and documented, unprotected exposure to ≥1 person with a case confirmed by reverse transcription PCR.

For the 7 probable case-patients, ELISA titers at 34 months ranged from <400 to 1,600, representing reduced antibody titers compared with the 13-month estimates

Table. Characteristics of Middle East respiratory syndrome patients and antibody titers at 13 and 34 months after 2012 outbreak, Jordan*

Patient no.	Age, y/sex	Underlying condition	Intensive care	Days hospitalized	Chest radiograph†	13-mo titer‡			34-mo titer‡		
						ELISA	IFA	MN	ELISA	IFA	MN
02	31/M	Atrial septal defect	Yes	16	Right lobar pneumonia	>6,400	Pos	160	1,600	Pos	80
03§	60/M	Hypertension	NA	NA	Bilateral consolidation; pneumonia	400	Pos	20	<400	Ind	20
04	35/M	Hypertension	Yes	8	Bilateral lobar pneumonia	>6,400	Pos	80	400	Pos	40
06	46/M	None reported	No	6	Right bronchial congestion with bronchovascular markings	1,600	Pos	20	1,600	Pos	20
09	45/M	None reported	No	10	Left consolidation; right infiltrate	400	Pos	40	<400	Neg	40
11¶	41/F	None reported	No	4	ND	1,600	Pos	<20	400	Neg	<20
HH303§	39/F	Pregnant	NA	NA	NA	1,600	Pos	80	400	Pos	80

*IFA, immunofluorescence assay; Ind, indeterminate; MN, microneutralization assay; NA, not applicable; ND, not documented; neg, negative; pos, positive.
†Taken within 3 days of presentation.
‡Antibodies against Middle East respiratory syndrome coronavirus.
§Symptomatic but refused hospitalization.
¶Classified overall as being serologically negative at 34 mo.

(400–6,400) for all but 1 person (Table). A nurse who worked in an intensive care unit (participant 06) and cared for confirmed case-patients during the outbreak was the only participant for whom ELISA indicated a consistent titer of 1,600 at both times.

Of these 7 participants, 6 (86%) had neutralizing antibody titers ranging from 20 to 80 at the 34-month follow-up evaluation, and only 2 (29%) had any decrease in neutralizing antibody titers over time. One participant had no detectable neutralizing antibodies (Figure). Of the 7 participants for whom IFA results were positive at 13 months, 4 (57%), had positive results at 34 months.

For the 8 surviving participants whose serologic results at 13 months were indeterminate, 6 (75%) consented to further testing. Of these, 3 (50%) reported having had no respiratory symptoms, 1 reported having had mild respiratory symptoms, and 2 had been hospitalized with respiratory infections; all 6 had had documented, unprotected exposure to ≥1 case-patient. Each of these 6 persons had negative serologic test results at 34 months and continue to be considered negative overall.

Conclusions

Antibodies against MERS-CoV, including neutralizing antibodies, persisted in 6 (86%) of 7 persons 34 months after the 2012 MERS-CoV outbreak in Jordan. The observed persistence of these antibodies contributes to the understanding of individual immune responses to MERS-CoV infection, of population-based immunity in regions where MERS-CoV outbreaks have occurred, and to efforts for developing effective vaccines and therapeutics to counter MERS-CoV infections.

Notwithstanding improvements in public health awareness and infection control practices in affected countries on the Arabian Peninsula and in the Middle East, emergence of the virus (e.g., its introduction to South Korea and the resultant epidemic of 2015 [*5*]) is ongoing. MERS-CoV continues to pose grave risks to international healthcare and socioeconomic systems (*6*).

It has been hypothesized that mild or asymptomatic MERS-CoV infections are potentially associated with lower levels of MERS-CoV neutralizing antibodies over time (*7*). All 7 case-patients reported here had respiratory symptoms, were relatively young, and had few underlying

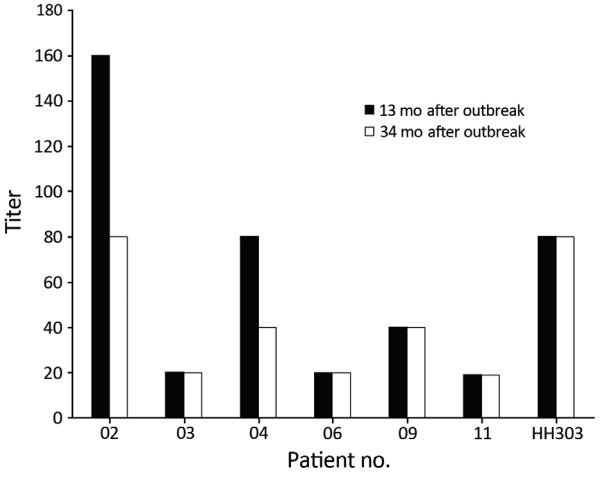

Figure. Neutralizing antibody titers against Middle East respiratory syndrome coronavirus (Hu/Jordan-N3/2012 strain) among 7 surviving case-patients at 13 and 34 months after the 2012 outbreak in Jordan. Patient numbers match those in the Table.

medical conditions (Table). Any association between our MERS-CoV antibody results and clinical severity is therefore difficult to assess. Nonetheless, of the 5 persons for whom chest radiographs showed substantial changes within 3 days of symptom onset, each remained positive by microneutralization (>20) 34 months after the outbreak.

Although some similarities in the short-term development of antibodies against MERS-CoV and SARS-CoV (e.g., seroconversion 2–3 weeks after illness onset) have been observed (*8*,*9*), longer term serum antibody kinetics of these infections have not yet been compared. After SARS-CoV infection, robust IgG titers were observed through the second year but declined substantially during the third year after infection (*10*). Our finding of generally reduced but persistent MERS-CoV antibody responses even at 34 months suggests the potential for longer lasting antibody-mediated protective immunity against reinfection. However, whether such long-lasting antibodies can prevent reinfection or affect clinical outcome has yet to be examined. Diverse individual antibody test results allude to a potential role of genetic factors in explaining observed differences in immunologic responses to MERS-CoV exposure and infection.

The times at which MERS-CoV antibodies were measured in our study were chosen because of logistics and field practicalities. Although limited outbreaks of MERS-CoV have occurred in Jordan since 2012, contact tracing efforts by investigators in Jordan lead us to believe that these persons were not subsequently exposed. The observed ELISA titers and neutralizing antibody titers support this supposition; otherwise, we would expect increases resulting from a booster effect after secondary exposure and infection. To further assess the duration and resiliency of MERS-CoV antibodies in human populations, continued follow-up serologic evaluations of these persons would be desirable.

Dr. Payne is the principal investigator and project officer for the Centers for Disease Control and Prevention study Enhanced Active Surveillance of Pediatric Infectious Diseases and Vaccines, and he conducts phase IV vaccine effectiveness evaluations. Additionally, he has led several MERS investigations and worked to establish post-Hajj acute respiratory illness surveillance in the Middle East.

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Address for correspondence: Daniel C. Payne, Centers for Disease Control and Prevention, 1600 Clifton Road NE, Mailstop A34, Atlanta, GA, 30329-4027, USA; email: dvp6@cdc.gov

Case-Fatality Rates and Sequelae Resulting from *Neisseria meningitidis* Serogroup C Epidemic, Niger, 2015

Matthew E. Coldiron, Halidou Salou, Fati Sidikou, Kadadé Goumbi, Ali Djibo, Pauline Lechevalier, Idrissa Compaoré, Rebecca F. Grais

We describe clinical symptoms, case-fatality rates, and prevalence of sequelae during an outbreak of *Neisseria meningitidis* serogroup C infection in a rural district of Niger. During home visits, we established that household contacts of reported case-patients were at higher risk for developing meningitis than the general population.

A novel strain of *Neisseria meningitidis* serogroup C has been circulating in parts of the African meningitis belt since 2013 (*1*), causing a large-scale epidemic in Nigeria and Niger in 2015. This novel strain appeared after the introduction of a conjugate vaccine (PsA-TT, MenAfriVac) against *N. meningitidis* serogroup A, previously the major cause of epidemic meningitis in the region. *N. meningitidis* serogroup A has been virtually eliminated as a result of PsA-TT, although the first cases of *N. meningitidis* serogroup C in Nigeria in 2013 predated MenAfriVac introduction there. We describe case-fatality rates and sequelae resulting from infection with *N. meningitidis* serogroup C in a rural health district of Niger.

The Study

During January 1–June 30, 2015, a total of 562 suspected cases of meningitis and 51 deaths (case-fatality ratio [CFR] 9.1%) from this disease were reported in the national surveillance system in the Dogondoutchi health district of Niger (estimated population 727,282) (*2*). Médecins Sans Frontières supported Niger's Ministry of Public Health in its epidemic response in Dogondoutchi by providing patient care with 5 days of injectable ceftriaxone, as described in recent recommendations (*3*); reinforcing surveillance by providing training on case definitions; collecting data; and collaborating in reactive vaccination campaigns. A patient-level database containing patient demographics, treatment, laboratory results, and outcome was maintained during January 2–May 17, 2015; during this period, the database compiled information for 473 case-patients. In September 2015, in collaboration with the Ministry of Health, teams consisting of a community health worker and a nurse attempted home visits to each of the 473 case-patients (or surviving family members) in the database. Team members verified patients' vital status and treatments received and assessed for presence of 6 major sequelae, offering treatment and referral when necessary. Paralysis and anosmia were noted on physical exam (anosmia was evaluated by asking participants to smell a pungent local food with their eyes closed). Persistent convulsions, hearing loss, loss of developmental milestones (among children <5 years of age), and persistent mental incapacity (among persons ≥5 years of age) were subjectively reported by patients (or family members). During the home visit, teams used a standardized questionnaire to collect information and conduct a household census (Table). Team members asked whether meningitis had been diagnosed in other family members during the epidemic and verified reported diagnoses by written records available from the patient or by verbal confirmation of the number of doses of intramuscular ceftriaxone received. A meningitis death was considered to be any death occurring ≤30 days of symptom onset.

The original patient database was anonymized during analysis, and no identifying information was collected. Niger's Ministry of Public Health approved the overall intervention, including the home visits conducted after the epidemic. All medical care was free of charge to patients.

Of the 473 meningitis patients in the database, 369 (78.0%) could be visited at home. In the original patient database, 54 deaths were recorded (CFR 11.4%). During the home visits, 6 patients that were reported as dead in the database were found to be alive; 22 patients reported as recovered had in fact died, for a total of 70 meningitis deaths (CFR 14.8%).

Collection of biologic samples for confirmation was emphasized during the epidemic; consequently, 406 (82.5%) of the 473 reported patients received a lumbar puncture, and 252 (63.6%) patient samples were positive for *N. meningitidis*, *Streptococcus pneumoniae*, or *Haemophilus influenzae* type b infection by PCR performed at the national reference laboratory for meningitis in Niamey. Among the positive samples, 189 (75.0%) were *N. meningitidis* serotype C; 47

Author affiliations: Epicentre, Paris, France (M.E. Coldiron, R.F. Grais); Epicentre, Niamey, Niger (H. Salou); Centre de Recherche Médicale et Sanitaire (CERMES), Niamey (F. Sidikou); Ministry of Public Health, Niamey (K. Goumbi, A. Djibo); Médecins Sans Frontières, Paris (P. Lechevalier); Médecins Sans Frontières, Niamey (I. Compaoré)

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Table. Characteristics of 369 suspected meningitis patients visited at home after the epidemic season, Dogondoutchi, Niger, September 2015

Characteristic	No. (%) patients
Sex	
M	220 (59.6)
F	149 (40.4)
Age, y	
<2	22 (6.0)
2–4	57 (15.5)
5–14	190 (51.5)
15–29	84 (22.8)
30–44	13 (3.5)
≥45	3 (0.8)
Positive by PCR	194 (62.2)
Neisseria meningitidis serogroup C	144 (74.2)
N. meningitidis serogroup W	36 (18.6)
Streptococcus pneumoniae	12 (6.2)
N. meningitidis serogroup unspecified	2 (1.0)
Delay between symptom onset and visit to health center, d*	
0	90 (24.4)
1	176 (47.7)
2	63 (17.1)
3	23 (6.2)
≥4	14 (0.8)

*Data missing for 3 patients.

(18.7%) were *N. meningitidis* serotype W; 13 (5.2%) were *S. pneumoniae*; and 3 (1.2%) were *N. meningitidis* serogroup unspecified.

Of the 189 patients with confirmed *N. meningitidis* serogroup C infection, 23 died (CFR 12.2%). A difference in death rates by sex was not statistically significant: 12 (9.8%) of 123 male patients died, compared with 11 (16.7%) of 66 female patients ($p = 0.16$ by χ^2 test). No difference appeared between recorded clinical signs and symptoms (i.e., headache, fever, neck stiffness, convulsions, vomiting, irritability, altered consciousness, bulging fontanelles, and focal neurologic symptoms) for patients with confirmed *N. meningitidis* serotype C infection and those for patients with all other suspected infections.

During the follow-up home visits, surviving patients were evaluated for sequelae. Among patients with suspected cases, prevalence of any sequela was 10.8%. The rate of sequelae among patients with confirmed *N. meningitidis* serogroup C infection was 15.1% (19/126), compared with 7.7% (3/39) for patients with all other confirmed infections and 7.9% (11/140) for all other patients (i.e., with suspected but unconfirmed cases or with suspected cases with negative test results) ($p = 0.06$). The 2 most common reported sequelae among patients with confirmed infections were hearing loss (15/126 [11.9%]) and persistent mental incapacity or loss of milestones (4/126 [3.2%]). Patients with these sequelae did not differ in sex or age from other patients.

The 369 case-patients (or their surviving family members) who received home visits after the epidemic lived in 346 households. For 298 households, only 1 case occurred; 48 (13.0%) households had multiple case-patients. Among these 48 households, all case-patients in 22 households appeared in the patient database. However, the home interviews revealed 26 households with additional case-patients (i.e., who confirmed receiving multiple doses of ceftriaxone) not found in the patient database. Taking into account the household census, the attack rate for subsequent case-patients in a household after notification of a first case was 1,760/100,000 population. During the same period, in the Dogondoutchi health district, the overall attack rate was 79/100,000 population. Median time between onset of the first case in a household and a subsequent case was 3 days (interquartile range 1–8).

Conclusions

By collecting detailed information on individual notified cases and then making home visits to ≈80% of case-patients 4 months after the epidemic ended, we gathered insights into this novel strain of *N. meningitidis* serogroup C. The CFR in the Dogondoutchi district was higher than the country's average and was also higher among women. Although slightly higher than CFRs seen in past *N. meningitidis* serogroup A epidemics, deaths recorded in the surveillance system were mostly in line with those occurring in historic epidemics. The prevalence of sequelae in confirmed cases of *N. meningitidis* serogroup C also falls in line with descriptions of sequelae caused by other meningococcus serotypes (*4*). Overall, the characteristics of this epidemic are similar to those of historic epidemics of *N. meningitidis* before the introduction of MenAfriVac (*4–6*).

Home visits enabled quantification of attack rates among members of households reporting a meningitis case. Because of the short time between the first and subsequent cases, subsequent cases should not be considered secondary cases, although household members are at higher risk for infection compared with the community at large. This finding could eventually support the use of antimicrobial drug prophylaxis for household members of meningitis patients, although more research on this topic is warranted. Available data did not permit subdistrict-level analyses. Given the heterogeneous distribution of cases across districts in other epidemics (*7*), subdistrict-level analyses would be preferable to district-level analyses.

Finally, even with dedicated resources to reinforce surveillance during the epidemic, we found unreported cases during the home visits and, after confirmation of patients' vital status, the case-fatality ratio differed from that reported. Our findings indicate that even reinforced surveillance systems are not perfectly sensitive, but incomplete reporting should not deter continued case-based surveillance.

Dr. Coldiron trained in internal medicine and works as a medical epidemiologist for Epicentre–Médecins Sans Frontières. His main interests include meningitis and malaria in the Sahel and neglected tropical diseases.

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Address for correspondence: Matthew E. Coldiron, Epicentre–Médecins sans Frontières, 8 rue Saint-Sabin, 75011 Paris, France; email: matthew.coldiron@epicentre.msf.org

Reemergence of *Mycobacterium chimaera* in Heater–Cooler Units despite Intensified Cleaning and Disinfection Protocol

Peter W. Schreiber, Stefan P. Kuster, Barbara Hasse, Cornelia Bayard, Christian Rüegg, Philipp Kohler, Peter M. Keller, Guido V. Bloemberg,[1] Francesco Maisano, Dominique Bettex, Maximilian Halbe, Rami Sommerstein, Hugo Sax

Invasive *Mycobacterium chimaera* infections after open-heart surgery have been reported internationally. These devastating infections result from aerosols generated by contaminated heater–cooler units used with extracorporeal circulation during surgery. Despite intensified cleaning and disinfection, surveillance samples from factory-new units acquired during 2014 grew nontuberculous mycobacteria after a median of 174 days.

Mycobacterium chimaera is an emerging pathogen causing disastrous infections of heart valve prostheses, vascular grafts, and disseminated infections after open-heart surgery (*1*,*2*). Growing evidence supports airborne transmission resulting from aerosolization of *M. chimaera* from contaminated water tanks of heater–cooler units (HCUs) that are used with extracorporeal circulation during surgery (*3*,*4*). HCUs were previously associated with surgical site infections caused by nontuberculous mycobacteria (NTM) (*5*). We describe the colonization dynamics of factory-new HCUs with NTM during regular use.

The Study

Identification of *M. chimaera* infection in 6 patients prompted an outbreak investigation at the University Hospital Zurich, a 900-bed tertiary-care hospital in Zurich, Switzerland, that performs ≈700 open-heart surgeries that use extracorporeal circulation per year. The investigation included microbiologic sampling of HCUs for NTM (*3*). Surveillance cultures of HCU water from the cardioplegia and patient circuits and airflow samples from running HCUs at ≈2–3 m distance were gathered in monthly intervals. Failure to eradicate *M. chimaera* and other NTM from older HCUs prompted the acquisition of 5 factory-new HCUs (model 3T; Sorin [now LivaNova, London, UK]) during 2014: 2 in January, 1 in April, and 2 in September.

Mycobacterial cultures were performed according to standard methods by using the mycobacteria growth indicator tube system (MGIT 960; Becton Dickinson, Sparks, MD, USA) or Middlebrook 7H11 agar plates (BD Difco Mycobacteria 7H11 Agar; Becton Dickinson) that were incubated at 37°C for 7 wks or until positive. Air specimens were gathered with a microbiologic air sampler (MAS-100 NT, MBV, Stäfa, Switzerland) running for 2.5 min at a rate of 100 L/min by using Middlebrook 7H11 agar plates. Mycobacterial species were identified by 16S rRNA gene sequencing, as described (*6*).

Before mid-April 2014, HCUs were serviced according to the manufacturer's recommendations. Before first use and every 3 months thereafter, a disinfection cycle was performed by adding 200 mL of 3% sodium hypochlorite (Maranon H; Ecolab, Northwich, UK) to the HCU water tanks filled with filtered tap water (Pall-Aquasafe Water Filter AQ14F1S; Pall, Portsmouth, UK) to a final concentration ≈0.045% sodium hypochlorite in the water tank and circuits. Water was changed every 14 days; 100 mL of 3% hydrogen peroxide was added to the initial water filling (final concentration ≈0.02% hydrogen peroxide in water tank and circuits); an additional 50 mL of 3% hydrogen peroxide was added every 5 days (*7*). In mid-April 2014, an intensified in-house cleaning and disinfection procedure was implemented, consisting of daily water changes with filtered tap water (Pall) and additions of 100 mL of 3% hydrogen peroxide combined with biweekly disinfection using sodium hypochlorite (Maranon H). In February 2015, with lack of availability of 3% sodium hypochlorite and in line with the manufacturer's recommendations, the disinfection solution was changed to a combination of peracetic acid and hydrogen peroxide (an additional 450 mL Puristeril 340; Fresenius Medical Care, Hamburg, Germany) in filled water tanks every 2 weeks. Also, stainless steel housings were custom built around the HCUs to ensure strict air separation between the exhaust air of the HCUs and the operating room air (Figure

Author affiliations: University Hospital Zurich, Zurich, Switzerland (P.W. Schreiber, S.P. Kuster, B. Hasse, C. Bayard, C. Rüegg, P. Kohler, F. Maisano, D. Bettex, M. Halbe, H. Sax); University of Zurich, Zurich (P.W. Schreiber, S.P. Kuster, B. Hasse, C. Bayard, C. Rüegg, P. Kohler, F. Maisano, D. Bettex, H. Sax); National Centre for Mycobacteria at University of Zurich, Zurich (P.M. Keller); Institute of Medical Microbiology at University of Zurich, Zurich (P.M. Keller, G.V. Bloemberg); University Hospital, Bern, Switzerland (R. Sommerstein); University of Bern, Bern (R. Sommerstein)

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[1]Current affiliation: Unilabs, Dübendorf, Switzerland.

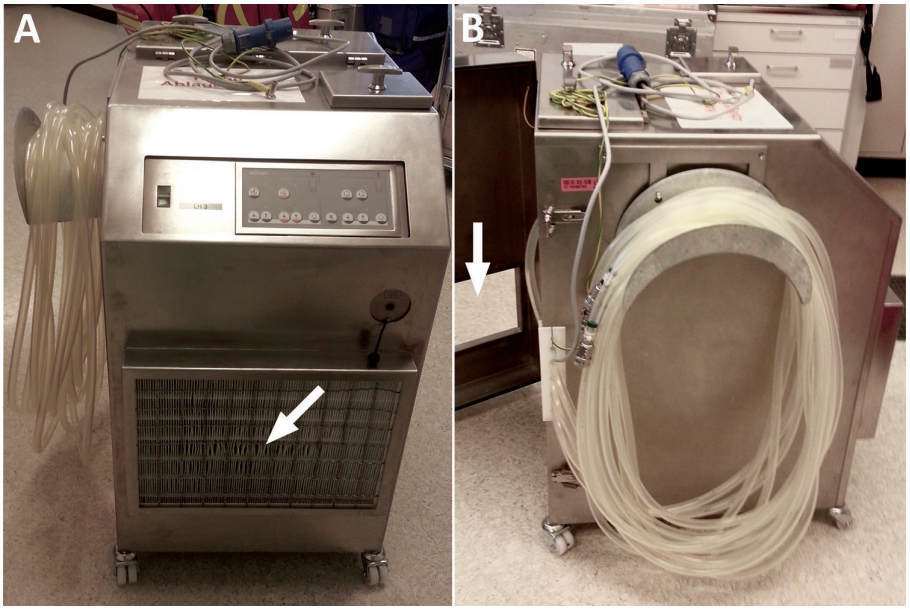

Figure 1. Custom-made stainless steel housing for heater–cooler units (model 3T; Sorin [now LivaNova, London, UK]) used at the University Hospital Zurich, Zurich, Switzerland. A) Front view shows the fine dust filter F7 over the air inlet (arrow). B) Side view shows the half-open back door with the rectangular opening (arrow), through which a duct connects the housing to the operating room ventilation exit. The negative pressure of the operating room ventilation system generates the necessary airflow.

1). These housings were directly connected to the operating room exhaust conduit.

A total of 134 water samples were obtained from the study HCUs, 127 after implementation of the intensified protocol. The first water sample tested positive for *M. chimaera* in August 2014, originating from a study HCU introduced 7 months earlier (Figure 2). Of all samples, 90 (67.2%) remained sterile for NTM; 6 (4.5%) were contaminated by bacterial overgrowth; and 38 (28.4%) yielded NTM. Of these NTM, 22 (57.9%) were *M. chimaera*; 12 (31.6%) were *M. gordonae*; 1 (2.6%) was *M. chelonae*; 1 (2.6%) was *M. paragordonae*; and 2 (5.3%) were a combination of *M. chimaera* and *M. gordonae*. NTM were found in both cardioplegia and patient HCU water circuits (Table).

Of 91 air samples, 90 (98.9%) had no mycobacterial growth. One sample grew *M. chelonae*, although no mycobacterium was detected simultaneously in the corresponding HCU water.

NTM growth was recorded after a median of 174 (range 158–358) days in HCU water samples. One of 5 HCUs remained permanently without growth of *M. chimaera*; 4 grew *M. chimaera* after a median of 250 (range 158–358) days.

Conclusions

HCUs seem to provide favorable environmental conditions for growth of NTM, in particular *M. chimaera*. An intensified cleaning and disinfection protocol failed to prevent growth of NTM entirely but succeeded in preventing detectable aerosolization of *M. chimaera*.

The contamination status of HCUs seems to be influenced by the intensity of maintenance, especially frequency of water changes. This hypothesis led to the development of the in-house maintenance protocol. The consistently negative air cultures for *M. chimaera* and the only intermittently positive water cultures support the benefits of our intensified protocol.

Our study design did not elucidate the origin of *M. chimaera* and other NTM in HCUs. The HCUs might have been already contaminated at time of delivery in a concentration below the detection threshold of mycobacterial cultures. A recent investigation confirmed the presence of environmental mycobacteria, including *M. chimaera*, in factory-new HCUs (*8*,*9*). In our study, 1 HCU (HCU 4, Figure 2) tested positive for *M. chimaera* for the first time after being returned from repair at the manufacturer. Contamination from tap water is unlikely because the study HCUs used only filtered water.

Previous studies indicated the durability of mycobacteria against several disinfectants, likely because of the organisms' complex cell wall (*10*). *M. avium* complex isolates were shown to have a high level of resistance against chlorine when grown in water (*11*). Corrosion, certain material characteristics, and dead-end spaces can favor biofilm formation and mycobacterial growth. Killing of NTM with heat may be promising; older studies reported a high efficacy with temperature exposure at 70°C (*10*,*12*). A recent report indicated complete suppression of *M. chimaera* in HCUs by intensified maintenance after a complex decontamination regimen, including replacement of plastic tubing; however, follow-up was limited to 3 months (*13*). Prolonged testing seems necessary for excluding presence of *M. chimaera* or other NTM.

Our report has limitations. First, some study HCUs were temporarily maintained according to the manufacturer's standard before the hospital adopted the intensified

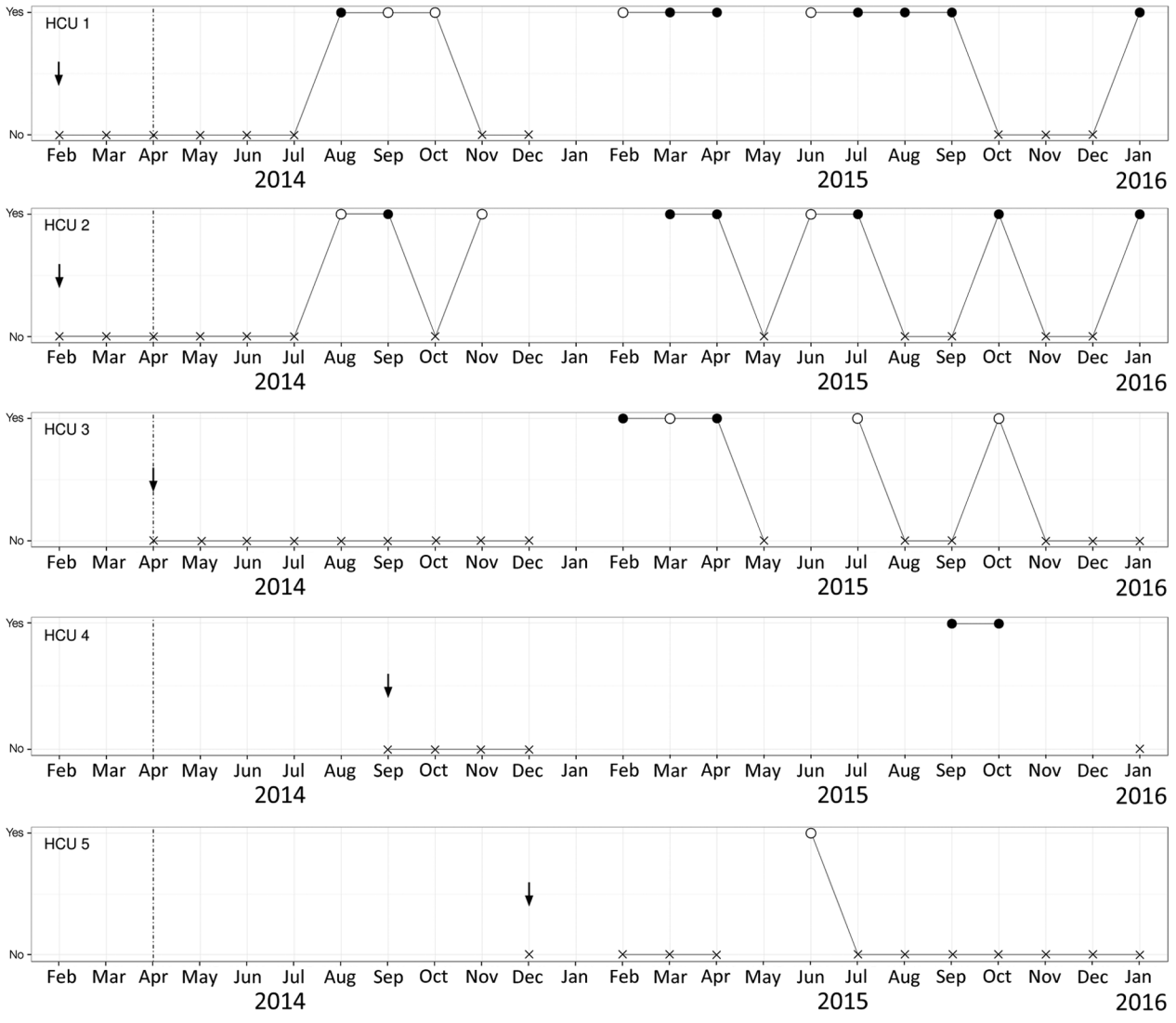

Figure 2. Results of heater–cooler unit (HCU) water surveillance cultures by year and month, University Hospital Zurich, Zurich, Switzerland. The dashed vertical line shows the date of implementation of the intensified protocol (i.e., mid-April 2014). The vertical arrows indicate the start of use of each factory-new HCU in the operating room. HCU 3, HCU 4, and HCU 5 were serviced with the intensified in-house maintenance from the time of delivery. HCU 4 was sent for repair at the manufacturer during December 2014–September 2015. Results of mycobacterial cultures are shown for each HCU. No indicates a negative culture for nontuberculous mycobacteria (NTM), indicated by X. Yes indicates a positive culture for NTM. Filled circles indicate *Mycobacterium chimaera*; empty circles indicate NTM other than *M. chimaera*. As per date indicated, cultures were reported as positive if ≥1 water sample (1 sample from cardioplegia and 1 sample from patient circuit were gathered at each date) grew NTM. For missing data points (i.e., no negative or positive results shown), mycobacterial cultures could not be tested because of bacterial overgrowth or lack of availability of the HCU.

protocol. Second, we did not include a control HCU with ongoing maintenance according to the manufacturer's recommendations. Third, the detection threshold of *M. chimaera* in water and air cultures remains to be identified. More sensitive culture methods might have produced different results.

Our findings challenge the effectiveness of the HCU manufacturer's maintenance recommendations, which were recently changed to disinfection with sodium hypochlorite before first use and every 14 days and water changes with all-bacteria–filtered tap water plus 150 mL 3% hydrogen peroxide every 7 days (*14*). To ensure patient safety until safe HCU technology is available, strict separation of the operating room and HCU air volumes is necessary. This separation can be achieved in several ways. One approach is placing the HCU outside the operating room. Nevertheless,

Table. Microbiology test results for heater–cooler unit water samples from University Hospital Zurich, Zurich, Switzerland*

Type of circuit	No. samples	Microbiology results, no. (%) samples		
		Any NTM growth	*Mycobacterium chimaera*	NTM other than *M. chimaera*
Cardioplegia†	48	14 (29.2)	10 (20.8)‡	5 (10.4)‡
Patient†	49	19 (38.8)	12 (24.5)‡	8 (16.3)‡
Circuit not specified	37§	5 (13.5)	2 (5.4)	3 (8.1)

*NTM, nontuberculous mycobacteria.
†No statistically significant different results for water samples from cardioplegia and patient circuit (Fisher exact test, p = 0.554).
‡One culture had growth of both *M. chimaera* and *M. gordonae*.
§Six (16.2%) samples had bacterial overgrowth.

with this measure, the airflow must be restrained from diffusion back into the OR (*15*). Because the maximum allowed length of water circuit tubing and the architectural layout prohibited this solution at our hospital, we produced airtight housings for the HCUs; however, this solution has less flexible placement of the HCUs within the OR. We continue both the in-house maintenance protocol and regular microbiologic surveillance.

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Dr. Schreiber is an internal medicine specialist working as an infectious disease fellow in the Division of Infectious Diseases and Hospital Epidemiology, University Hospital Zurich, Switzerland. His research interests are infections of immunocompromised hosts and infection prevention.

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Address for correspondence: Hugo Sax, Division of Infectious Diseases and Hospital Epidemiology, University Hospital and University of Zurich, Rämistrasse 100, CH-8091 Zurich, Switzerland; email: hugo.sax@usz.ch

Synovial Tissue Infection with *Burkholderia fungorum*

Shih Keng Loong, Yih Harng Soh,[1] Nur Hidayana Mahfodz, Jefree Johari, Sazaly AbuBakar

Author affiliations: University of Malaya, Kuala Lumpur, Malaysia (S.K. Loong, N.H. Mahfodz, J. Johari, S. AbuBakar); University Malaya Medical Centre, Kuala Lumpur (Y.H. Soh)

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To the Editor: The genus *Burkholderia*, first proposed in 1992, has now expanded to consist of many species (*1*). Bacteria of this genus exhibit extensive ubiquity; they have been isolated from human, animal, and environmental sources (*1*,*2*). Although rare, *Burkholderia fungorum* has been implicated in several human infections (*2*–*4*). We isolated *B. fungorum* from the synovial tissue of a patient's knee.

In February 2011, a 26-year-old man had a meniscal tear on the left knee but deferred surgery. Two years later, the same knee was injured after a fall. On November 1, 2013, the patient underwent anterior cruciate ligament reconstruction. On November 28, 2013, he underwent arthroscopy because of decreased range of motion in the knee. The patient did not have a fever, and leukocyte count and erythrocyte sedimentation rate were within reference range. The patient was obese but did not have diabetes, hypertension, or any autoimmune disease.

Arthroscopy indicated that the anterior cruciate ligament graft was intact. However, the synovial fluid was turbid, and synovial tissue infection was suspected. A synovial tissue biopsy was sent to the microbiology laboratory for culture. Test results for HIV, hepatitis B, and hepatitis C infection were negative. Fungal culture was also negative. After the tissue biopsy sample was incubated on blood agar for 48 h at an ambient temperature of 37°C, 2 different colonies grew; both showed big, yellow colonies of gram-negative rods. Vitek 2 (bioMérieux, Marcy l'Etoile, France) identified the isolates as *Pseudomonas fluorescens* (91% probability) and *Brucella melitensis* (94% probability). Because of the possible *B. melitensis* infection, all excess specimens and agar plates were sealed and discarded after being autoclaved. Later DNA amplification and sequencing of the bacterial 16S rDNA identified the isolates previously identified as *P. fluorescens* and *B. melitensis* as *Mesorhizobium amorphae* and *Burkholderia fungorum* (isolate BF370), respectively. The 16S rDNA sequences of BF370 (GenBank accession no. LN868266) displayed 99% similarity to *B. fungorum* strain DBT1 (GenBank accession no. HM113360).

[1]Current affiliation: Hospital Kuala Lipis, Pahang, Malaysia.

Disk-diffusion tests performed according to the Clinical and Laboratory Standards Institute interpretive standards for *Burkholderia cepacia* (*5*) revealed that BF370 was sensitive to ceftazidime, meropenem, and trimethoprim/sulfamethoxazole. Additional characterization of BF370 with multilocus sequence typing (*6*) revealed a novel sequence type, 868. Novel alleles were found for 7 loci: *atpD* (allele 347), *gltB* (allele 409), *gyrB* (allele 609), *recA* (allele 367), *lepA* (allele 418), *phaC* (allele 319), and *trpB* (allele 406). Intravenous ampicillin/sulbactam (1.5 g, 3×/d) was administered to the patient from November 29, 2013, through December 13, 2013. No pus was observed at the wound, and the patient was afebrile. He did not report discomfort, and physiotherapy commenced on day 8 after surgery.

Initial identification of the isolate as *B. melitensis* raised concern for possible laboratory transmission of this pathogen (*7*). The possibility of finding brucellae in the synovial tissue was not unexpected because *Brucella* spp. have been reported to cause joint infections (*8*). However, 16S rDNA sequencing confirmed the identity of the isolate as *B. fungorum*. This different finding suggests that commercial phenotypic identification system could sometimes be unreliable. The isolation of *B. fungorum* from the synovial tissue of this patient, however, is similar to an earlier case of leg tissue infection in a girl (*3*). A striking similarity shared between the isolates was their antimicrobial drug susceptibility profiles; each isolate was susceptible to ceftazidime, meropenem, and trimethoprim/sulfamethoxazole. A literature search revealed that *B. fungorum* had first been recovered from vaginal secretions and cerebrospinal fluid of 2 women (*2*). However, no clinical information was presented, leading to the absence of clinical descriptions of the infections.

Recently, *B. fungorum* was detected in a granuloma specimen from a 26-year-old patient (*4*). The bacteria were suspected to have been transmitted to the patient through a rose thorn puncture. Swelling did not appear until 3 years after the initial puncture, and the patient did not report pain or fever and was otherwise healthy (*4*). Although the clinical implication of *B. fungorum* infection remained unclear, recent cases (*3,4*) seem to suggest that the bacteria can induce fever in young children and has a lengthy incubation period before erupting.

We suspect that the patient reported here acquired *B. fungorum* during his initial injury, perhaps from the environment. The concurrent isolation of *M. amorphae*, a plant bacterium (*9*), is consistent with possible environmental origin of the infection. Our observations and those reported earlier (*4*) suggest the ability of *B. fungorum* to act as a slowly replicating opportunistic pathogen associated with tissue injuries. The slow replication rate of *B. fungorum* presumably mimics the growth characteristic

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of *Burkholderia mallei*, essential for the bacterium's survival, proliferation, and evasion of host adaptive immune responses (*10*). Our findings suggest that an approach combining culture, 16S rDNA sequencing, and multilocus sequence typing be considered for the accurate identification of uncommon bacterial infection.

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Address for correspondence: Sazaly AbuBakar, Tropical Infectious Diseases Research & Education Centre, Block N & O, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia; email: sazaly@um.edu.my

Naegleria fowleri Meningoencephalitis Associated with Public Water Supply, Pakistan, 2014

Najia K. Ghanchi, Erum Khan, Azam Khan, Wali Muhammad, Faisal Riaz Malik, Afia Zafar

Author affiliations: Aga Khan University, Karachi, Pakistan (N.K. Ghanchi, E. Khan, F.R. Malik, A. Zafar); Karachi Water and Sewerage Board, Karachi (A. Khan); World Health Organization, Karachi (W. Muhammad)

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To the Editor: *Naegleria fowleri*, a free-living ameba, causes acute, fulminant, fatal primary amebic meningoencephalitis (PAM) in persons with history of recreational activities in warm freshwater (*1,2*). During 2008–2009, thirteen case-patients with PAM and no history of recreational water activity were reported from Karachi, Pakistan (*3*). Since then, PAM caused by domestic water exposure, nasal cleansing by using neti pots, and ablution has been reported globally (*4–6*). During 2014–2015, the Aga Khan University Hospital clinical laboratory in Karachi confirmed 19 PAM case-patients without history of recreational activities in warm freshwater.

Karachi has a subtropical, arid climate and long summers (March–October). The increasing number of PAM cases might be attributable to rising environmental temperatures and a dysfunctional water supply system in Karachi (*7*). Data indicating direct evidence of *N. fowleri* amebae in Karachi's water supply are limited, but consistent annual reemergence of PAM in patients without history of recreational water exposure raises concerns about Karachi's water supply.

In August 2014, a previously healthy 34-year-old man living in Karachi and having no recreational water exposure was admitted to the Aga Khan University Hospital with multiple episodes of vomiting, severe headache, and fever. Cerebrospinal fluid culture showed a low glucose level (46 mg/dL [reference 45–80 mg/dL]) and high levels of protein (216 mg/dL [reference 20–40 mg/dL]), erythrocytes (30 cells/mm^3 [reference 0–10 cells/mm^3]), and leukocytes (1,440 cells/mm^3 [reference 0–5 cells/mm^3]; 65% lymphocytes and 35% neutrophils). PCR confirmed presence of *N. fowleri*. The patient died 4 days after admission, and cerebrospinal fluid and blood cultures were negative for bacterial and fungal growth.

We investigated for presence of N. fowleri amebae in domestic water and for the patient's possible exposure. In September 2014, we collected 23 samples from 2 water treatment plants (plants A and B), their pumping

stations, and catchment areas (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/10/15-1236-Techapp1.pdf). Plant A supplied 13 of the samples from its water distribution system, which provided water to the patient's residence and neighborhood mosque (Table). The other 10 samples were from Plant B and its water distribution system (online Technical Appendix Table), for which the local government had initiated additional chlorine enhancement because of previously occurring PAM cases. Plant A had no chlorine enhancement. Real-time PCR, as described (8), confirmed presence of *N. fowleri* amebae in the plant's water supply distribution. Both plants are monitored for quality control by using World Health Organization water treatment procedures guidelines (http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf). The plants' water distribution exit points had the highest residual chlorine levels (0.5 ppm) (9), and levels gradually decreased beyond the plants. Residual chlorine was undetectable in plant A's water distribution to the patient's residential area (12 km from the plant); however, with plant B's additional chlorine enhancement stations, chlorine levels were detectable in all households tested.

Residual chlorine and specimen positivity for free-living amebae were inversely correlated. Differences were most noticeable in samples collected from plant A's distributed water, compared with plant B's water distribution samples. PCR confirmed *N. fowleri* amebae in 2 water samples collected from the patient's household overhead storage tanks and neighborhood mosque. The samples, taken from plant A's distributed water, showed no residual chlorine and a temperature >30°C. Lack of detectable chlorine and water temperature >25ºC might have provided favorable conditions for *N. fowleri* amebae to thrive in domestic water (*5*); water temperatures 25°–40°C are favorable for *N. fowleri* growth. Absence of

Table. Characteristics of 13 water samples collected from water treatment plant A and its distribution system, including water supplied to the apartment and neighborhood mosque of a patient with primary amebic meningitis, Karachi, Pakistan, 2014*

Water supply	Sample location	Sample type	Total chlorine, mg/L	Temperature, °C†	Culture positivity for FLAs	PCR results for *Naegleria fowleri* amebae‡	Distance relationships of water samples
Reservoir§	Water from Kinjhar	Untreated	ND	30	++	–	From reservoir to plant A, ≈100 km
Water treatment plant A	Filtration unit¶	Treatment underway	<0.5	29.5	++	–	
	Plant A exit point	Filtered and chlorinated	0.5	30	++	–	
Pumping station#	Pumping station, site 1	Filtered and chlorinated	ND	30	++	–	From plant A to pumping station, ≈11 km
	Pumping station, site 2	Filtered and chlorinated	ND	30	++	–	
Catchment areas: patient's apartment and neighborhood	Mainline	Filtered and chlorinated	ND	30	–	–	From pumping station to patient's house, 1km
	Underground boring well water**	Untreated	ND	28.5	+	–	Within patient's house, ≈10 m
	Underground tank	Mixed††	ND	28	+	–	Within patient's house, ≈10 m
	Overhead tank	Mixed	ND	31	++	+	Within patient's house, ≈10 m
	In-house storage tank‡‡	Mixed	ND	30	++	–	Within patient's house, ≈5 m
	Bathroom	Mixed	ND	29	+	–	Within patient's house, ≈5 m
	Neighborhood mosque	Filtered and chlorinated	ND	31	++	+	From patient's house to mosque, ≈100 m

*FLAs, free-living amebae; ND, not detected; ++, >3 amebae seen with 40x magnification; +, 1–3 amebae seen with 40 x magnification; –, no amebae detected.
†Water temperatures of 25°C–40°C are conductive for flourishing of *Naegleria fowleri* amebae.
‡PCR was negative for other pathogenic FLAs such as *Balamuthia* or *Acanthamoeba* spp.
§Kinjhar Lake, located in Sindh province, Pakistan, is the main reservoir that supplies water to Karachi.
¶The filtration unit was the only site for which 2 samples were taken; only 1 sample was taken from all other specific sample locations.
#Samples were taken from a single pumping station at 2 separate sites.
**Underground wells provide additional water supplies locally known as boring wells.
††Water from these wells is not treated and is mixed with treated water from the main water supply in storage tanks.
‡‡Underground and overhead tanks are shared by >100 households, so for continuous water supply, residents keep small tanks in their homes.

the amebae in plant B's water suggests the importance of enhanced chlorine pumping at distribution points beyond water treatment plants for maintain residual chlorine in Karachi's domestic water supply.

Because water supply can be intermittent, underground and overhead storage tanks are essential for Karachi homes. To ensure continuous domestic supply, water is stored in overhead tanks and pumped from tanks into homes as needed. Water storage in tanks perhaps facilitated propagation of *N. fowleri* amebae in domestic and mosque water. During the summer, ambient temperatures reach 44°C, leading to increased water temperatures in overhead tanks. We found water temperatures up to 34°C, which may facilitate excystation of *N. fowleri* amebae to infective forms. Slime, dirt, and high ambient temperatures likely explain *N. fowleri* multiplication in storage tanks, the possible source of infection for this patient in Karachi.

Presence of *N. fowleri* amebae in mosque water is alarming. Ablution (Wudhu) is a ritual performed by Muslims before offering prayers and involves thorough cleaning of mouth, ears, face, arms, feet, and nasal passages, the latter by inhaling water forcefully up the nostrils. Performing this activity with contaminated water could be a communal source for potential outbreaks.

Karachi water supply authorities have initiated chlorine enhancement at various sites beyond plant B, and our findings support the need for this enhancement. We recommend that the government implement measures to maintain appropriate chlorine levels in the domestic water supply and at recreational sites and to develop effective amebae-monitoring programs. The public should use boiled or filtered water for nasal cleansing, regularly clean storage tanks, and add supplemental chlorine to water in homes, especially during the summer.

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Address for correspondence: Afia Zafar, Section of Microbiology, Department of Pathology and Laboratory Medicine, Aga Khan University, Stadium Rd, PO Box 3500, Karachi 74800, Pakistan; email: afia.zafar@aku.edu

Unmet Needs for a Rapid Diagnosis of Chikungunya Virus Infection

Elisa Burdino, Guido Calleri, Pietro Caramello, Valeria Ghisetti

Author affiliation: Amedeo di Savoia Hospital, Torino, Italy

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To the Editor: Chikungunya virus (CHIKV) has become a global health problem. Clinical manifestations are not specific and are difficult to differentiate from those of similar viral diseases (e.g., dengue and Zika virus disease). Diagnostic laboratories must be prepared to meet the changing epidemiology of viral diseases. CHIKV infection is currently identified by viral genome detection, using reverse transcription PCR (RT-PCR), viral culture, and serologic testing for IgG and IgM by indirect immunofluorescence (IFA) or ELISA. RT-PCR is most sensitive during the early phase of CHIKV infection (within 5–7 days of symptom onset), but its use is limited by the short viremic phase of the disease. After the acute phase, serologic testing for IgG and IgM is a more accurate indicator of disease.

Molecular and serologic tests are complementary, reliable, and sensitive methods, but they require special equipment and a medium-to-high level of technical skill that may not be available in many laboratories, especially those in rural areas, where outbreaks usually occur.

Accurate and rapid detection of CHIKV infection by reliable point-of-care (POC) assays has been recommended to facilitate outbreak control. To meet this need, rapid CHIKV IgM POC tests are now available, but little information exists regarding their performance. The sensitivity of these tests evaluated in settings with a high prevalence of CHIKV infection is poor (range 1.9%–50.8%) compared with that for reference assays, especially in the acute phase of disease (*1*–*5*). In low-prevalence settings, CHIKV infection generally occurs as imported cases in travelers returning from disease-endemic countries. Diagnosis of such cases requires discrimination between CHIKV, dengue, Zika, and other febrile diseases in the differential diagnosis; this discrimination could be facilitated by the use of a reliable POC assay. The recent Zika virus disease outbreak in South America also highlights the worldwide need for rapid reliable POC tests.

From June 2014 through November 2015, eight patients who had returned to Italy from the Caribbean and Latin America were referred to the regional Center for Infectious Diseases, Amedeo di Savoia Hospital, in Turin for travel-associated CHIKV infection. These cases were the first in the region after 3 years without imported cases. We used IFA (Euroimmun AG, Lubek, Germany) and real-time RT-PCR (TIB MOLBIOL GmbH, Berlin, Germany) for CHIKV diagnosis. In addition, we evaluated the OnSite Chikungunya IgM Combo Rapid Test CE (CTK Biotech, San Diego, CA, USA) for CHIKV infection.

The rapid test identified IgM in only 3 of 8 patients (sensitivity 37.5%). All patients were negative for viral RNA, probably due to the time elapsed between symptom onset and serum sample collection, as confirmed by the presence of CHIKV IgG in most patients. No false-positive or invalid results were recorded with the rapid test on 30 CHIKV-negative serum samples (specificity 100%; positive and negative predictive values 37.5% and 100%, respectively).

Rapid and appropriate diagnostic tools are needed to slow or stop the worldwide spread of CHIKV. Rapid POC tests are highly cost-effective because they are easy to perform and can be disseminated to many laboratories for differentiating between diseases that are similar. Moreover, their results can easily be evaluated and shared within networks of reference laboratories.

However, our findings, in agreement with those of others, show that current rapid CHIKV tests perform poorly and need major improvement (Table) (*1*–*5*). This poor performance might have several explanations. For example, CHIKV patients do not often seek medical care in the early course of the disease. Most patients in our study were no longer in the acute phase of illness: the diagnosis was made a mean of 16.8 (range 7–30) days after fever onset, and when tested, all patients were viral RNA–negative by real-time RT-PCR. POC reactivity generally increases in patients with illness duration of >1 week (*1*–*5*), but this

Table. Reported sensitivity and specificity of rapid point-of-care tests for detecting chikungunya virus, 2008–2015*

Reference and test(s)	Time from symptom onset to testing, d	Sensitivity, %‡	Specificity, %‡	Test reference standard
(*1*)				
OnSite Chikungunya IgM Rapid Test	1 to >21	20.5	100	Capture ELISA IgM (in house) with Asian lineage virus; rRT-PCR
SD BIOLINE Chikungunya IgM test	1 to >21	50.8	89.2	Capture ELISA IgM (in house) with Asian lineage virus; rRT-PCR
(*2*)				
SD BIOLINE Chikungunya IgM test	<7; 8 to >14§	22; 83	88; 71	ELISA IgM; rRT-PCR
(*3*)				
OnSite Chikungunya IgM Rapid Test	3.75 to >7	12.1	100	IgM IFA; capture ELISA IgM (in house); rRT-PCR
(*4*)				
SD BIOLINE Chikungunya IgM test	3–8	1.9–3.9	92.5–95.0	Capture ELISA IgM; rRT-PCR
(*5*)				
OnSite Chikungunya IgM Combo Rapid Test CE	NA	20	93	Capture ELISA IgM/IgG (in house); plaque reduction neutralization test
SD BIOLINE Chikungunya IgM test	NA	30	73	Capture ELISA IgM/IgG (in house); plaque reduction neutralization test
This study				
OnSite Chikungunya IgM Combo Rapid Test CE	7 to 30	37.5	100	IFA IgM/IgG (commercial); rRT-PCR

*IFA, indirect immunofluorescence assay; NA, not applicable; rRT-PCR, real-time reverse transcription PCR.
†Manufacturers: CTK Biotech, San Diego, CA, USA (OnSite Chikungunya IgM Combo Rapid Test CE and OnSite Chikungunya IgM Rapid Test); Standard Diagnostics, Inc., Seoul, South Korea (SD BIOLINE Chikungunya IgM test).
‡Values are those reported in the original publications.
§Testing was done at 2 different time points after symptom onset.

was not the case in our study. Genetic differences in circulating CHIKV lineages could also explain poor testing performance. Furthermore, the OnSite Chikungunya IgM Combo CE POC test uses a recombinant antigen covering the 226 residues of the E1 gene from CHIKV variant A226; recent studies on CHIKV protein characterization showed that more sensitive serologic assays can be obtained using specific early-phase E2 glycoprotein as antigens (*3*).

The successful use of rapid immunochromatography-based assays with monoclonal antibodies to detect viral diseases (e.g., dengue) has encouraged the development of rapid immunoassays for CHIKV antigens, and preliminary results for these assays seem promising (*6*). External quality assessment programs for POC tests and quality controls consisting of standardized positive serum could also be helpful for improving the performance of diagnostic tests.

In conclusion, returning travelers are sentinels of the rapidly changing epidemiology of CHIKV; thus, they require a prompt diagnosis and careful surveillance for their possible role in subsequent autochthonous disease transmission. Implementation of user-friendly, rapid, and easily deliverable POC tests for a prompt and accurate laboratory diagnosis is therefore needed to improve patient management and disease control measures.

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Address for correspondence: Elisa Burdino, Laboratory of Microbiology and Virology, Amedeo di Savoia Hospital, Corso Svizzera 164, I-10149 Torino, Italy: email: elisa.burdino@libero.it

Novel Single-Stranded DNA Circular Viruses in Pericardial Fluid of Patient with Recurrent Pericarditis

Sébastien Halary, Raja Duraisamy, Laura Fancello, Sonia Monteil-Bouchard, Priscilla Jardot, Philippe Biagini, Frédérique Gouriet, Didier Raoult, Christelle Desnues

Author affiliations: Institut Hospitalo-Universitaire Méditerranée-Infection, Marseille, France (S. Halary, R. Duraisamy, L. Fancello, S. Monteil-Bouchard, P. Jardot, P. Biagini, F. Gouriet, D. Raoult, C. Desnues); Aix-Marseille Université, Marseille (S. Halary, R. Duraisamy, L. Fancello, S. Monteil-Bouchard, P. Jardot, P. Biagini, F. Gouriet, D. Raoult, C. Desnues); Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Marseille (S. Halary, R. Duraisamy, L. Fancello, S. Monteil-Bouchard, P. Jardot, F. Gouriet, D. Raoult, C. Desnues); KU Leuven, Leuven, Belgium (L. Fancello); Unité Mixte de Recherche, Marseille (P. Biagini); Etablissement Français du Sang, Marseille (P. Biagini); Centre National de la Recherche Scientifique, Marseille (P. Biagini, C. Desnues)

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To the Editor: Circular replication initiation protein (Rep)–encoding single-stranded DNA (ssDNA) (CRESS-DNA) genomes are found in diverse group II virus families, which all possess a conserved Rep-encoding gene and a nonenveloped icosahedral capsid, except geminiviruses, which have twinned particles (*1*). Gemycircularvirus (GcV) were initially discovered in fungi, but a growing number of new species has been characterized by metagenomics in air, sewage, insects, and feces from a broad range of vertebrates (*1*–*5*). GcVs have also been found in the brain and serum of humans with multiple sclerosis; in the cerebrospinal fluid of a patient with encephalitis; and in several blood samples, including those from an HIV-positive blood donor (*6*–*8*). We report the presence of 2 divergent GcVs and a novel CRESS-DNA virus (CV) in 2 pericardial fluid samples from a patient with idiopathic recurrent pericarditis.

The patient, a 14-year-old girl who had thoracic scoliosis surgery in 2007, was admitted to the hospital in 2009 for pleuropneumonia and pericarditis, which required pericardial drainage twice within 3 weeks (samples PF_1 and PF_2, respectively). She had thrombocytopenia, a leukocyte count within the normal range, and a high C-reactive protein level. Biochemical and cytologic testing, bacterial cultures, and PCR of pericardial fluid samples for cytomegalovirus, varicella zoster and herpes simplex viruses, parvovirus B19, fungal 18S rRNA, bacterial 16S rRNA, and *Mycobacterium tuberculosis* were negative. Upon approval from the Institut Fédératif de Recherche IFR48 Ethics Committee (Marseille, France) and written informed consent from the patient's parents, we submitted the drainage samples for further investigation.

Virus particles in 0.45-μm filtrates were purified and analyzed by metagenomics as described (*9*); resulting contigs were aligned against the National Center for Biotechnology Information nonredundant protein database using blastx (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Three contigs were of viral origin (viral first hit, E-value ≤1E-03), all belonging to the ssDNA circular viruses. We obtained complete genomic sequences by PCR with ad hoc primer pairs and Sanger sequencing technology (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/10/16-0052-Techapp1.pdf).

We annotated genomes using GeneMark (heuristic parameters; http://exon.gatech.edu/GeneMark/) and EMBOSS palindrome (http://emboss.bioinformatics.nl/cgi-bin/emboss/palindrome). Analysis of PF1 enabled characterization of HV-GcV1 (GenBank accession no. KU343136) (Figure). This genome displayed a typical GcV architecture, with a 2,264-nt circular DNA molecule carrying a capsid gene on 1 strand and 2 genes on the opposite strand, which coded for Rep1 (involved in replication initiation) and Rep2 (involved in replication termination), respectively. A putative hairpin structure showed a nanonucleotide motif, which was thought to be the Rep target, TAATGTTAT. A fourth gene with no homologs in databases was predicted upstream of the capsid gene. Phylogenetic inference from concatenated Rep placed this virus close to another GcV (found in sewage) in a clade comprising 2 other human-associated GcVs (online Technical Appendix Figure 1).

PF_2 contained 2 other viruses: HV-GcV2 (GenBank accession no. KU343137), another GcV, and HV-CV1 (GenBank accession no. KU343138), a novel CRESS-DNA virus. HV-GcV2 (2,262 nt) shares the same stem-loop motif and genomic structure with HV-GcV1, with the exception of the *rep* gene, which is coded by a single open reading frame. HV-GcV2 proteins share low sequence similarity with HV-GcV1 proteins (33% for capsid and 46% for Rep, as determined by blastp [https://blast.ncbi.nlm.nih.gov/Blast.cgi]). HV-GcV2 belongs to another clade of the phylogenetic tree that also contains sewage- and bird feces–associated viruses (online Technical Appendix Figure 1). HV-CV1 (2,951 nt) possesses characteristics of CRESS-DNA genomes. Phylogenetic analysis of REP sequences showed

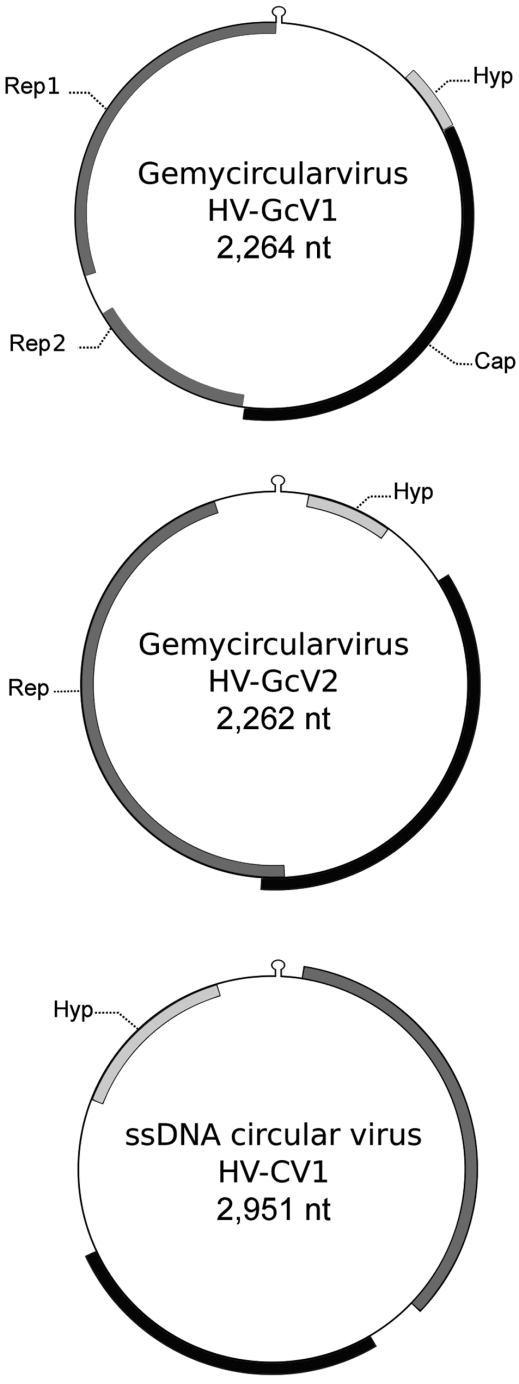

Figure. Genomic features of gemycircularviruses HV-GcV1 and HV-GcV2 and of a novel circular single-stranded DNA (ssDNA) virus, HV-CV1, including hairpin structure and predicted open reading frames. Cap, capsid; Hyp, hypothetical protein with unknown function; Rep, replication initiation protein.

that HV-CV1 and its closest homologue, an ssDNA circular virus of unknown taxon discovered in an Antarctic shelf pond, are distantly related to other CRESS-DNA viruses (online Technical Appendix Figure 2). HV-GcV2 and HV-CV1 displayed no capsid protein similarity between them or with any other virus, as determined by blastp. Annotation of the HV-CV1 capsid gene required use of HHBlits (https://toolkit.tuebingen.mpg.de/hhblits), a more sensitive algorithm (E-value 1.2E-06, probability of 97.2%).

PCR confirmed the absence of HV-GcV1 in PF_2 and HV-GcV2 and HV-CV1 in PF_1, suggesting multiple infections before each pericarditis event or a rapid fluctuation in the load of all 3 persisting viruses. An additional blastx search on 53 other virus metagenomes sequenced from pericardial fluids after pericarditis events failed to retrieve these sequences. To exclude the possibility of sample contamination during procedures, we simultaneously treated a sample with the same reagents and kits used for PF_1 and PF_2 and surveyed it by PCR; results were negative. All metagenomes are publically available in the METAVIR (http://metavir-meb.univ-bpclermont.fr) directory under the pericardial fluids heading.

No relationship between these viruses and pericarditis was established. However, the fact that some CRESS-DNA viruses are animal pathogens (*10*) and the growing number of GcVs found in human samples in pathologic contexts (*6*,*7*) indicate that the viral genomes described here might replicate in human cells, possibly as opportunistic pathogens (*8*). On the other hand, although diagnostic tests ruled out fungal or bacterial infections, we should still consider the possibility that these viruses infect other uncharacterized organisms. The genomes described here will assist further studies of the prevalence of these viruses in human populations.

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Address for correspondence: Christelle Desnues, URMITE, UM63, CNRS 7278, IRD 198, INSERM 1095, Aix-Marseille Université, Marseille, France; email: christelle.desnues@univ-amu.fr

Reemergence of Japanese Encephalitis in South Korea, 2010–2015

Jun-Sang Sunwoo,[1] Keun-Hwa Jung, Soon-Tae Lee, Sang Kun Lee, Kon Chu

Author affiliation: Seoul National University Hospital, Seoul, South Korea

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To the Editor: Japanese encephalitis (JE) is caused by a virus transmitted by *Culex tritaeniorhynchus* mosquitoes. JE was the major public health concern in South Korea until the late 1960s, with several thousand cases reported annually. The national vaccination program with the inactivated mouse brain–derived Nakayama strain was initiated in 1983 and targeted children <15 years of age. During 1983–2000, annual booster vaccinations were given to children <15 years of age, but in 2000, the booster schedule was changed to 2 doses (1 dose each) for children 6 and 12 years of age. The live attenuated JE vaccine SA

[1]Current affiliation: Soonchunhyang University Seoul Hospital, Seoul, South Korea.

14-14-2 was introduced in 2002 and included in the national immunization program in 2014. After introduction of the mandatory immunization program, JE was nearly eliminated; during most of the past 3 decades, <0.02 cases per 100,000 population have been reported annually (*1*). However, since 2010, JE has reemerged in South Korea. We describe epidemiologic data for JE, focusing on the recent increase in number of cases in South Korea. We accessed demographic information from the disease web statistics system provided by the Korea Centers for Disease Control and Prevention (*2*). Our study was exempted from review by the Institutional Review Board at Seoul National University Hospital (E-1602-053-739).

During 2010–2015, South Korea reported 129 JE cases (*2*). JE was diagnosed on the basis of clinical signs and symptoms and laboratory examination that showed either the presence of JE virus (JEV)–specific IgM in serum or cerebrospinal fluid samples or identification of a ≥4-fold increase in neutralizing antibody titers between the acute and convalescent stages. Laboratory procedures were conducted by the Korea Centers for Disease Control and Prevention, as described (*3*). Clinical features suggesting JEV infection were acute encephalitis syndrome (defined as altered consciousness with fever or seizures) and focal neurologic deficits. Reports excluded clinically suspected but serologically unconfirmed cases. Among the 129 confirmed cases, only 1 (0.78%) case-patient had documented evidence of JEV vaccination. Domestic or international travel history was evident in 18 (14.0%) case-patients; 16 (12.4%) were found to live in proximity to a pigsty; and 8 (6.2%) were foreign-born residents.

Annual incidences of JE have increased markedly since 2010, except for 2011, when only 3 cases were reported (Figure, panel A). Incidence was highest during 2015, when 40 cases were reported. A total of 19 patients died during 2010–2014 (overall case-fatality rate 21.3%), whereas during the previous 25 years (1985–2009), only 5 deaths were attributable to JE (*4*).

Median age of the 129 patients with JE was 53 years (interquartile range 46.5–62.0). Most (73 [56.6%]) patients were male; 56 (43.4%) were female. On average, affected female patients were older than male patients (mean 56.6 ± 16.3 years vs. 51.4 ± 12.8 years; p = 0.017 by Mann-Whitney U test). When patients were stratified by age, those 50–59 years of age (37.2%) were the most affected group, followed by those 40–49 years of age (24%). Patients <19 years of age accounted for only 3.1% of cases (Figure, panel B). Analysis of the monthly incidence of JE revealed a distinctive summer peak; 109 (84.5%) cases occurred during August–October, suggesting a temporal association with activity of mosquito vectors (*5*). Analysis of geographic distribution showed that 58 (45%) cases originated in Seoul, the capital of South Korea, or in Gyeonggi Province, the area surrounding Seoul (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/10/16-0288-Techapp.pdf).

Our findings indicated that reemerging JE predominantly affects unvaccinated adults >40 years of age. Shifts in age distribution toward older groups after initiation of vaccination programs were also evident in Japan and Taiwan (*6*,*7*). Many researchers believe that prolonged periods with near elimination of JE over the past 3 decades and an unvaccinated adult population have contributed to older adults' high vulnerability to JE. However, recent JEV seroprevalence data showed that 98.1% of persons in high-risk age groups had neutralizing antibodies, with no differences appearing among age groups (*8*). Although those findings conflict with the assumption that lack of vaccination among older adults contributes to vulnerability to infection, high seroprevalences could be explained by natural infection resulting from the large epidemics of the 1950s and 1960s.

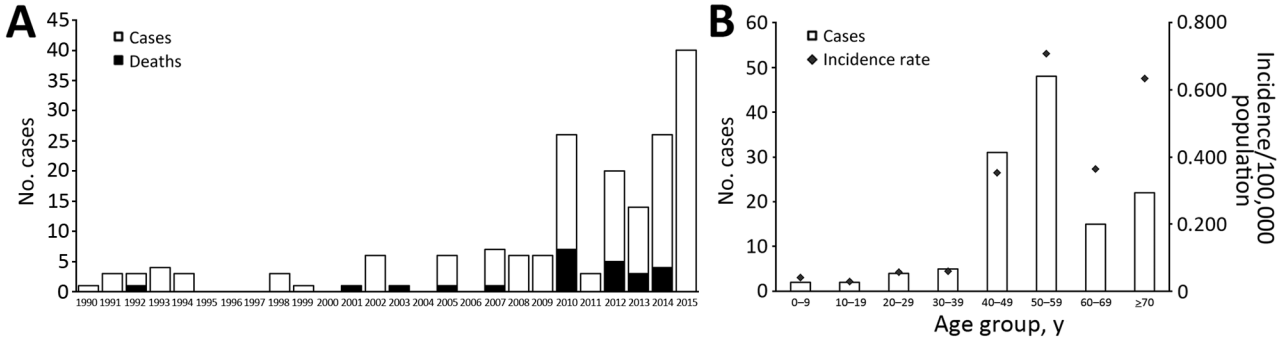

Figure. Reemergence of Japanese encephalitis in South Korea. A) Number of cases and deaths caused by Japanese encephalitis by year, 1990–2015. Number of deaths for 2015 is not shown because final data were unavailable. B) Number of cases and incidence of Japanese encephalitis by age group, 2010–2015. Population data for the denominator originated from the future population projection data of the Korea Statistical Information Service through 1992 and from the mid-year population data from the resident registration system as of 1993. All disease data shown in the figure were provided by the Korea Centers for Disease Control and Prevention, Infectious Disease Statistics System (http://is.cdc.go.kr/dstat/index.jsp).

Our study has several limitations. For example, information on clinical features and outcomes of patients, except for death, was unavailable, and we could not determine prognostic factors for recent JE cases. Because details of each patient's travel history was not identified, we could not clearly understand the mechanism of JEV transmission. In addition, we do not explore the possible cause of JE reemergence. Moreover, although JE incidence was detected by the national surveillance system, incidence might be underestimated because the database identified only serologically confirmed cases.

JE vaccination is presumed to have failed to induce lifelong immunity so that older age groups become susceptible again. Further research is warranted to determine the long-term protection against JEV after primary vaccination. Moreover, future studies should address the need for booster vaccination for adults to maintain immunity against JEV.

Acknowledgments

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Address for correspondence: Kon Chu, Department of Neurology, Seoul National University Hospital, 101 Daehak-ro, Jongno-gu, Seoul, 03080, South Korea; email: stemcell.snu@gmail.com

Recombinant Enterovirus A71 Subgenogroup C1 Strains, Germany, 2015

Sindy Böttcher, Patrick E. Obermeier, Katrin Neubauer, Sabine Diedrich; the Laboratory Network for Enterovirus Diagnostics[1]

Author affiliation: Robert Koch Institute, Berlin, Germany

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To the Editor: Enterovirus A71 (EV-A71) strains circulate worldwide, and numerous outbreaks have been reported from Asia, Australia, Europe, and America (*1*). Symptomatic infections range from mild febrile illness or characteristic diseases such as hand, foot and mouth disease to severe neurologic disorders such as meningitis/encephalitis and acute flaccid paralysis. EV-A71 infections are usually asymptomatic and self-limiting but can also result in life-threatening complications such as pulmonary edema and cause death, predominantly in children <5 years of age. On the basis of viral protein 1 (VP1) sequences, 3 genogroups (A, B, C), including different subgenogroups (B0–B5, C1–C5), have been defined (*2*,*3*). Additional genogroups (D, E, F, G) have been proposed (*4*,*5*). In Europe, C1 and C2 strains have circulated predominantly within the past 2 decades, and recent introduction of C4 strains has been reported (*6*,*7*). Within subgenogroup C1, a lineage is replaced by the subsequent lineage over time (*8*).

National enterovirus surveillance (EVSurv) in Germany monitors polio-free status by testing fecal or cerebrospinal fluid (CSF) samples from hospitalized patients with suspected meningitis/encephalitis or acute flaccid paralysis. Enterovirus typing, using molecular and virologic methods, is performed within a laboratory network for enterovirus diagnostics. Since 2006, ≈2,500 samples have been tested annually; 25%–30% were enterovirus positive. Of the typed strains, 0.8%–12.7% were identified as EV-A71 (2006, 0.8%; 2007, 6.8%; 2008, 0.9%; 2009, 3.4%; 2010, 12.7%; 2011, 2.3%; 2012, 2.8%; 2013, 8.6%; 2014, 2.7%), indicating peaks with increased EV-A71 detection rates. Molecular characterization based on the VP1 region of a subset of EV-A71–positive samples revealed that C2 was the predominant subgenogroup in Germany from 2006 to 2014. Subgenogroups B5, C1, and C4 have also been identified, but less frequently (online Technical Appendix Table 3, http://wwwnc.cdc.gov/EID/article/22/10/16-0357-Techapp1.pdf).

In 2015, a total of 419 samples tested enterovirus positive within EVSurv. Of these, 43 fecal specimens and

[1]Contributing members of the Laboratory Network for Enterovirus Diagnostics are listed at the end of this article.

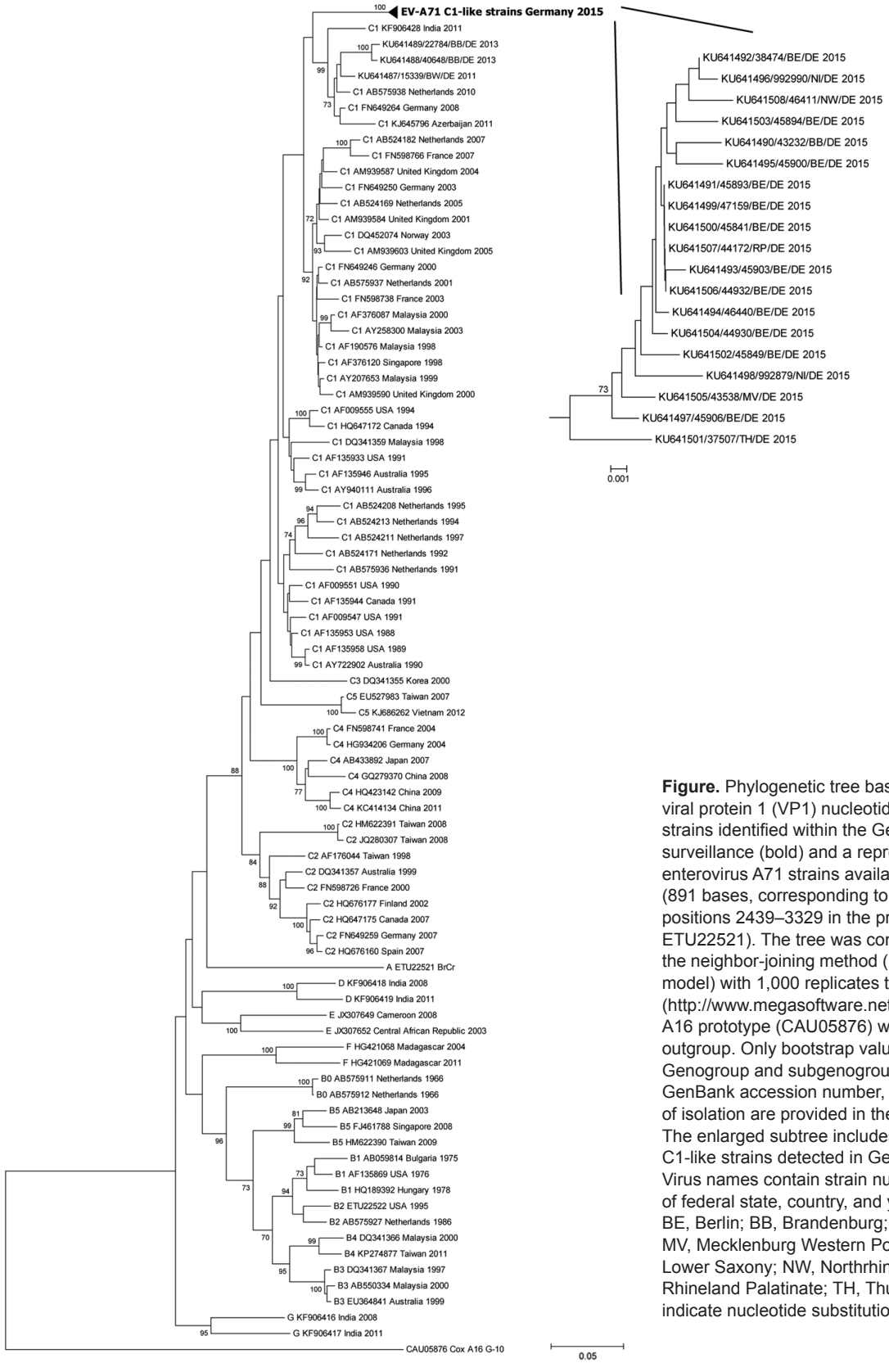

Figure. Phylogenetic tree based on complete viral protein 1 (VP1) nucleotide sequences of the strains identified within the German enterovirus surveillance (bold) and a representative set of enterovirus A71 strains available from GenBank (891 bases, corresponding to nucleotide positions 2439–3329 in the prototype BrCr ETU22521). The tree was constructed by using the neighbor-joining method (Kimura 2-parameter model) with 1,000 replicates through MEGA 6.06 (http://www.megasoftware.net/). Coxsackievirus A16 prototype (CAU05876) was used as the outgroup. Only bootstrap values >70 are shown. Genogroup and subgenogroup assignment, GenBank accession number, country and year of isolation are provided in the virus names. The enlarged subtree includes enterovirus A71 C1-like strains detected in Germany in 2015. Virus names contain strain number, abbreviation of federal state, country, and year of isolation. BE, Berlin; BB, Brandenburg; DE, Germany; MV, Mecklenburg Western Pomerania; NI, Lower Saxony; NW, Northrhine-Westphalia; RP, Rhineland Palatinate; TH, Thuringia. Scale bars indicate nucleotide substitution per site.

1 CSF specimen tested EV-A71 positive (11.2% of the typed enteroviruses); these samples were obtained from patients with signs of meningitis/encephalitis hospitalized in 25 secondary and tertiary care hospitals from 13 of 16 federal states of Germany. Thirty-six strains were further characterized at the National Reference Centre for Poliomyelitis and Enteroviruses (online Technical Appendix Table 2). Seventeen strains were identified as C2 by using the RIVM Enterovirus Genotyping Tool Version 1.0 (http://www.rivm.nl/mpf/enterovirus/typingtool) based on the VP1 region sequences (*9*). Sequence analyses of the remaining 19 strains revealed highest nucleotide identity (90%–93%) with recently circulating C1 strains from GenBank. Phylogenetic analysis that used the neighbor-joining tree algorithm showed separate clustering of these strains within the C1 subgenogroup (Figure). In contrast to the VP1 tree, phylogenetic analyses based on the 5′ untranslated region (UTR) and the P2 and P3 regions revealed different clustering of the German 2015 EV-A71 C1-like group (online Technical Appendix Figure). In line with these phylogenetic tree topologies, we found highest nucleotide identities to subgenogroup B3 and C2-like strains for 5′ UTR (both 90%), whereas P2 and P3 regions showed highest nucleotide identity of 82% and 84%, respectively, with C4 strains identified in China. We found no specific amino acid changes within the conserved major antigenic sites of the capsid proteins. However, we observed a V16M change in VP1. Few EV-A71 strains (all B1) also carry a methionine at this position, including the outbreak strains from Bulgaria (1975) and Hungary (1978). Also, we identified a valine residue at position 262 in VP1. Tee et al. have proposed that toggling of amino acids isoleucine and valine at this position in recent C1 lineages generates antigenic novelty (*8*). Within the 5′ UTR, a C526U change has been proposed to affect replication efficiency (*10*). All isolates belonging to the new EV-A71 C1 variant carried uracil at this position. In addition, we found a 2-nucleotide deletion within the spacer 2 region between the internal ribosome entry site and the coding region, similar to the EV-A71 prototype BrCr and the C2-like strains (GenBank accession nos. HM622392, HM622391, JQ280307) (data available on request).

Our findings highlight the need for molecular surveillance of enteroviruses to identify new variant strains with potential for increased virulence and pathogenicity. One limitation of the EVSurv is the lack of detailed clinical data because the request form deliberately asks for only basic cardinal symptoms justifying the clinical suspicion of meningitis/encephalitis or acute flaccid paralysis. Nevertheless, all patients had been hospitalized, suggesting severe disease. Besides characteristic symptoms (including nuchal rigidity, headache, fever, and vomiting), cerebral seizures, myoclonia, ataxia, petechiae, and stomatitis were also mentioned for some patients tested for the new variant C1-like strains described here. All but 1 patient was <5 years of age (online Technical Appendix Table 1). Therefore, pediatricians, in particular, should be aware of this new recombinant, potentially more pathogenic, strain and intensify diagnostic work-ups to better monitor EV-A71 circulation. In addition to CSF samples, fecal samples, throat swab specimens, and samples related to other clinical prodromes (e.g., vesicle fluids in cases of hand, foot and mouth disease) should be obtained. Particular attention should be paid to measures in daycare centers to prevent large outbreaks of enterovirus-associated meningitis/encephalitis.

The following authors contributed to this work through the national laboratory network for enterovirus diagnostics (LaNED): Jörg Hofmann, Department of Virology, Labor Berlin–Charite Vivantes GmbH, Berlin, Germany; Harald Heider, Department of Virology, Labor Berlin–Charite Vivantes GmbH, Berlin; Ursula Reif, Saxony State Laboratory of Health and Veterinary Affairs Department of Medical Microbiology and Hygiene, Dresden, Germany; Klaus Korn, Virology Institute–Clinical and Molecular Virology University Hospital Erlangen and Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany; Holger F. Rabenau, Institute of Medical Virology University Hospital Frankfurt, Frankfurt, Germany; Andreas Wille, Institute for Hygiene and Environment, Medical Microbiology, Hamburg, Germany; Armin Baillot, Governmental Institute of Public Health of Lower Saxony, Hannover, Germany; Aleksandra Pettke, Institute of Medical Microbiology–Clinical Virology University Hospital, Muenster, Germany; Joachim Kühn, Institute of Medical Microbiology–Clinical Virology University Hospital, Muenster; Nikolaus Ackermann, Bavarian Health and Food Safety Authority, Oberschleissheim, Germany; Christiane Ramelow, Ernst von Bergmann, Diagnostic GmbH, Potsdam, Germany; Ralph Hahn, Laboratory Prof. G. Enders MVZ and Institute of Virology, Infectious Diseases and Epidemiology e.V., Stuttgart, Germany; Martin Enders, Laboratory Prof. G. Enders MVZ and Institute of Virology, Infectious Diseases and Epidemiology e.V., Stuttgart; Maja Adam, Governmental Institute of Public Health of Baden-Wuerttemberg (LGA), Stuttgart; Detlef Michel, Institute of Virology, University Medical Center Ulm, Ulm; Axel Schubert, Institute of Virology, University Medical Center Ulm, Ulm; Benedikt Weißbrich, Institute of Virology and Immunobiology University of Wuerzburg, Wuerzburg, Germany; Christiane Prifert, Institute of Virology and Immunobiology University of Wuerzburg, Wuerzburg.

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Address for correspondence: Sabine Diedrich, National Reference Centre for Poliomyelitis and Enteroviruses, Robert Koch Institute, Seestraße 10, 13353 Berlin, Germany; email: DiedrichS@rki.de

Cerebral Syphilitic Gumma within 5 Months of Syphilis in HIV-Infected Patient

Motoyuki Tsuboi, Takeshi Nishijima, Katsuji Teruya, Yoshimi Kikuchi, Hiroyuki Gatanaga, Shinichi Oka

Author affiliations: National Center for Global Health and Medicine, Tokyo, Japan (M. Tsuboi, T. Nishijima, K. Teruya, Y. Kikuchi, H. Gatanaga, S. Oka); Kumamoto University, Kumamoto, Japan (H. Gatanaga, S. Oka)

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To the Editor: Tertiary syphilis, including cerebral syphilitic gumma, usually occurs >10 years after contracting syphilis (*1*) and is a rare manifestation since the introduction of penicillin (*2*). However, progression of syphilis is reported to be faster in HIV-infected patients than in those without such infections (*3*). We report a case of cerebral syphilitic gumma in an HIV-1–infected patient for whom serum samples obtained as recently as 5 months earlier showed negative results for syphilis.

A 21-year-old man infected with HIV came to the AIDS Clinical Center, National Center for Global Health and Medicine (Tokyo, Japan), because of a 2-hour loss of consciousness. He reported an uncomfortable feeling at the back of his head and neck and eye fatigue that lasted for 1 week. His HIV-1 infection was well-controlled with an antiretroviral combination of tenofovir, emtricitabine, and dolutegravir. The patient had a CD4 count of 565 cells/mL and a viremia level below detectable limits (<20 copies/mL). He was not using any other medications.

At examination, his vital signs were within reference ranges. Apart from a tongue bite, physical and neurologic examinations showed no abnormal findings. Results for chest radiograph, Holter electrocardiogram, and electroencephalogram were unremarkable. There were no abnormal ophthalmologic findings. Computed tomography of the brain showed a hypodense lesion at the left frontal lobe (Figure, panel A). Subsequent magnetic resonance imaging showed that the lesion (mass) was hypointense by gadolinium-enhanced, axial, T1-weighted imaging (Figure, panel B), hyperintense by T2-weighted imaging, and surrounded by extensive cerebral edema (Figure, panel C).

Symptomatic epilepsy caused by the mass was suspected to have caused the loss of consciousness. This conclusion was based on the intracranial mass, long duration of loss of consciousness, increase in creatine kinase level (471 U/L), and tongue bite.

When the HIV-1 infection was diagnosed in the patient 15 months earlier, results of serum rapid plasma reagin (RPR) and *Treponema pallidum* hemagglutination test (TPHA) were negative. However, during this examination, serum RPR and TPHA titers were 1:32 and 1:10,240, respectively. Results of cerebrospinal fluid (CSF) analysis were compatible with neurosyphilis (*4,5*) and showed a leukocyte count of 35 cells/mL (2 neutrophils/mL, 33 lymphocytes/mL), a total protein level of 30 mg/dL, a glucose level of 59 mg/dL (serum glucose level 92 mg/dL), an RPR titer of 1:<1, a TPHA titer of 1:160, and a fluorescent treponemal antibody-absorption titer of 1:32.

Cerebral syphilitic gumma was suspected on the basis of neurosyphilis and compatible imaging findings (*6*) and because other conditions, such as meningioma, primary central nervous system lymphoma, toxoplasmosis,

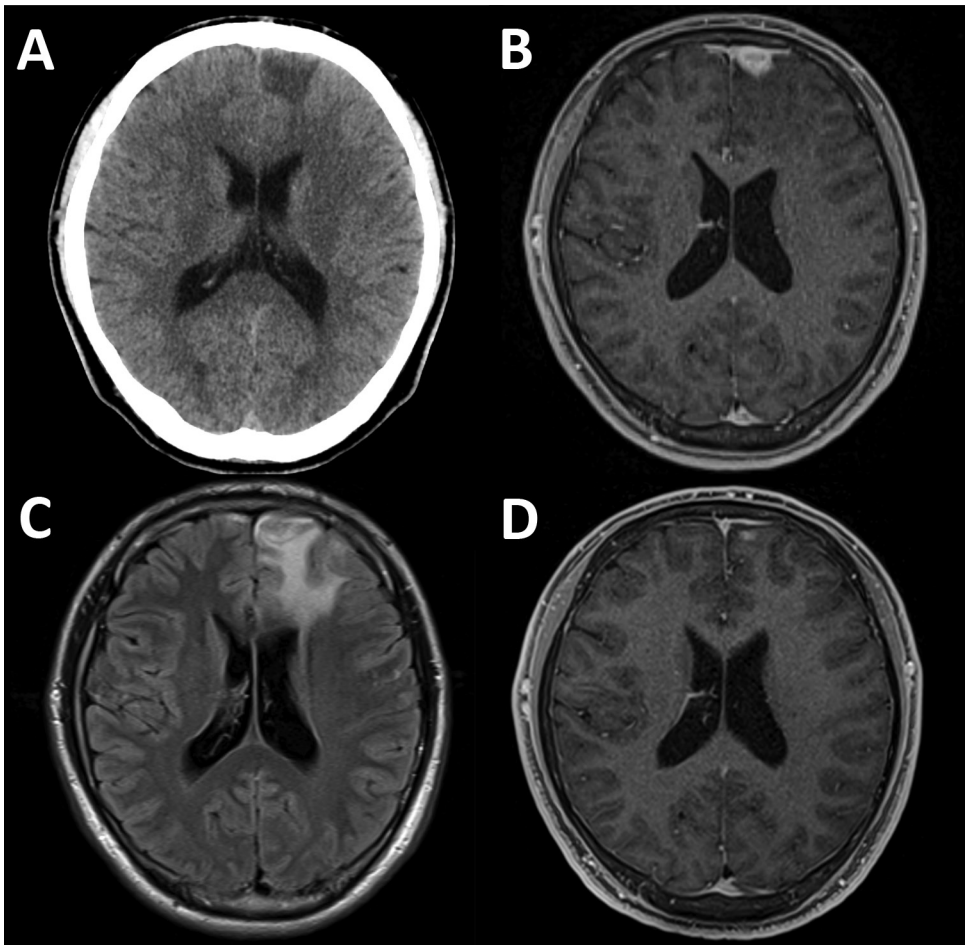

Figure. Diagnostic imaging results for a brain mass in a 21-year-old HIV-positive man with cerebral syphilitic gumma in Tokyo, Japan, for whom serum samples obtained as recently as 5 months earlier showed negative results for syphilis. A) Noncontrast, cranial computed tomography showing a hypodense lesion in the left frontal lobe. B) Gadolinium-enhanced, axial, T1-weighted magnetic resonance imaging (MRI) showing an enhanced lesion (mass) (14 × 14 × 12 mm) adjacent to the enhanced dura in the left frontal lobe. C) Axial, fluid-attenuated inversion recovery MRI showing extensive left frontal edema. D) Two months after treatment for syphilis, gadolinium-enhanced, axial T1-weighted MRI showing substantial resolution of the lesion.

cryptococcoma, tuberculoma, or brain abscess, were unlikely. These results were based on radiologic findings; high CD4 cell counts; and negative results for Epstein-Barr virus and malignant cells in CSF; serum cryptococcal antigen; CSF culture for bacteria, mycobacteria, and fungi; and interferon-γ release assay.

We did not perform a biopsy for the patient because of presumed high pretest probability of cerebral syphilitic gumma, the invasiveness of this complication, and the young age of the patient. However, successful therapy confirmed the diagnosis (*3*).

We empirically treated the patient with intravenous benzylpenicillin (24 million units/d for 14 consecutive days). Clinical symptoms improved shortly after treatment. At 2-month follow-up, all clinical symptoms and signs had resolved, and a 4-fold decrease in RPR titer was observed (RPR titer 1:8, TPHA titer 1:5,120) (*7*). The brain mass was substantially reduced, which confirmed the diagnosis of cerebral syphilitic gumma (Figure, panel D).

Gumma is a complication of long-term infection with *T. pallidum*, which develops 1–46 years after healing of secondary lesions; most cases develop within 15 years (*1*). However, for our patient, cerebral syphilitic gumma developed within 5 months after he contracted syphilis. After written informed consent was obtained, serum samples were obtained from the patient at his first and subsequent clinic visits and stored. Samples obtained at 11 months, 10 months, 9 months, 5 months, 11 weeks, and 5 weeks before detection of the brain mass were then tested retrospectively for RPR and TPHA titers. Results were negative at 11, 10, 9, and 5 months before detection of the brain mass, but TPHA titers became positive (1:80) at 11 weeks before presentation, and RPR titers became positive (1:16) at 5 weeks before presentation. Because RPR and TPHA titers can become positive as late as 6 weeks after infection (*8*), we believe that the patient contracted syphilis within 5 months before documentation of the cerebral mass.

In conclusion, we report an HIV-1–infected patient in whom cerebral syphilitic gumma developed within 5 months after contracting syphilis. Cerebral syphilitic gumma should be considered in the differential diagnosis of a cerebral lesion in sexually active patients even if they had recently contracted syphilis. Moreover, as guidelines

recommend (*9*), screening of HIV-infected patients who are sexually active with multiple partners should be conducted every 3–6 months for early detection of syphilis and initiation of proper treatment to prevent transmission and progression to late syphilis.

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Address for correspondence: Takeshi Nishijima, AIDS Clinical Center, National Center for Global Health and Medicine, 1-21-1, Toyama, Shinjuku, Tokyo 162-8655, Japan; email: tnishiji@acc.ncgm.go.jp

African Tick-Bite Fever in Traveler Returning to Slovenia from Uganda

Petra Bogovic, Stanka Lotric-Furlan, Misa Korva, Tatjana Avsic-Zupanc

Author affiliations: University Medical Center Ljubljana, Ljubljana, Slovenia (P. Bogovic, S. Lotric-Furlan); Institute of Microbiology and Immunology, Ljubljana (M. Korva, T. Avsic-Zupanc)

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To the Editor: African tick-bite fever (ATBF) is a well known disease in travelers to sub-Saharan Africa (*1*). The causative agent, *Rickettsia africae*, is transmitted to humans by ticks of the genus *Amblyomma* (*1*,*2*). *R. africae* has been isolated or detected in ticks, humans, or both in 22 sub-Saharan countries (*3*). Most ATBF cases have been described in tourists returning from countries to which it is endemic, most often from South Africa, Zimbabwe, and Botswana (*4*). We report a case of ATBF in a Slovenian traveler returning from Uganda.

In June 2015, a 29-year-old Slovenian man without underlying illnesses sought care at the Department of Infectious Diseases, University Medical Centre Ljubljana (Ljubljana, Slovenia). He had a 1-day history of fever up to 38°C without chills, 5 days after returning from a 2-week trip to Uganda. He had received vaccines against yellow fever and viral hepatitis A before traveling and did not use antimalarial prophylaxis during his stay in southwestern Uganda. A day before he left Uganda, he noticed a tick bite on his left upper abdomen.

At initial examination, he appeared well. He had a temperature of 37.8°C, pulse rate 75 beats/min, and blood pressure 120/80 mm Hg. Skin examination was remarkable for a solitary papular lesion at the site of tick bite surrounded by a small erythematous halo associated with discrete lymphangitic streaking and painful enlarged left axillar lymph nodes. Results of initially performed routine laboratory tests were normal.

On day 5 of illness, the man was still febrile, with a temperature up to 39°C. Papular skin lesion had developed a dark brown crusted center (compatible with a tache noire), and some new discrete asymptomatic pale papular skin lesions appeared on his left leg and arm. Repeat laboratory testing indicated only mildly increased serum C-reactive protein (16.0 mg/L [reference <5 mg/L]).

The clinical course improved rapidly after treatment began with doxycycline. Fever resolved in 2 days, and enlarged lymph nodes and skin lesions resolved completely within 14 days.

Microbiological procedures to detect for infection with tick-transmitted pathogens were performed to elucidate the

cause of the illness. The PCR for amplification of a 470-bp fragment of citrate synthase gene was performed according to a previously published protocol (*5*). DNA was extracted with QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) from whole blood and the crust of the eschar collected on day 5 of illness. In addition, serum samples were tested by indirect immunofluorescent assay for specific IgG and IgM against *Francisella tularensis* and *Rickettsia* spp. (spotted fever and typhus group) 5 days and 10 weeks after onset of fever.

Diagnosis of ATBF was affirmed by positive PCR result from the crust of the eschar; further sequence analysis revealed the infection with *R. africae*. Serologic testing demonstrated seroconversion of IgG to *R. conorii* and *R. rickettsii*, which cross-reacts with *R. africae* (negative immunofluorescent assay IgG titer at initial testing and 1:1,024 for *R. conorii* and *R. rickettsii* 10 weeks later) (*6*). Thick and thin blood smears were negative for malaria.

ATBF is the second most well-established cause of febrile illness among travelers to sub-Saharan Africa, after malaria. Usually it manifests by fever (59%–100% of cases), headache (62%–83%), eschar (53%–100%), lymphadenopathy (43%–100%), and rash (15%–46%). The clinical and laboratory findings in the patient reported here were similar to those previously reported among *R. africae*–infected patients (*1*). In the acute phase of illness, a biopsy and culture from an eschar, as well as PCR, are the most suitable methods to confirm the ATBF diagnosis. In this case, ATBF was proven by PCR and subsequent sequencing from a crust sample but not from whole blood and seroconversion of IgG.

The first information about *R. africae* in ticks in Uganda was published in 2013 by Lorusso et al. (*7*), but previously *R. conorii* also was found (*8*). The prevalence rate of *R. africae* infection among *Amblyomma variegatum* ticks in Uganda was 97.1% (*9*). Recently, Proboste et al. established the presence of previously undetected tickborne pathogens in rural dogs and associated ticks in Uganda. Tick species *Haemaphysalis leachi*, *Rhipicephalus* spp., and *A. variegatum* were infected by *Rickettsia* spp. (18.9%), including *R. conorii* and *R. massiliae*; by *Ehrlichia* spp. (18.9%), including *E. chaffeensis*; and by *Anaplasma platys* (*10*).

Our MEDLINE literature search found no previous descriptions of human *R. africae* infection in Uganda. This case indicates that ATBF should be included as a possible diagnosis in persons with febrile illness who have traveled to Uganda, a well-known tourist destination.

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Address for correspondence: Petra Bogovic, Department of Infectious Diseases, University Medical Center Ljubljana, Japljeva 2, 1525 Ljubljana, Slovenia; email: petra.bogovic@kclj.si

Polymyxin B Resistance in Carbapenem-Resistant *Klebsiella pneumoniae*, São Paulo, Brazil

Flávia Bartolleti, Bruna Mara Silva Seco, Carla Capuzzo dos Santos, Carolina Bragança Felipe, Mara Elisa Borsato Lemo, Tatiane da Silva Alves, Lilian F. Passadore, Marcelo J. Mimica, Suely Carlos Ferreira Sampaio, Alexandre Prehn Zavascki, Jorge Luiz Mello Sampaio

Author affiliations: University of São Paulo School of Pharmaceutical Sciences, São Paulo, Brazil (F. Bartolleti,

B.M.S. Seco, J.L. Mello Sampaio); Santa Casa de São Paulo School of Medical Sciences, São Paulo (F. Bartolleti, M.J. Mimica, S.C.F. Sampaio); Fleury Medicine and Health, São Paulo (C.C. dos Santos, C.B. Felipe, M.E.B. Lemo, T.S. Alves, J.L.M. Sampaio); University of São Paulo, São Paulo (L.F. Passadore); Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil (A.P. Zavascki)

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To the Editor: Infections caused by carbapenem-resistant *Enterobacteriaceae* have been associated with higher death rates than infections caused by carbapenem-susceptible strains, and resistant infections are mostly treated with polymyxins (*1*). Several outbreaks caused by carbapenem- and polymyxin-resistant *Klebsiella pneumoniae* (CPRKp) have been reported, mainly from Europe, and represent an emerging threat.

Carbapenem-resistant *K. pneumoniae* (CRKp) are endemic to Brazil, where polymyxin B (PMB) has been largely used against infections caused by these microorganisms. We evaluated PMB resistance rates and clonal diversity among CRKp isolates from patients in São Paulo, Brazil. The study was approved by the Research Review Board of Fleury Institute in São Paulo.

All *K. pneumoniae* isolates, except those from urine and active surveillance samples, recovered from inpatients during January 1, 2011–December 31, 2015, at 10 private tertiary-care hospitals in São Paulo were included in the study. *K. pneumoniae* isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; we analyzed only the first isolate from each patient, unless other isolates were recovered after a 90-day interval. To determine antimicrobial drug MICs, we used nonautomated broth microdilution (*2*) polystyrene plates with cation-adjusted Mueller–Hinton broth (Becton Dickinson, Franklin Lakes, NJ, USA) for PMB and tigecycline; the Etest (bioMérieux, Marcy l'Etoile, France) for fosfomycin; and the disk-diffusion method (*3*) for all other antimicrobial drugs. Isolates with a MIC of ≤2 mg/L for PMB were considered susceptible; this value is the EUCAST (European Committee on Antimicrobial Susceptibility Testing) breakpoint for colistin in *Enterobacteriaceae* (*2*). Tigecycline and fosfomycin MICs were interpreted according to EUCAST guidelines (*2*). Imipenem and meropenem MICs were determined using the Etest for all isolates that were nonsusceptible to at least 1 carbapenem (ertapenem, meropenem, or imipenem) by disk-diffusion (*3*). We phenotypically detected class A carbapenemases as previously described (*4*).

We used convenience sampling to select 62 CPRKp isolates that were detected during 2014–2015 and used pulsed-field gel electrophoresis (PFGE) to evaluate their genomic DNA macrorestriction profiles after *Xba*I digestion. Dice similarity indexes were calculated using the UPGMA method with 1.25% tolerance and optimization (*5*). The minimal Dice index for a clonal group was defined as 80%.

We performed multilocus sequence typing as described (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html) for 11 isolates that represented the 2 major PFGE clonal groups, CPRKp1 and CPRKp2. The full bla_{KPC} nucleotide sequence was determined for these isolates (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/11/16-0695-Techapp1.pdf).

We included a total of 3,085 *K. pneumoniae* isolates in the analysis (online Technical Appendix Table 2). A significant increase in carbapenem resistance ($p<0.001$) was seen from 2011 (6.8%) to 2015 (35.5%) (Figure, panel A). During the last year of analysis, we detected *K. pneumoniae* carbapenemase (KPC) in 96.2% of CRKp isolates.

PMB MICs showed a bimodal distribution that was clearly differentiated by a 2 mg/L MIC (Figure, panel B). When we stratified MICs by year and carbapenem resistance, a significantly increasing trend of resistance was seen among CRKp isolates from 2011 (0%) to 2014 (24.8%) to 2015 (27.1%) ($p<0.001$) (Figure, panel C). Resistance among carbapenem-susceptible *K. pneumoniae* varied from 0.7% in 2011 to 3.9% in 2014 ($p = 0.002$).

We did not evaluate the mechanism of PMB resistance. However, this resistance in KPC-producing *K. pneumoniae* is probably caused by the loss of *mgrB* function or the presence of nonsynonymous substitutions in *pmrB* that upregulate the *pmrCAB* and *arnBCADTEF-pmrE* operons, resulting in modification of lipid A. All these genes are located on the bacterial chromosome. Susceptibility testing showed that amikacin and tigecycline were the most active non–β-lactam antimicrobial agents against CPRKp isolates (amikacin 73.8%, tigecycline 69.4%) and CRKp isolates (amikacin 79.9%, tigecycline 72.2%) (online Technical Appendix Table 3).

PFGE identified 2 major clonal groups. The largest group, CPRKp1 (n = 30), belonged to sequence type (ST) 11, and the other group, CPRKp2 (n = 17), belonged to ST437. Both STs belonged to clonal complex (CC) 258. Interhospital and intrahospital dissemination among private and public hospitals was observed. All isolates tested had the bla_{KPC-2} gene (online Technical Appendix Figure).

In a previous study, the PMB resistance rate was 27% among 22 CRKp isolates from patients at a tertiary hospital in São Paulo during 2008–2010 (*6*). This rate is much higher than the rate we obtained for 2010, possibly because the previous study had a small number of isolates. CPRKp has been reported in various European countries at rates similar to those we report (*7*).

The predominance of CC258 among KPC-2-producing *K. pneumoniae*, but not among CPRKp, was reported

in Brazil (*8*), and ST11, a variant of ST258, has occasionally been detected in colistin-resistant KPC-producing isolates in Spain (*9*). The ST437 clone has been reported in KPC-2 producers in China (*10*), but we found no reports of CPRKp among this clonal group. Our findings show an alarming yearly increase in the rate of PMB resistance among CRKp isolates, mostly KPC-2 producing, and the occurrence of interhospital and intrahospital dissemination of CPRKp from CC258 in São Paulo.

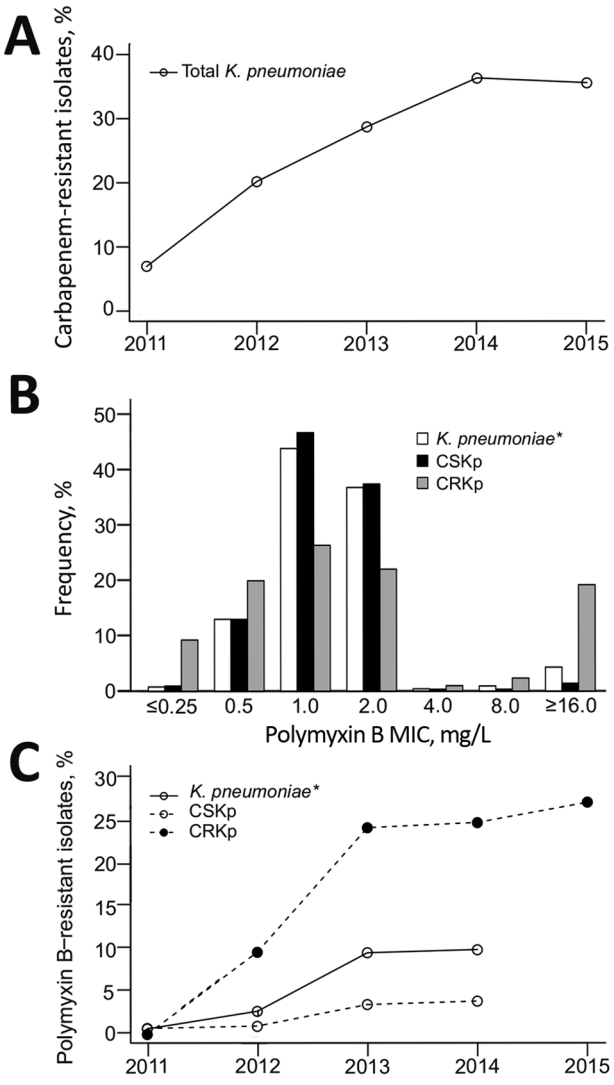

Figure. Antimicrobial resistance profile of *Klebsiella pneumoniae* isolated from hospital inpatients in São Paulo, Brazil. A) Carbapenem resistance trend among all *K. pneumoniae* isolates cultured during January 1, 2011–December 31, 2015 (n = 3,085; p<0.001). B) Polymyxin B MIC distribution stratified by carbapenem susceptibility. C) Polymyxin B resistance trend stratified by carbapenem susceptibility, 2011–2015. B, C) Carbapenem-susceptible *K. pneumoniae* (CSKp) isolated during January 1, 2011–June 30, 2014 (n = 1,511) and carbapenem-resistant *K. pneumoniae* (CRKp) isolated during January 1, 2011–December 31, 2014 (n = 436); *during July 1, 2015–December 31, 2015, only CRKp were tested for polymyxin B susceptibility (n = 377). All statistical analyses were conducted using SAS Studio 3.4 (SAS Institute, Inc., Cary, NC, USA). The statistical significance of a trend in resistance rates was evaluated using the χ^2 test, in which p values <0.05 were considered significant: *K. pneumoniae*, p<0.001; CSKp, p = 0.004; CRKp, p = 0.003.

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Address for correspondence: Jorge Luiz Mello Sampaio, Faculdade de Ciências Farmacêuticas, Avenida Professor Lineu Prestes, 580, Butantã, São Paulo, SP, Brazil, 05508-900; email: sampaio@usp.br

Resolution of a Chikungunya Outbreak in a Prospective Cohort, Cebu, Philippines, 2012–2014

Anon Srikiatkhachorn, Maria Theresa Alera, Catherine B. Lago, Ilya A. Tac-An, Daisy Villa, Stefan Fernandez, Butsaya Thaisomboonsuk, Chonticha Klungthong, Jens W. Levy, John Mark Velasco, Vito G. Roque, Jr., Ananda Nisalak, Louis R. Macareo, In-Kyu Yoon[1]

Author affiliations: University of Rhode Island, Providence, Rhode Island, USA (A. Srikiatkhachorn); Armed Forces Research Institute of Medical Sciences Virology Research Unit, Cebu City, Philippines (M.T. Alera, C.B. Lago); Cebu City Health Department, Cebu City (I.A. Tac-An, D. Villa); Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand (S. Fernandez, B. Thaisomboonsuk, C. Klungthong, J.W. Levy, J.M. Velasco, A. Nisalak, L.R. Macareo, I.K. Yoon); Philippines Department of Health National Epidemiology Center, Manila, Philippines (V.K. Roque, Jr.)

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To the Editor: Chikungunya is a reemerging, mosquitoborne infectious disease caused by chikungunya virus (CHIKV). Its classic manifestations include fever and joint inflammation, which can develop into chronic joint disease. Infections in immunocompromised persons can lead to severe organ involvement and death. Chikungunya outbreaks appear to occur in 2 patterns: 1) spatially and temporally restricted outbreaks in endemic areas (*1*,*2*); and 2) large epidemics that occur periodically every 40–50 years affecting multiple geographic areas (*3*). The mechanisms associated with the initiation of these large outbreaks are not well understood. An A226V amino acid substitution in the virus envelope, which enhances replication in *Aedes albopictus*, a mosquito vector for CHIKV, and expansion of vectors into areas with previously immunologically naive populations are thought to be responsible for some recent epidemics (*4*). In chikungunya-endemic areas, environmental factors such as changes in rainfall and vector densities have been implicated in smaller scale outbreaks. The mechanisms underlying outbreak resolution are not well understood. Herd immunity afforded by exposed persons might play an important role in preventing ongoing virus transmission. Reported seroprevalence rates in affected areas have ranged from 10% to ≈40% after an outbreak (*5*–*7*).

We established an age-structured prospective cohort consisting of persons ≥6 months of age in Cebu, Philippines, and conducted active surveillance for acute febrile illnesses by making weekly telephone calls or home visits during 2012–2014 (Table). We defined symptomatic chikungunya as an acute febrile illness with CHIKV RNA detected in an acute-phase blood sample or seroconversion detected by CHIKV IgM/IgG ELISA in paired acute/convalescent-phase serum samples. We tested serum samples collected at enrollment, 12 months, and 24 months for neutralizing antibodies by using a CHIKV plaque-reduction neutralization test. Persons identified during active surveillance who had a >4-fold rise in neutralizing antibody titers in the absence of symptomatic chikungunya were considered to have subclinical infection. As reported previously (*8*), the overall incidence of CHIKV infection during the first year of surveillance was 12.32/100 person-years among all cohort members and 16.9/100 person-years among immunologically naive members (defined by the absence of CHIKV neutralizing antibodies at baseline) (Table). Only 19% of infections were symptomatic, and most were accompanied by reported or documented fever without severe joint symptoms. Persons with detectable neutralizing antibodies at the beginning of the surveillance period exhibited no clinical or serologic evidence of CHIKV infection during active surveillance.

During the second year of surveillance, 765 cohort members completed all study activities, including undergoing collection of blood samples at the beginning of the study and at the end of the first and second year. The overall incidence of CHIKV infection during the second year (2.84 cases/100 person-years) decreased significantly ($p<0.05$) compared with the first year (12.32 cases/100 person-years). This change was attributable primarily to a decline in subclinical infections and was observed equally in all age groups. We also observed a decline in incidence of symptomatic infections; however, this difference was not significant, possibly because of the small number of symptomatic cases. The decline in incidence during the second year was also observed when chikungunya–immunologically naive persons were analyzed separately. The prevalence of neutralizing antibodies increased significantly from 28% at the beginning of the first year to 42% at the beginning of the second year. No persons with detectable baseline neutralizing antibodies were infected during the 2-year surveillance period.

Our study documented the resolution of a chikungunya outbreak in a prospective cohort in an endemic setting during 2 years of active surveillance. The duration of this outbreak is consistent with a previous model suggesting that chikungunya outbreaks in the Philippines last ≈1–3 years (*9*). The decline in incidence during the second year coincided with an increase in chikungunya-immune

[1]Current affiliation: International Vaccine Institute, Seoul, South Korea.

Table. Incidence of subclinical and symptomatic chikungunya virus infection during 2 years of active surveillance in an age-stratified cohort, Cebu, Philippines, 2012–2014*

Cohort	No. persons/prevalence of neutralizing antibodies at beginning of year, %	No. cases/no. cases per 100 person-years (95% CI)		
		Acute symptomatic infections	Subclinical infections	All infections
Year 1				
All persons with paired serum samples, by age				
6 mo–5 y	203/0.7	5/3.23 (1.23–7.08)	10/6.46 (3.23–11.47)	15/9.69 (5.66–15.59)
6–15 y	201/1.1	8/4.23 (1.99–7.98)	24/12.68 (8.23–18.55)	32/16.91(11/.8–23.56)
16–30 y	200/20.24	2/1.13 (0.23–3.63)	20/11.32 (7.13–17.4)	22/12.45 (8.02–18.51)
31–50 y	204/52.9	4/2.38 (0.79–5.65)	21/12.48 (7.95–18.7)	25/14.85 (9.85–21.57)
>50 y	200/61.06	1/0.56 (0.05–2.6)	12/6.7 (3.66–11.34)	13/7.25 (4.06–12.05)
All ages	1,008/28.0	20/2.3 (1.5–3.49)	87/10.02 (8.08–12.3)	107/12.32 (10.15–14.83)
Only persons with negative neutralizing antibodies at beginning of 1st year, by age				
6 mo–5 y		5/3.26 (1.23–7.4)	10/6.51 (3.34–11.55)	15/9.77 (5.71–15.7)
6–15 y		8/4.27 (2.02–8.6)	24/12.82 (8.43–18.76)	32/17.09 (11.91–23.82)
16–30 y		2/1.42 (0.28–4.56)	20/14.22 (8.96–21.52)	22/15.64 (10.08–23.25)
31–50 y		4/5.02 (1.68–11.94)	21/26.37 (16.81–39.54)	25/31.39 (20.81–45.59)
>50 y		1/1.41(0.13–6.6)	12/16.98 (9.27–28.75)	13/18.39 (10.3–30.55)
All ages		20/3.17 (1.99–4.79)	87/13.77(11.1–16.9)	107/16.94 (13.95–20.38)
Year 2				
All persons with paired serum samples				
6 mo–5 y	148/8.6	2/1.68 (0.33–5.37)	**1/0.84 (0.08–3.91)**	**3/2.51 (0.7–6.71)**
6–15 y	184/18.6	**1/0.63 (0.06–2.93)**	**3/1.88 (0.52–5.03)**	**4/2.51 (0.84–5.97)**
16–30 y	168/35.04	1/0.74 (0.1–5.36)	**4/2.98 (1.0–7.08)**	**5/3.72 (1.41–8.16)**
31–50 y	172/70.4	1/0.62 (0.06–2.87)	**4/2.46 (0.82–5.85)**	**5/3.08 (1.17–6.74)**
>50 y	182/69.7	1/0.61 (0.06–2.86)	**3/1.84 (0.51–4.9)**	**4/2.45 (0.82–5.83)**
All ages	854/42.0	6/0.81 (0.34–1.67)	15/2.03 (1.19–3.27)	**21/2.84 (1.81–4.26)**
Only persons with negative neutralizing antibodies at beginning of 2nd year, by age				
6 mo–5 y		2/1.84 (0.37–5.89)	**1/0.92 (0.08–4.28)**	**3/2.76 (0.76–7.35)**
6–15 y		1/0.77 (0.07–3.6)	**3/2.32 (0.64–6.18)**	**4/3.09 (1.03–7.35)**
16–30 y		1/1.15 (0.1–5.36)	**4/4.6 (1.54–10.94)**	**5/5.75 (2.18–12.6)**
31–50 y		1/2.11 (0.19–9.82)	**4/8.43 (2.82–20.03)**	**5/10.53 (3.99–23.09)**
>50 y		1/2.05 (0.19–9.55)	3/6.15 (1.7–16.4)	4/8.2 (2.74–19.49)
All ages		6/1.42 (0.59–2.93)	**15/3.56 (2.08–5.72)**	**21/4.98 (3.18–7.47)**

Bold indicates significantly different (p<0.05) from incidence observed among the corresponding age groups during the first year of surveillance.
Prevalence of neutralizing antibodies at the beginning of the first year was 28% and at the beginning of the second year was 42%.

persons at the beginning of the second year, which approached 50%. This seroprevalence rate is higher than the 10%–30% rate reported after major chikungunya outbreaks on the island of Mayotte in the Indian Ocean, on the island of St. Martin in the Caribbean, and in Italy (*5*,*6*,*10*). The higher sensitivity of the neutralization assay in this study compared with the IgM/IgG ELISA used in other studies might have contributed to the higher rate of detection of chikungunya-immune persons. Neutralizing antibodies against chikungunya appear to be long lasting, as indicated by the higher seroprevalence in the older age group in our cohort. A study in Thailand demonstrated the presence of neutralizing antibodies more than a decade after infection (*1*). Although other environmental factors might contribute to outbreak resolution, the absence of infection in cohort members with baseline neutralizing antibodies in our study suggests the protective role of antibodies. A high prevalence of neutralizing antibodies has been documented in a community without any previously reported outbreaks (*1*), suggesting that immunity elicited by subclinical or mildly symptomatic infections might play a role in conferring protection against further transmission, leading to resolution of an outbreak.

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Address for correspondence: Anon Srikiatkhachorn, Institute for Immunology and Informatics, University of Rhode Island, 80 Washington St, Room 302F, Providence, Rhode Island 02903, USA; email: anons.gst@afrims.org

Resurgence of Yellow Fever in Angola, 2015–2016

Antoinette A. Grobbelaar, Jacqueline Weyer, Naazneen Moolla, Petrus Jansen van Vuren, Francisco Moises, Janusz T. Paweska

Author affiliation: National Institute for Communicable Diseases of the National Health Laboratory Service, Johannesburg, South Africa (A.A. Grobbelaar, J. Weyer, N. Moolla, P. Jansen van Vuren, J.T. Paweska); National Public Health Institute, Luanda, Angola (F. Moises)

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To the Editor: Yellow fever virus (YFV) is endemic in tropical and subtropical Africa and South America, and it is transmitted to humans and nonhuman primates through the bites of infected mosquitoes. The virus, a member of the family *Flaviviridae*, causes yellow fever, which in severe cases manifests as fulminant hemorrhagic fever. Outbreaks of yellow fever in humans occur mostly in the urban cycle of the virus, which involves its transmission through the bites of the day-feeding infected *Aedes aegypti* mosquitoes (*1*). As many as 130,000 cases with fever and jaundice or hemorrhage may occur annually with a concomitant 78,000 deaths (*2*). A low capacity for yellow fever diagnosis and lack of surveillance in disease-endemic countries likely contribute to case underreporting (*1*).

Although wide-scale yellow fever vaccination, which began in the 1940s and continued through 1960, resulted in a dramatic decrease in the number of outbreaks, waning population immunity and lapse of continued high coverage vaccination in yellow fever–endemic countries have led to the disease's resurgence in Africa (*1–3*). Between 1980 and 2012, the World Health Organization received reports of 150 outbreaks in 26 countries in Africa (*2*). In the past 5 years (2011–2016), outbreaks have been documented in Democratic Republic of Congo, Sudan, Cameroon, Chad, Senegal, Côte d'Ivoire, Uganda, and Sierra Leone (*3*). During 2005–2016, Sudan was the most affected country; 3 outbreaks were reported, resulting in 1,508 cases and 368 deaths (*3,4*).

Yellow fever was first recognized in Angola in the 1930s, but not until 1971 (65 cases) and 1988 (37 cases) were sizeable outbreaks reported (*5–7*). As of July 1, 2016, a total of 3,552 suspected cases, including 875 laboratory-confirmed cases and 355 deaths, had been reported from all 18 provinces of Angola, with most cases occurring in Luanda Province (*8*). In this account, we provide laboratory confirmation that the first suspected viral hemorrhagic fever cases in Angola were YFV infections and report preliminary sequencing data.

On January 14, 2016, we received whole blood samples from 3 patients who resided in Luanda, Angola, and were suspected of having viral hemorrhagic fever. All were men, two 22 and one 30 years of age. Clinical manifestations in all patients were fever, headache, nausea, and vomiting. Myalgia, malaise, reduced consciousness, and jaundice each occurred in 2 patients; abdominal pain, back pain, ecchymosis, conjunctivitis, and bleeding gums each occurred in 1 patient. Two of the patients died 7 days after disease onset (online Technical Appendix Table, http://wwwnc.cdc.gov/EID/article/22/10/16-0818-Techapp1.pdf). Laboratory diagnosis consisted of testing for filoviruses, arenaviruses, and bunyaviruses, as well as for chikungunya and dengue viruses by using reverse transcription PCR (RT-PCR). All results of RT-PCRs were negative. A real time RT-PCR for YFV, targeting the 5′ noncoding region (*9*), produced positive results for samples from all 3 patients. The samples were then tested by using a pan-flavivirus RT-PCR targeting the flavivirus NS5 gene region using primers FU1 8993F and cFD2 9258R (*10*). Resulting amplicons of expected size (266 bp) obtained from 2 of the 3 samples were subjected to conventional Sanger sequencing. Phylogenetic

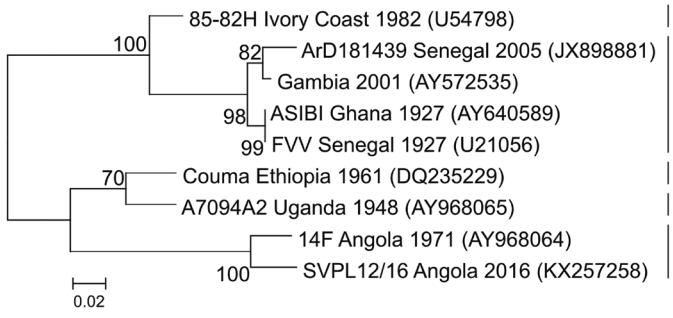

Figure. Maximum-likelihood phylogenetic reconstruction of 201 nt of the NS5 gene of yellow fever virus in Angola and other recognized genotypes of the virus in Africa. Node values indicate bootstrap confidence values generated from 1,000 replicates. GenBank accession numbers are indicated in brackets. Scale bar indicates substitutions per site.

analysis was performed by using a maximum-likehood method in MEGA version 6 (http://www.megasoftware.net) based on the general time reversible model under 1,000 bootstrap iterations, and sequence divergence was determined to calculate the p-distances between sequences. Phylogenetic inference of the sequence data demonstrated 95% nucleotide sequence similarity between the virus from this outbreak and the 14F YFV strain isolated in Angola in 1971 (Figure). PCR and sequencing results were reported to Angolan Public Health Institute on January 19, 2016.

The identification of the outbreak prompted cordon vaccination in Luanda in February 2016, followed by mass vaccination in other areas (*8*). The initially localized outbreak in Angola developed into the biggest and most widespread yellow fever epidemic recorded in Africa for decades (*3,8*). Sequencing and phylogenetic analysis indicate that the outbreak virus is highly similar to that identified during the epidemic in Angola in 1971. This finding reiterates the endemicity of yellow fever in Angola and emphasizes the need for consistent routine mass vaccination of the at-risk population to prevent future outbreaks.

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Address for correspondence: Janusz T. Paweska, National Institute for Communicable Diseases of the National Health Laboratory Service, 1 Modderfontein Road, Sandringham 2131, Johannesburg, South Africa; email: januszp@nicd.ac.za

Sexual Transmission of Zika Virus and Persistence in Semen, New Zealand, 2016

Jay Harrower, Tomasz Kiedrzynski, Simon Baker, Arlo Upton, Fahimeh Rahnama, Jill Sherwood, Q. Sue Huang, Angela Todd, David Pulford

Author affiliations: Auckland Regional Public Health Service, Auckland, New Zealand (J. Harrower, S. Baker); Ministry of Health, Wellington, New Zealand (T. Kiedrzynski); Labtests and Northland Pathology Laboratory, Whangarei, New Zealand (A. Upton); Auckland District Health Board, Auckland (F. Rahnama); Institute of Environmental Science and Research Limited, Wallaceville, New Zealand (J. Sherwood, Q. Sue Huang, A. Todd); Ministry for Primary Industries, Wallaceville (D. Pulford)

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To the Editor: Zika virus infection is an emerging arboviral disease linked to an increased risk for severe neurologic outcomes and devastating adverse fetal complications, including pregnancy loss and congenital microcephaly (*1*). Most Zika virus infections result from bites from mosquito vectors; however, increasing evidence indicates that Zika virus infection can be sexually transmitted (*1*–*3*). Probable sexual transmission from male to female (*3*–*5*) and male to male (*6*) have been reported, and transmission through vaginal, anal (*6*), and oral (*5*) sexual intercourse has been implicated. Studies also have demonstrated the presence of Zika virus RNA in semen 2 weeks after symptom onset, with viral loads ≈100,000 times greater than those detected concurrently in serum (*7*), and Zika virus RNA has been detected in semen while being undetectable in serum (*2*,*5*,*8*). The presence of Zika virus RNA in semen up to 62 days after symptom onset of Zika virus infection has been reported (*8*). Here we report a case of locally acquired Zika virus infection that was almost certainly the result of sexual transmission and our findings indicating the duration of Zika virus RNA persistence in semen.

A 51-year-old man (patient 1) who regularly traveled to Samoa, a Pacific island with known Zika virus transmission, returned to New Zealand in late January 2016. He reported having been sexually abstinent while overseas. After 1 week he experienced onset of fever, rash, arthralgia, and ankle edema. Aware of the symptoms of mosquito-borne illnesses prevalent in his region of travel, he assumed he had a mild case of chikungunya and sought no medical attention.

A 53-year-old woman (patient 2) who was the sexual partner of patient 1 experienced onset of sore throat, fever, arthralgia, and rash 17 days after her partner's return to New Zealand (10 days after he had onset of symptoms). She had had no recent overseas travel, had no blood transfusions, and was unaware of having any mosquito bites. Four days after symptom onset, she visited a general practitioner, whose investigations revealed mild neutropenia, normal liver and renal function, no evidence of streptococcal throat infection, and a demonstrated immunity to measles.

The rash subsequently spread, and small joint arthralgia worsened. The patient returned to the general practitioner 9 days after symptom onset. Disclosing her partner's travel history and symptoms and reporting having unprotected sexual intercourse with him after his return to New Zealand (before and during the time he was symptomatic), she enquired about the possibility of Zika virus infection. After discussing the matter with a clinical microbiologist and securing the consent of the woman and her partner, the general practitioner ordered tests for arboviruses common in the male partner's region of travel.

All laboratory tests for chikungunya and dengue viruses were negative for both patients. Testing for Zika virus RNA was performed by using real-time reverse transcription PCR (rRT-PCR) in accordance with published methods (Table) (*9*). Serum from patient 2 tested negative for Zika virus RNA when first tested on day 9 after symptom onset, although subsequent retrospective testing on the serum sample collected on day 4 returned a positive result. A urine sample collected on day 9 was positive for Zika virus RNA. These tests were repeated 3 days later and returned identical results. Testing of serum collected on day 9 detected Zika IgM and IgG (IgG titer >1:640), and similar results were obtained on serum collected on day 12.

Laboratory investigations for Zika virus RNA in patient 1 demonstrated negative rRT-PCR results for serum (first tested 19 days after symptom onset) and for urine (first tested 21 days after symptom onset). Serologic testing performed on day 21 detected Zika IgM and IgG (IgG titer 1:320). Semen collected on day 23 tested positive for Zika virus RNA (cycle threshold [C_t] 25). Semen collected on days 35 and 76 also tested positive (C_t values 29 and 35, respectively; duplicate testing on the sample

Table. Timeline and results of rRT-PCR testing on specimens from 2 patients with suspected Zika virus infection, New Zealand, 2016*

Patient no. (age, y/sex)	Days after initial return of patient 1 to New Zealand	Days after symptom onset	Specimen type	Result of rRT-PCR for Zika virus
Patient 1 (51/M)	26	19	Serum	–
	28	21	Urine	–
	28	21	Serum	–
	30	23	Semen	+
	42	35	Semen	+
	83	76	Semen	+
	106	99	Semen	–
	124	117	Semen	–
Patient 2 (53/F)	21†	4†	Serum	+
	26	9	Serum	–
	26	9	Urine	+
	29	12	Serum	–
	29	12	Urine	+

*rRT-PCR, real-time reverse transcription PCR; +, positive; –, negative.
†Performed retrospectively on stored serum after observation of positive results obtained on urine samples collected on days 9 and 12 after symptom onset.

from day 76 performed at the national arbovirus reference laboratory indicated a C_t value of 35.82). Semen samples collected on days 99 and 117 tested negative for Zika virus RNA. Attempts at virus isolation from the semen sample collected on day 23 failed to cultivate infectious particles.

It is very unlikely that transmission of Zika virus infection to patient 2 occurred through a mosquito bite. Although occasional interceptions of exotic mosquito species have occurred at international ports of entry into New Zealand, neither of the *Aedes* species of mosquito capable of transmitting Zika virus infection is established in the country (*10*). This case report and results of research into the duration of infectivity of Zika virus in semen can inform the evolving guidelines concerning the recommended duration of abstinence from sexual intercourse and the practice of barrier protection methods to prevent sexual transmission of Zika virus infection.

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Address for correspondence: Jay Harrower, Auckland Regional Public Health Service, Cornwall Complex, Bldg 15, Level 2, Rm 247, Greenlane Clinical Centre, Auckland, New Zealand; email: jay.harrower@kinect.co.nz

Culex pipiens and *Aedes triseriatus* Mosquito Susceptibility to Zika Virus

Matthew T. Aliota, Stephen A. Peinado, Jorge E. Osorio, Lyric C. Bartholomay

Author affiliation: University of Wisconsin, Madison, Wisconsin, USA

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To the Editor: Zika virus, genus *Flavivirus*, has spread nearly uncontrolled since its introduction into the Western Hemisphere; autochthonous spread has occurred in ≥39 countries and territories, including several US territories. Transmission of Zika virus is usually by the bite of infected mosquitoes, and potential for emergence in areas with competent mosquito vectors is high (*1*). Future spread of Zika virus is unpredictable; however, eventual local spread in the United States is possible. As of July 13, 2016, a total of 1,306 travel-associated cases had been reported (ArboNET, https://www.cdc.gov/zika); substantial populations of *Aedes (Stegomyia) aegypti* (Linnaeus) mosquitoes exist in ≥16 states in the eastern, southeastern, and southwestern United States; and *Ae. (Stegomyia) albopictus* (Skuse) mosquitoes inhabit ≥28 states and continued expansion throughout the northern United States is probable (*2*). Mosquitoes of these 2 species have demonstrated the ability to transmit Zika virus (*1*).

The recent epidemic spread of Zika virus suggests that *Ae. aegypti* mosquitoes are the main vector; however, information about the role of other species in driving and maintaining Zika virus transmission is lacking. Of particular concern this summer (2016) is emergence and establishment of Zika virus in previously unaffected geographic areas; with the advent of mosquito season commencing in most of the continental United States, the likelihood of mosquitoborne transmission of Zika virus in states without populations of *Ae. aegypti* and *Ae. albopictus* mosquitoes remains unknown. To understand the potential risk for spread of Zika virus in temperate US states, we compared the relative abilities of *Culex pipiens* and *Ae. triseriatus* mosquitoes to transmit Zika virus in the laboratory. We used *Ae. aegypti* and *Ae. albopictus* mosquitoes as positive controls.

Laboratory colonies of mosquitoes used in this study were maintained at the University of Wisconsin–Madison, and vector competence for Zika virus was evaluated by using established procedures (*3*,*4*). Mosquitoes from each group were incapacitated (exposed to trimethylamine); legs were removed and collected. Salivary secretions were collected in capillary tubes containing a 1:1 ratio of fetal bovine serum and 50% sucrose. Mosquitoes were then placed

Table. Competence of mosquitoes, by species, as Zika virus vectors, 14 days after peroral infection, United States*

	No. virus-positive/no. tested (%)								
	Biological replicate 1, mean 6.02 $\log_{10}$ PFU/mL ± SD 0.67			Biological replicate 2, mean 4.74 $\log_{10}$ PFU/mL ± SD 0.06			Biological replicate 3, mean 6.83 $\log_{10}$ PFU/mL ± SD 0.45		
Mosquito species	I	D	T	I	D	T	I	D	T
Culex pipiens†	0/20 (0)	0/20 (0)	0/20 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/30 (0)	0/30 (0)	0/30 (0)
Aedes triseriatus‡	ND	ND	ND	0/20 (0)	0/20 (0)	0/20 (0)	4/13 (31)	0/4 (0)	0/4 (0)
Ae. albopictus§	9/9 (100)	6/9 (67)	2/9 (22)	1/6 (17)	0/1 (0)	0/1 (0)	ND	ND	ND
Ae. aegypti¶	ND	ND	ND	ND	ND	ND	17/17 (100)	12/17 (71)	4/17 (24)

*Zika virus strain PRVABC59 (GenBank accession no.KU501215) was originally isolated from a traveler to Puerto Rico in December 2015. I, infected; D, disseminated; ND, no data; T, transmitted.
†Originated from egg rafts collected in Iowa in 2002 and colonized at the Iowa State University Medical Entomology Laboratory.
‡Originated from eggs collected in Iowa in 2002 and 2003 and colonized at the Iowa State University Medical Entomology Laboratory.
§Originated from eggs collected in Missouri in 2002 and colonized at the Illinois Natural History Survey.
¶Black-eyed Liverpool strain.

in individual tubes; their bodies and legs were homogenized, clarified by centrifugation, and screened for virus infection. Dissemination was indicated by virus-positive legs, and transmission potential was indicated by virus-positive salivary secretions. All samples were screened by plaque assay on Vero cells. Mosquitoes were exposed to Asian lineage Zika virus strain PRVABC59 (GenBank accession no. KU501215) (*5*) by feeding on Zika virus-infected *Ifnar*−/− mice (*4*). Mice (n = 4/replicate) yielded infectious blood meal concentrations of 6.02 $\log_{10}$ PFU/mL ± 0.67 (mean ± SD; biological replicate no. 1), 4.74 $\log_{10}$ PFU/mL ± 0.06 (replicate no. 2), and 6.83 $\log_{10}$ PFU/mL ± 0.45 (replicate no. 3). Blood meal concentrations in mice were consistent with viremia concentrations of humans in the field (*4*).

All samples from *Cx. pipiens* mosquitoes and all replicates were negative for Zika virus by plaque assay (Table). In contrast, *Ae. triseriatus* mosquitoes were susceptible to infection when exposed to mice with the highest viremia concentrations (Table). However, none of these infected mosquitoes disseminated virus and none were capable of transmitting the virus. Data from *Ae. albopictus* and *Ae. aegypti* mosquitoes that had been exposed to the same mice demonstrated that the viremia concentrations used could productively infect mosquitoes. Of note, *Ae. albopictus* mosquito infection rates were dose dependent (i.e., infection rates increased with blood meal titer). Furthermore, data generated from exposure to the same mice demonstrated productive mosquito infection with these viremia concentrations (*4*). It therefore seems likely that if Zika virus circulation in the United States occurs, it will be driven by *Ae. albopictus* or *Ae. aegypti* mosquitoes (*6*). However, we cannot rule out that anthropophilic mosquitoes of other species in this country could be competent vectors.

These data argue for continued studies (experimental and epidemiologic) assessing interactions between differing mosquito–Zika virus combinations in the United States because of geographic variations that may exist in oral susceptibility of mosquitoes of the same or different species. The few vector competence studies conducted to date have focused primarily on *Ae. aegypti* and *Ae. albopictus* mosquitoes (*8*), but mosquitoes of other species may be vectors, depending on geographic location. We focused on *Cx. pipiens* mosquitoes because they are ubiquitous (*7*), they are considered one of the principal vectors of West Nile virus in the northern half of the United States, and a recent report from Brazil suggests *Cx. quinquefasciatus* mosquitoes as potential Zika virus vectors (*8*). We chose *Ae. triseriatus* mosquitoes because they are the natural vector and overwintering host of La Crosse virus, they are extremely tolerant to a range of temperatures, they are distributed from Florida to eastern Canada (*9*), and they have been implicated as potential enzootic vectors for West Nile virus (*10*). To determine the risk for Zika virus transmission in the United States, surveillance of different human-biting mosquito species will be paramount. Although we expected that *Cx. pipiens* and *Ae. triseriatus* mosquitoes would not be competent Zika virus vectors, our experimental verification helps exclude uncertainties surrounding the potential vectors of this emerging pathogen.

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Address for correspondence Matthew T. Aliota, Department of Pathobiological Sciences, University of Wisconsin-Madison, 1656 Linden Dr, Madison, WI 53706, USA; email: mtaliota@wisc.edu

Yellow Fever—More a Policy and Planning Problem than a Biological One

Charles H. Calisher, John P. Woodall

Author affiliations: Colorado State University, Fort Collins, Colorado, USA (C.H. Calisher); Program for Monitoring Emerging Diseases, Brookline, Massachusetts, USA (J.P. Woodall)

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To the Editor: The recent and ongoing outbreak (epidemic) of yellow fever (YF) in Angola is cause for concern, not only in West Africa, but also in contiguous and other nearby countries (*1*). As of June 21, 2016, the World Health Organization (WHO) had reported 3,137 cases (847 laboratory-confirmed) and 350 deaths (*2*), but at the present time, it is not cause for panic or for extravagant claims of an "impending global health threat" (*3*). As long as the Angola Ministry of Health reports that there have been <400 deaths from YF since it declared the outbreak 4 months ago, and because there is an effective vaccine against this disease, it is difficult to understand dire warnings of a global threat.

WHO considers the situation of high concern because of the inadequate surveillance system in Angola, one that is incapable of identifying new foci or areas where cases might emerge (*2*). Such an inability suggests that many more cases and deaths have already occurred (*4*). What is needed now, without further unconscionable delays, is a proper vaccination campaign, one that has been unachievable to now. We here reaffirm what has been suggested by the WHO Strategic Advisory Group of Experts on Immunization and by others, but as of June 20, 2016, not applied by WHO: that the YF vaccine administered at one fifth of the regular dose could be used until the epidemic ends (*5*).

Despite vaccination campaigns in various provinces in Angola, circulation of YF virus (YFV; family *Flaviviridae*, genus *Flavivirus*) in some districts persists. Attempts to control this epidemic are being made by application of the effective YFV 17D vaccine that has been used for many decades worldwide. Whereas recognition of cases of YF has decreased in Angola, cases continue to occur there, and isolated cases have been detected in persons who have visited Angola as tourists or for business purposes. Furthermore, cases in nearby Democratic Republic of the Congo have increased. Because YF is not endemic to Asia, such patients have the potential to serve as primary sources of YFV and as index sources for subsequent clusters, outbreaks, or epidemics, not only in China, but elsewhere in Asia, which is a nightmare scenario.

The principal mosquito vector of YFV is *Aedes aegypti*, which is found in southern China and elsewhere in Asia, as are *Ae. albopictus* mosquitoes (*6*), which can also transmit YFV and serve as a bridging vector between jungle and urban cycles of YFV in a variety of ecosystems (*7*). These mosquitoes feed on humans and are found peridomestically. Mosquitoes of these species also are capable of transmitting dengue viruses, chikungunya virus, Zika virus, and other human pathogens. Their presence should serve as a warning to local health authorities of potential arbovirus disease outbreaks and, therefore, to maintain or initiate mosquito vector control programs. Most industrialized countries are aware of these warnings; the 40 YF-endemic countries, predominantly tropical areas in Africa and Central and South America (≈90% of cases reported every year occur in sub-Saharan Africa), maintain diagnostic competence and surveillance systems, including clinical findings, testing of sick nonhuman primates and arthropods, and other indicators. Four countries that produce YF vaccine have purchased stocks or have arrangements in place to obtain sufficient doses in instances of immediate need.

Because destinations of an increasing number of travelers include YF-endemic areas, national and international regulations require a recent (<10 years) verified history of vaccination against this virus; China does not have such regulations. If a person traveling to a recognized YF-endemic area is not required to be vaccinated in advanced, then they are essentially on their own with regard to self-protection, but the greater threat is to their own country, if and when they return.

Of ostensibly great concern has been 11 unvaccinated YF-infected Chinese residents and workers who returned

from Angola to China; others probably returned elsewhere, including to the Democratic Republic of the Congo, where 1,400 suspected cases (53 imported from Angola), including 82 deaths, have occurred, including some secondary cases, and to Kenya (*1*). Several YF cases have been detected recently in Uganda but are not related to the Angola outbreak. In China, all recognized YF patients have been isolated and given appropriate medical care; secondary cases have not been detected. Thus, all cases of YF in China were imported. Nonetheless, should secondary cases begin to be detected in areas in Asia to which *Ae. aegypti* or *Ae. albopictus* mosquitoes are endemic, a door would open for more widespread extension of YFV and for establishment of enzootic and endemic situations, which are unheard of in most parts of the temperate world. The last thing China in particular and Asia in general need is to become endemic for YF.

The question "why has there been no YF in Asia?" has been asked for decades, even before global climate change was recognized, and various hypotheses have been put forward. *Ae. aegypti* mosquitoes from various geographic sources and with corresponding genetic variations at different isozyme loci were infected orally with YF virus (*8*). Subsequent infection rates suggested that *Ae. aegypti* mosquito populations with genetic similarities had similar rates of infection with the virus. Tabachnick et al. concluded that there are differences between New World and Asian mosquito populations in this regard (*8*). Whether such differences might account for preclusion of YFV from Asia has not been proven. Antibodies against dengue viruses, which are widely present in Southeast Asia, might be protective against YFV infections, but studies by Izurieta et al. (*9*), Weiland et al. (*10*), and Agampodi and Wickramage (*11*) suggest otherwise. Because YFV is a zoonotic agent whose natural cycle involves nonhuman primates and mosquitoes other than *Ae. aegypti*, it is not possible to eradicate these mosquitoes by using available tools; thus, we rely on application of YF vaccine to protect humans.

Countries in YF-endemic areas or with a history of YF and that do not have adequate resources to enable them to set aside funds for controlling diseases, or that do not request assistance in formulating expanded childhood immunization programs, or that spend the funds they have available on other projects must be clearly informed that they are potential dangers to the rest of us. Obviously, not all countries need to vaccinate their residents against all viruses for which vaccines are available. However, countries not vaccinating when there are potential risks might seriously consider reevaluating their requirements for persons traveling to or returning from YF-endemic areas and for protecting key population groups, such as health and essential services workers, the adult work force, and adult females (to keep families together and take care of orphans) for postepidemic recovery.

It is self-evident to us that dose-sparing (diluting the vaccine) and postponing the next round of vaccinations (until more vaccine is produced), are the best solutions to the YF vaccine shortage. Even the 17 million doses WHO projects to be produced over the next few months will not fill current demand. From an abundance of caution, we recommend authorizing the use of a one fifth dose (*12*), before any more of the inadequate stocks are irrevocably depleted. WHO has indicated its approval to use such fractional dosage; application of this plan should be instituted immediately.

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Addresses for correspondence: Charles H. Calisher, Arthropod-borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Foothills Campus, Fort Collins, CO 80523-1690, USA; email: calisher@cybersafe.net

Pandemic

Sonia Shah, St. Martin's Press, Sarah Crichton Books/ Farrar, Straus & Giroux, New York, NY, USA, 2016; ISBN-13: 9780374122881 (hardback); ISBN-13: 9780374708740 (ebook); Pages: 288; Price: US $39.99

During the past 2 years, back-to-back epidemics of Ebola and Zika have stunned even seasoned public health officials with unprecedented levels of illness and death that have been associated with these events. Both viruses, previously thought to cause only limited outbreaks, surprised the people of the world with a seemingly sudden ability to spread across multiple countries and cause illness in new, previously unthinkable ways. As we struggle to understand both the scale and impact of the Zika and Ebola crises, we are forced to once again ask: how did this happen and what can we do to prevent the next one?

Given these events, Pandemic: Tracking Contagions, From Cholera to Ebola and Beyond by Sonia Shah makes for a very timely read. Although there have been several other books that have examined the origins of pandemics, Shah attempts to differentiate her analysis by using Rita Colwell's description of the "Cholera Paradigm" as a framework for discussing the categories of social and environmental change; for example, locomotion, filth, crowds, and social dynamics that can explain how the microbes can emerge and spread disease around the world (*1*). In doing so, Pandemic takes its reader from the initial "spillover" of cholera from copepods in the Sundarbans wetlands of the Bay of Bengal through a series of historical outbreaks, including the recent outbreaks in Haiti.

While tracking cholera's journey, Shah discusses other pathogens that have emerged and progressed down cholera's path from isolated microorganism to pandemic pathogen: severe acute respiratory syndrome, Ebola, Middle East respiratory syndrome, West Nile, and Lyme disease, as well as pathogens that have acquired antimicrobial resistance. In doing so, Shah offers useful accounting of the history of the most consequential epidemic events that have occurred in the past 50 years. Shah's personal experience in battling methicillin-resistant *Staphylococcus aureus* within her family serves as a particularly compelling illustration of how challenging even seemingly simple infections can be.

Scientists and practitioners who have been grappling with these diseases may quibble with Shah's simplified explanations. The book's central metaphor, the cholera paradigm, although somewhat instructive, is an imperfect model for explaining the rise and spread of pandemics. While some dimensions of cholera can be stretched to fit aspects of recent epidemics, there is scant evidence supporting how the central thesis of the cholera paradigm, that transmission can be in part explained by climate, applies to pandemics caused by other organisms, such as influenza. Additionally, that cholera today is largely a problem for countries without modern sanitation may create false assurances about all countries' risks for pandemics.

Seasoned practitioners who know that there are almost no magic bullets in public health will also likely take issue with Shah's discussion of potential ways to prevent and mitigate pandemics. Although real progress toward a pandemic-free world will likely require a long list of changes: some big, many of which are mundane, Shah's attempts to offer up salvation in a single chapter comes across as more naive than inspiring.

Although Pandemic doesn't offer a simple recipe for how to prevent the next pandemic, those who devote their careers to these issues should appreciate Shah's attempt to elevate public understanding of these events. Rather than portray pandemics as freak, science-fiction–esque occurrences that can be quickly resolved and then forgotten, in its extensive discussion of the common conditions that create them, Shah illustrates why we should view pandemics as expected, recurring events and, therefore, should plan to invest in systemic, long-lasting preparedness. At a time when the current public debate about responding to Zika appears to be focused on the reallocation of Ebola funding despite the ongoing challenges posed by that not-yet-over epidemic, this is a message that is worth repeating.

Jennifer B. Nuzzo

Author affiliation: University of Pittsburgh Medical Center/Center for Health Security, Baltimore, Maryland, USA

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Reference

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Address for correspondence: Jennifer B. Nuzzo, UPMC Center for Health Security, 621 E Pratt St, Ste 210, Baltimore, MD 21202, USA; email: jnuzzo@upmc.edu

Correction: Vol. 22, No. 4

The authors of Microevolution of Monophasic *Salmonella* Typhimurium during Epidemic, United Kingdom, 2005–2010 (L. Petrovska al.) have provided the following Addendum: "It has come to the authors' attention that the designation '*Salmonella* Genetic Island 3 (SGI-3)' has been previously assigned to a 31-kb genomic island in a strain of *Salmonella* Mississippi (http://dx.doi.org/10.1371/journal.pone.0041247). To avoid confusion in the literature, we propose that the SGI-3 referred in our manuscript be designated SGI-4 in future reference." In addition, some of the accession numbers listed in online Technical Appendix 1 were incorrect, and funding sources and author contact information for the article have also been updated. The article has been corrected online (http://wwwnc.cdc.gov/eid/article/22/4/15-0531_article).

Correction: Vol. 22, No. 6

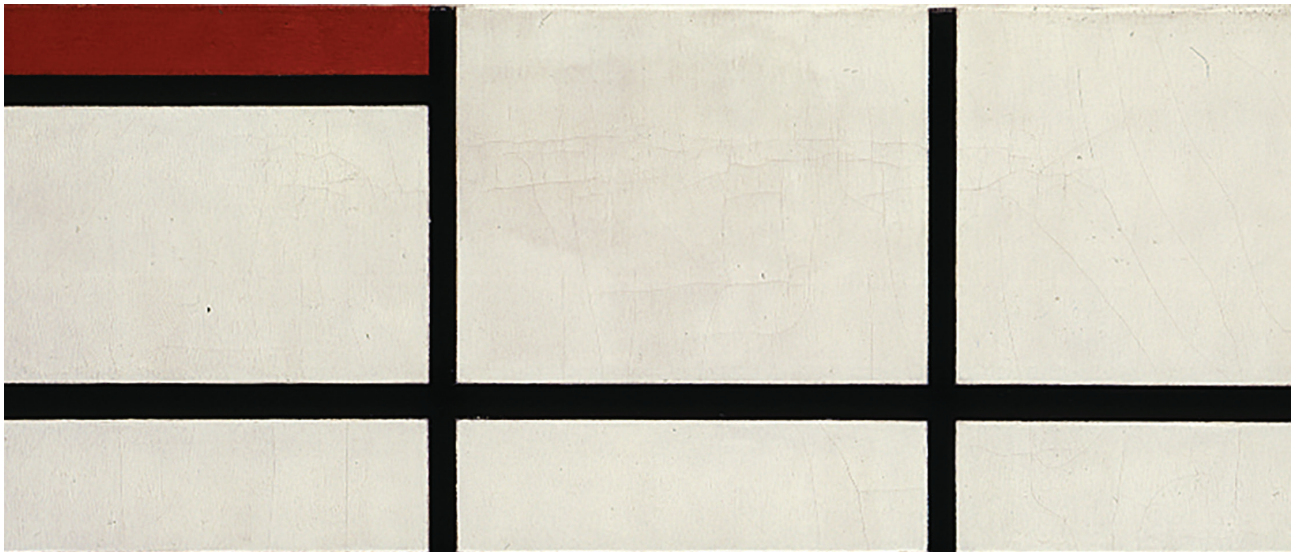
The increase in monkeypox cases in the Bokungu Health Zone of the Democratic Republic of the Congo during the second half of 2013 should have been listed as >600% in the abstract of the article in Extended Human-to-Human Transmission during a Monkeypox Outbreak in the Democratic Republic of the Congo (L.D. Nolen al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/22/6/15-0579_article).

Correction: Vol. 22, No. 6

The name of Wenqing Zhang was misspelled in the acknowledgments of Improved Global Capacity for Influenza Surve (L.S. Polansky et al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/22/6/15-1521_article).

Correction: Vol. 22, No. 6

The descriptions of pneumonia diagnoses and hospitalizations were unclear in Changes in Childhood Pneumonia Hospitalizations by Race and Sex Associated with Pneumococcal Conjugate Vaccines (A.D. Wiese al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/22/6/15-2023_article).



Piet Mondrian (1872–1944), Composition in White, Red, and Yellow (1936). Oil on Canvas, 31 1/2 in x 24 1/2 in / 80.01 cm x 62.23 cm. Public domain digital image courtesy of Los Angeles County Museum of Art, 5905 Wilshire Boulevard, Los Angeles, CA 90036.

Geometric Abstract Art and Public Health Data

Salaam Semaan

In his "Composition in White, Red, and Yellow," Dutch artist Piet Mondrian illustrated geometric abstraction. He accomplished this effect via two primary-colored units embedded in a grid of vertical and horizontal black lines that draw viewers to a flat-structured polished web. Mondrian distilled direction to perpendicular columns and rows, color to bright red and yellow and neutral white and black, shape to squares and rectangles, and form to intersecting lines and outlined units. He methodically pared down form to indispensable lines and stacked rich colors in a few blocks. Influenced by analytical cubism, which is renowned for breaking objects and images into components, Mondrian pioneered reductive form and color, through an abbreviated pictorial vernacular. In creating this seemingly simplified painting he championed a distinctive relationship between color and blocks, rendering asymmetric balance and harmony.

Black, bordering pigmented zones, creates a maze reflecting mathematical accuracy and distinct complexity. Moving a single line or changing one color might disrupt subtle accord and structural delineations. The painting portrays minimal, yet essential, information through blatant intersections, geometric shapes, and interlocking planes. While thick black lines separate blocks, the arrangement and colors radiate energy that is both kinetic and serene, stimulating and restful, dynamic and static. Mondrian imparted a delicate balance between unequal and equivalent counterparts by using indispensable and contrasting positive and negative energies of solid and void, horizontal and vertical.

Similar to the balance in Mondrian's painting, corresponding energies of time and space, art and science, and medicine and public health create momentum and energy in our compartmentalized and integrated personal and professional experiences. As public health specialists, we analyze microscopic images as well as gigantic amounts of clinical, public health, and geospatial data to develop interventions that enhance individual and population health. Through intricate scientific and geostatistical methods, scientists and healthcare providers assess relationships between risk factors and disease and between behaviors

Author affiliation: Centers for Disease Control and Prevention, Atlanta, Georgia, USA

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and well-being. We frame prevention and control of health conditions—infectious or chronic, environmental or hazardous—in units and blocks of person, place, and time.

By pioneering geoanalysis of data and using a dot map, Dr. John Snow traced the 1854 cholera outbreak in Soho, London, England, to a public water pump on Broad Street (now Broadwick Street). Disabling a well pump by removing its handle, a reductive and a seemingly simple public health intervention, credited Snow with ending an outbreak, providing advocacy for public health changes in London water and waste systems, and contributing to modern epidemiology. Then and now, visual representations of public health data, including graphs and maps, help in understanding disease causation by showing locations of cases and highlighting similarities or differences between blocks, regions, and countries with and without infection. The web of disease causation, a core public health concept, pinpoints theories on population patterns of health and disease and embodies multiple and complex factors influencing disease dynamics and differentials between and within populations.

Well into the twenty-second century, public health professionals continue to track infection, direct prevention, advance treatment, and predict trends by culling information on disease transmission. By scrutinizing mazes and grids of public health data and leveraging digital technologies, we provide insights into causes and manifestations of disease, reduce data to strategies crucial for prevention and treatment, and strive to translate science into public health. Through distilling complex epidemiologic, molecular, and geospatial data, we address also disease syndemics—two or more conditions that interact synergistically and increase morbidity and mortality. Through the media, the public receives crucial information and graphics about affected populations and locations and about health conditions that are physiologically, socially, or clinically linked.

Geospatial data, virtual grid meta-databases, grid computing concepts, spatial analytical methods, visualization or data-display techniques, and color-coded geographic visualizations: these all enhance our understanding of public health threats and facilitate control of outbreaks, endemic diseases, epidemics, and pandemics. Such methods inform research and programs on the effectiveness of vaccination programs, whole-genome sequencing analysis, and cluster detection of infections and diseases. Similar to how artists craft and reveal their expertise in color, form, and perspective—in cubist and abstract art—public health professionals leverage their expertise in epidemiology, statistics, and communication and in behavioral, social, and laboratory sciences to integrate clinical care and public health. Mondrian's network of black lines and colored blocks focuses on essence, equilibrates white-painted blocks with primary colored blocks, enunciates focal and peripheral information, and juxtaposes structural and dynamic equilibrium. We perceive similar reductive and essential processes and pictorials in public health. Art and science balance our health and enhance our lives.

Dr. Semaan is a behavioral scientist in the Division of HIV/AIDS Prevention, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, GA. She has served as volunteer docent at the Philadelphia Museum of Art and at the Atlanta High Museum. As a volunteer, she also serves on the board of directors of the Oglethorpe University Museum of Art, Atlanta, GA.

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Address for correspondence: Salaam Semaan, Division of HIV/AIDS Prevention, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop E37, Atlanta, GA 30329-4027, USA; email: svs5@cdc.gov

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Savannah, GA, USA
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October 28–30, 2016
ID Week
New Orleans, LA, USA
http://www.idweek.org/

October 29–November 2, 2016
American Public Health Association
Denver, Colorado, USA
https://www.apha.org/events-and-meetings/annual/past-and-future-annual-meetings

November 4–7, 2016
IMED
International Meeting on Emerging Diseases and Surveillance
Vienna, Austria
http://imed.isid.org/

November 13–17, 2016
ASTMH
American Society of Tropical Medicine and Hygiene
Atlanta, GA, USA
https://www.astmh.org/annual-meeting?utm_source=ASTMH%2DInformz&utm_medium=email&utm_campaign=default

November 29–December 2, 2016
Institut Pasteur International Network Scientific Symposium
Paris, France
http://www.pasteur-network-meeting2016.org/

December 3–8, 2016
ASLM
African Society for Laboratory Medicine
Cape Town, South Africa
http://aslm2016.org/

March 29–31, 2017
SHEA
Society for Healthcare Epidemiology 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/

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
Infection-Related Death among Persons with Refractory Juvenile Idiopathic Arthritis
CME Questions

1. You are seeing an 8-year-old girl with a history of polyarticular juvenile idiopathic arthritis (poly-JIA) that failed to improve after treatment with corticosteroids and methotrexate. She also had a poor response to infliximab. What should you consider regarding the management of JIA in this patient and its prognosis?

A. More than 70% of all patients with JIA will have ongoing active disease into adulthood
B. Patients with systemic JIA (So-JIA) and poly-JIA have the worst prognosis
C. The recommended practice after failure of a tumor necrosis factor-alpha antagonist is initiation of a biologic agent that blocks the action of interleukin
D. The recommended practice for this patient would be to combine 2 biologic agents with different mechanisms of action

2. The patient has a central venous catheter placed to facilitate treatment. One week later, she experiences a fever and malaise. Which of the following statements regarding characteristics of the 4 patients with juvenile idiopathic arthritis (JIA) and severe infection in the current study is most common?

A. Antibiotics were significantly delayed in all cases
B. There was a prolonged course of all infections with a slow progression toward death
C. Affected patients had no previous history of serious infections
D. Bacteria implicated in the infections included coagulase-negative Staphylococcus and alpha-hemolytic Streptococcus

3. What should you consider regarding the association between infections and the use of biologic disease-modifying antirheumatic drugs (DMARDs) for juvenile idiopathic arthritis (JIA)?

A. Treatment that targets B- and/or T-cell lymphocyte function results in immune dysfunction that mimics very rare and specific primary immunodeficiencies
B. Treatment that blocks specific inflammatory pathways such as interleukin-1 and tumor necrosis factor-alpha is associated with infections from a broad spectrum of organisms
C. Patients receiving biologic DMARDs may not present with high fever and elevated serum C-reactive protein levels
D. Deaths related to the use of biologic DMARDs are now well categorized in international patient registries

4. You are concerned regarding the potential for macrophage activation syndrome (MAS) in this patient. Which of the following is consistent with the clinical picture of MAS?

A. Central nervous system involvement is very uncommon
B. Triglyceride and D-dimer levels are elevated
C. Leukocyte count is elevated
D. Erythrocyte sedimentation rate is elevated

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

Ebola Virus Disease in Children, Sierra Leone, 2014–2015

CME Questions

1. You are advising an Ebola Holding Unit regarding anticipated outcomes from the next outbreak. According to the retrospective cohort study by Fitzgerald and colleagues, which of the following statements about mortality for patients with pediatric Ebola virus disease (EVD) is correct?

A. Case-fatality rate was 25%
B. Most deaths occurred after treatment was initiated in treatment centers
C. Median time from Ebola Holding Unit admission to death was 3 days (interquartile range 1–5 days)
D. Death was less rapid than in mixed-age cohorts

2. According to the retrospective cohort study by Fitzgerald and colleagues, which of the following statements about morbidity for patients with pediatric EVD is correct?

A. Diarrhea was more common in this study than in the international cohort study
B. Hypoglycemia affected 20% of children
C. Elevated white cell count showed lymphocytic predominance
D. Blood test results showed hepatic and renal dysfunction

3. According to the retrospective cohort study by Fitzgerald and colleagues, which of the following statements about characteristics associated with outcomes in patients with pediatric EVD is correct?

A. Mortality was associated with older age
B. Diarrhea at presentation was associated with 15% increase in the risk for death
C. The study proved that proper management can improve survival time
D. Efficacy assessment of pediatric interventions in future EVD epidemics will require robust, rapid data collection

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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Emerging Infectious Diseases is a peer-reviewed journal established expressly to promote the recognition of new and reemerging infectious diseases around the world and improve the understanding of factors involved in disease emergence, prevention, and elimination.

The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles, see below and visit http://wwwnc.cdc.gov/eid/pages/author-resource-center.htm.

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. Starting with the January 2017 volume, EID will publish 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

Synopses

Research

Dispatches

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