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EMERGING INFECTIOUS DISEASES™

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Sexually Transmitted Infections

January 2016

EMERGING INFECTIOUS DISEASES

Pages 1–168

George Caleb Bingham (1811–1879), The Jolly Flatboatmen, 1846. Oil on canvas, 38 1/8 in x 48 1/2 in/96.8 cm x 123.2 cm.
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On the Cover

George Caleb Bingham (1811–1879), *The Jolly Flatboatmen*, 1846.

Oil on canvas, 38 1/8 in × 48 1/2 in/96.8 cm × 123.2 cm. Open access digital image courtesy of the National Gallery of Art, Washington, DC, USA.

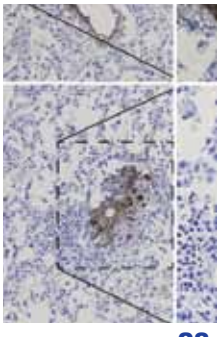
About the Cover p. 162

Synopsis

Medscape EDUCATION ACTIVITY

Epidemiology of *Haemophilus ducreyi* Infections 1
C. González-Beiras et al.

Infections are at their lowest levels worldwide, but nongenital cutaneous infections have increased.



p. 28

Research

Waterborne *Elizabethkingia meningoseptica* in Adult Critical Care..... 9
L.S.P. Moore et al.

This outbreak might reflect improved diagnostic testing, indicating that *E. meningoseptica* is a pseudo-emerging pathogen.

Human Papillomavirus Vaccination at a Time of Changing Sexual Behavior..... 18
I. Baussano et al.

Early vaccination may prevent infections in populations undergoing changes related to age and sexual activity.

p. 34

Multiorgan WU Polyomavirus Infection in Bone Marrow Transplant Recipient 24
E.A. Siebrasse et al.

Virus was detected in the lung and trachea of a deceased patient.

Multifacility Outbreak of Middle East Respiratory Syndrome in Taif, Saudi Arabia 32
A. Assiri et al.

Enhanced surveillance and infection-control practices are needed to prevent outbreaks in healthcare settings.

Medscape EDUCATION ACTIVITY

Falling *Plasmodium knowlesi* Malaria Death Rate among Adults despite Rising Incidence, Sabah, Malaysia, 2010–2014..... 41
G.S. Rajahram et al.

The decreased notification-fatality rate is likely associated with improved use of intravenous artesunate for severe malaria.

Risk Factors for Primary Middle East Respiratory Syndrome Coronavirus Illness in Humans, Saudi Arabia, 2014 49
B.M. Alraddadi et al.

Direct exposure to camels, diabetes mellitus, heart disease, and smoking were independently associated with this illness.

Human Papillomavirus Prevalence and Herd Immunity after Introduction of Vaccination Program, Scotland, 2009–2013 56
R.L. Cameron et al.

Prevalence was reduced, and early evidence indicates herd immunity.

EMERGING INFECTIOUS DISEASES™
January 2016

Dispatches

65 **Decline in Decreased Cephalosporin Susceptibility and Increase in Azithromycin Resistance in *Neisseria gonorrhoeae*, Canada**
I. Martin et al.

68 **Rapid Emergence and Clonal Dissemination of CTX-M-15–Producing *Salmonella enterica* Serotype Virchow, South Korea**
J.S. Kim et al.

71 **Avian Influenza A(H7N9) Virus Infection in 2 Travelers Returning from China to Canada, January 2015**
D.M. Skowronski et al.

75 **Surveillance of Bacterial Meningitis, Ethiopia, 2012–2013**
W. Mihret et al.

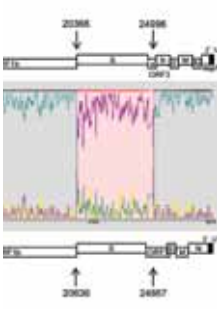
79 **Identification of Source of *Brucella suis* Infection in Human by Using Whole-Genome Sequencing, United States and Tonga**
C. Quance et al.

83 **Porcine Epidemic Diarrhea Virus and Discovery of a Recombinant Swine Enteric Coronavirus, Italy**
M.B. Boniotti et al.

88 **Increase in Sexually Transmitted Infections among Men Who Have Sex with Men, England, 2014**
H. Mohammed et al.

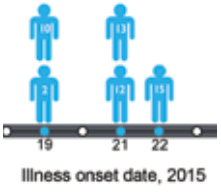
92 **Seroepidemiology of Human Enterovirus 71 Infection among Children, Cambodia**
P.F. Horwood et al.

96 **Outbreak of Panton-Valentine Leukocidin–Associated Methicillin-Susceptible *Staphylococcus aureus* Infection in a Rugby Team, France, 2010–2011**
E. Couvé-Deacon et al.



p. 86

p. 101



100 **Variations in Spike Glycoprotein Gene of MERS-CoV, South Korea, 2015**
D.-W. Kim et al.

105 **Effectiveness of Ring Vaccination as Control Strategy for Ebola Virus Disease**
A.J. Kucharski et al.

109 **Autochthonous *Nocardia cerradoensis* Infection in Humans, Spain, 2011 and 2014**
M. Ercibengoa et al.

112 **Asymptomatic Lymphogranuloma Venereum in Men who Have Sex with Men, United Kingdom**
C. Saxon et al.

117 **Increased Risk for ESBL-Producing Bacteria from Co-administration of Loperamide and Antimicrobial Drugs for Travelers' Diarrhea**
A. Kantele et al.

121 **Hemagglutinin Gene Clade 3C.2a Influenza A(H3N2) Viruses, Alachua County, Florida, USA, 2014–15**
J.A. Lednicky et al.

124 **Factors Related to Fetal Death in Pregnant Women with Cholera, Haiti, 2011–2014**
E. Schillberg et al.

128 **Rift Valley Fever Virus among Wild Ruminants, Etosha National Park, Namibia, 2011**
A.C. Dondona et al.

131 **Legionnaires' Disease in South Africa, 2012–2014**
N. Wolter et al.

134 **Severe Community-Acquired Bloodstream Infection with *Acinetobacter ursingii* in Person who Injects Drugs**
H.J.F. Salzer et al.

EMERGING INFECTIOUS DISEASES™
January 2016

Letters

138 Highly Pathogenic Avian Influenza Virus, Midwestern United States

140 Widespread Bat White-Nose Syndrome Fungus, Northeastern China

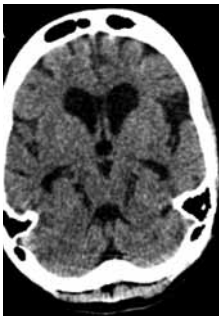
142 New Clinical Strain of *Neisseria gonorrhoeae* with Decreased Susceptibility to Ceftriaxone, Japan

144 Measles Outbreak among Adults, Northeastern China, 2014

146 Objective Determination of End of MERS Outbreak, South Korea, 2015

148 Surveillance for Coronaviruses in Bats, Lebanon and Egypt, 2013–2015

150 Ebola Virus Disease Complicated by Late-Onset Encephalitis and Polyarthritis, Sierra Leone



p. 151

152 Louseborne Relapsing Fever in Young Migrants, Sicily, Italy, July–September 2015

154 Anticipated Negative Responses by Students to Possible Ebola Virus Outbreak, Guangzhou, China

156 Multiple Fungicide-Driven Alterations in Azole-Resistant *Aspergillus fumigatus,* Colombia, 2015

157 Azole Resistance of *Aspergillus fumigatus* in Immunocompromised Patients with Invasive Aspergillosis

159 Schistosomiasis Screening of Travelers to Corsica, France

About the Cover

162 Flatboats, Travelers, Infectious Diseases, and Other River Thoughts

Etymologia

17 *Elizabethkingia*

162 Corrections
Vol. 21, No. 11
Vol. 21, No. 12

Epidemiology of *Haemophilus ducreyi* Infections

Camila González-Beiras, Michael Marks, Cheng Y. Chen, Sally Roberts, Oriol Mitjà

Medscape EDUCATION ACTIVITY

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Release date: December 17, 2015; Expiration date: December 17, 2016

Learning Objectives

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

- Distinguish the clinical presentation of genital ulcer disease with *Haemophilus ducreyi*
- Assess the means used to diagnose *H. ducreyi* infection
- Identify global areas disproportionately affected by *H. ducreyi*–related genital ulcer disease
- Assess worldwide trends in the epidemiology of infection with *H. ducreyi*

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Disclosures: ***Camila González-Beiras, BSc, MSC; Michael Marks, MBBS; Cheng-Yen Chen, PhD; Sally Roberts, MBChB, FRACP, FRCPA;*** *and* ***Oriol Mitjà, MD, PhD,*** *have disclosed no relevant financial relationships.*

The global epidemiology of *Haemophilus ducreyi* infections is poorly documented because of difficulties in confirming microbiological diagnoses. We evaluated published data on the proportion of genital and nongenital skin ulcers caused by *H. ducreyi* before and after introduction of syndromic management for genital ulcer disease (GUD). Before 2000, the proportion of GUD caused by *H. ducreyi* ranged from 0.0% to 69.0% (35 studies in 25 countries). After 2000, the proportion ranged from 0.0% to 15.0% (14 studies in 13 countries). In contrast, *H. ducreyi* has been recently identified as a causative agent of skin ulcers in children in the tropical regions; proportions ranged from 9.0% to 60.0% (6 studies in 4 countries). We conclude that, although there has been a sustained reduction in the proportion of GUD caused by *H. ducreyi*, this bacterium is increasingly recognized as a major cause of nongenital cutaneous ulcers.

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Haemophilus ducreyi, a fastidious gram-negative bacterium, is the causative agent of chancroid, a genital ulcer disease (GUD). The organism is usually spread during sexual intercourse through microabrasions, and the disease usually manifests as multiple painful superficial ulcers associated with inguinal lymphadenitis (*1*). As a result of the painful nature of the lesions, patients usually seek immediate treatment, and asymptomatic carriage is therefore uncommon (*2*). In addition to causing GUD, *H. ducreyi* has been found in several recent studies to be a major cause of chronic skin ulceration in children from developing countries (*3*–*5*).

The global epidemiology of chancroid is poorly documented, and it is not included in World Health Organization estimates of the global incidence of curable sexually transmitted infections (STIs). There are some key challenges in interpreting data on the epidemiology of *H. ducreyi* as a causative agent of GUD. First, genital herpes cases are easily misdiagnosed as chancroid on clinical examination. Thus, reports based only on clinical diagnosis can be erroneous. Second, laboratory culture is technically difficult, and the highly sensitive and specific nucleic acid amplification tests, such as PCR, are rarely available outside national reference laboratories or specialized STI research settings, which makes it difficult to confirm clinical diagnoses.

Determination of the true global incidence of chancroid is made more difficult by widespread adoption of syndromic management for bacterial GUD (i.e., treatment with antimicrobial drugs effective against syphilis and chancroid) without microbiological confirmation in many countries. Therefore, countries often report only the total number of GUD cases. In addition, identification of GUD etiology is rarely conducted in resource-poor countries to validate syndromic management for which chancroid could also be common.

Earlier studies of tropical skin ulcers did not generally test for *H. ducreyi,* with the exception of a small number of case reports. There are major limitations in describing the prevalence of causative agents in tropical skin lesions that typically occur in children in rural areas where there is no access to laboratory facilities. Pathogens such as *Fusobacterium fusiforme, Staphylococcus aureus*, and *Streptococcus pyogenes* have been reported from Gram staining of exudative material collected from tropical ulcers (*6*). However, cultures or PCR testing for definitive identification of fastidious pathogens involved has not been traditionally conducted. The purpose of this study was to improve our understanding of the epidemiology of *H. ducreyi* infection through a systematic review of published data on the proportion of genital and skin ulcers caused by this bacterium.

Methods

Search Strategy and Selection Criteria

A systematic review was conducted to identify all relevant studies that examined the etiology of GUD and nongenital skin ulcers involving *H. ducreyi*. We searched the National Library of Medicine through PubMed for "*H. ducreyi*," "chancroid," "genital ulcer," OR "skin ulceration" AND "proportion" OR "prevalence." The search was limited to studies published during January 1, 1980–December 31, 2014. In addition, we searched references of identified articles and other databases for other articles, and we reviewed abstracts, titles, and selected studies potentially containing information on chancroid epidemiology. We contacted researchers who were working with *H. ducreyi* to identify unpublished literature for inclusion. No language restrictions were set for searches.

The decision tree for inclusion or exclusion of articles is shown in Figure 1. We included studies if the proportion of etiologic agents in genital ulcers and nongenital skin ulcers, including *H. ducreyi*, was confirmed by laboratory techniques. Clinical diagnosis of chancroid is often based on the appearance of the ulcer, which is characteristically painful, purulent, and deep with ragged, undermined edges (Figure 2). However, because the appearance of these ulcers is similar to ulcers caused by other bacteria, clinical diagnosis can be nonspecific or insensitive and often requires laboratory confirmation (*1*). In addition, microscopy identification of typical morphologic features and serologic detection lack sensitivity and specificity (*7*,*8*). Thus, we only considered the following diagnostic methods as providing acceptable evidence of *H. ducreyi* infection: 1) isolation and identification by culture; or 2) PCR/real-time PCR.

Data Extraction and Synthesis

For all qualifying studies, extracted data included study country, year of study, diagnostic test used for confirmation, total number of *H. ducreyi*–positive cases, and sample size. Descriptive analyses of extracted data were conducted, and the number of *H. ducreyi*–confirmed cases was divided by the total number of cases to calculate the proportion of cases caused by *H. ducreyi.* Studies qualifying for data extraction were grouped into 2 categories: studies conducted before 2000 and studies after 2000. This date separates studies before and after widespread implementation of syndromic management of GUD. Study sites were also plotted by geographic region. No quantitative metaanalysis was undertaken.

Results

We identified 277 records in which we found 46 articles describing 49 studies on GUD that met our inclusion criteria

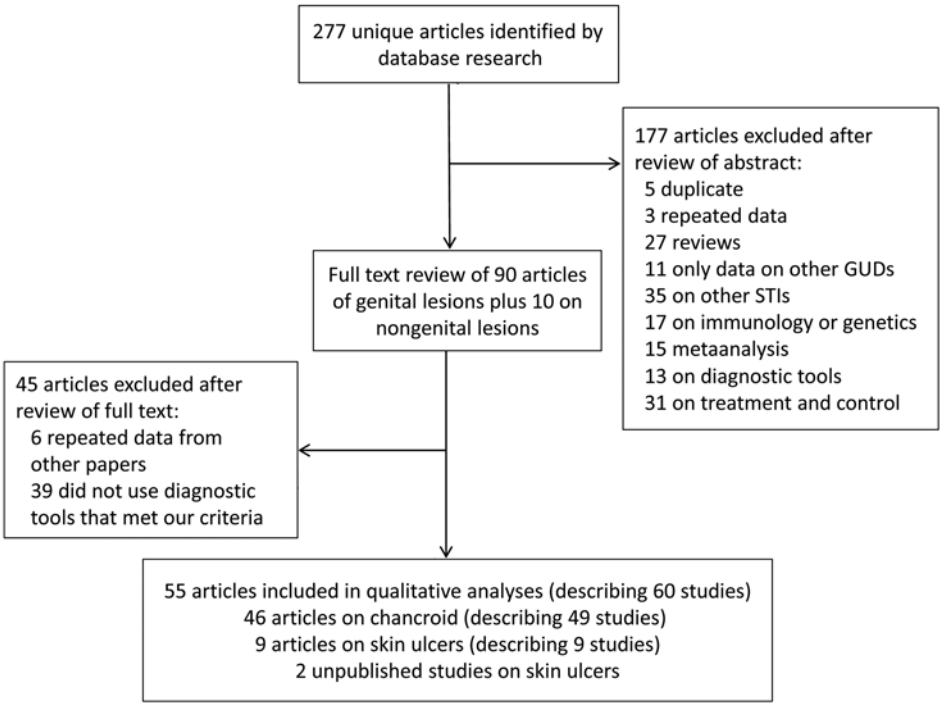

Figure 1. Procedure for selecting eligible references on the epidemiology of *Haemophilus ducreyi* as a causative agent of genital ulcers. GUDs, genital ulcer disease; STI, sexually transmitted infections.

(Tables 1, 2; online Technical Appendix, http://wwwnc.cdc.gov/EID/article/21/1/15-0425-Techapp1.pdf). All identified studies were based on cohorts of patients attending STI clinics, including 3 studies that enrolled only commercial sex workers. The age group for all cases was adults >18 years of age, except for 3 studies in Zambia, South Africa, and China, which included patients >16 years of age, and 1 study in Madagascar, which included patients >14 years of age. A total of 9 published studies and 2 unpublished reports that described nongenital skin ulcers caused by *H. ducreyi* were also included in our systematic review.

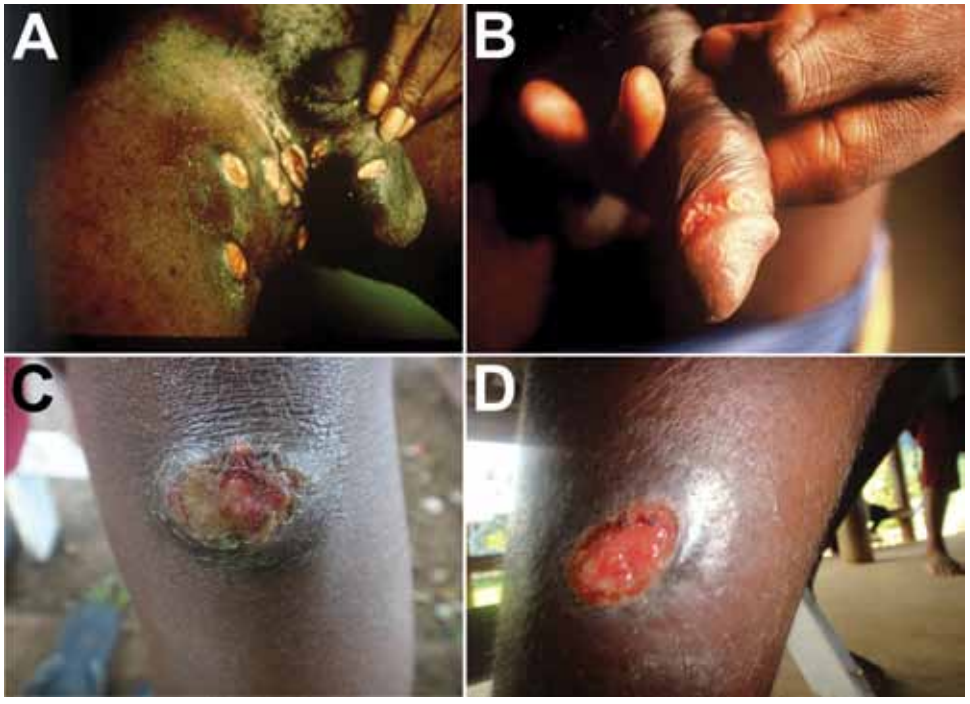

Figure 2. Ulcers caused by infection with *Haemophilus ducreyi*. A, B) Genital ulcers in adult patients from Ghana (provided by David Mabey). C, D) Skin ulcers in children from Papua New Guinea (provided by Oriol Mitjà).

Table 1. Characteristics of 35 studies of genital ulcers caused by *Haemophilus ducreyi*, 1980–1999*

Area, reference†	Country	Year of study	Diagnostic method	No. patients with GUD	No. cases *H. ducreyi* infection	% (95% CI)
Africa						
Paz-Bailey et al. (*16*)	Botswana	1993	Culture	108	27	25.0 (17.7–33.9)
Steen (*17*)	Côte d'Ivoire	1996	PCR	NA	NA	47
Mabey et al. (*18*)	Gambia	1987	Culture	104	54	51.9 (42.4–61.2)
Hawkes et al. (*19*)	Gambia	1995	M-PCR	18	8	44.4 (24.5–66.2)
Nsanze et al. (*20*)	Kenya	1980	Culture	97	60	61.8 (51.9–70.9)
Kaul et al. (*21*)	Kenya	1997	Culture	189	54	28.5 (22.6–35.3)
Morse et al. (*22*)	Lesotho	1994	M-PCR	105	55	53.3 (43.8–62.6)
Harms et al. (*23*)	Madagascar	1992	Culture	12	61	19.6 (11.6–31.3)
Behets et al. (*24*)	Madagascar	1997	M-PCR	196	64	32.6 (26.4–39.5)
Behets et al. (*25*)	Malawi	1995	M-PCR	778	204	26.2 (23.2–29.4)
Hoyo et al. (*26*)	Malawi	1999	M-PCR	137	41	29.0 (22.8–38.0)
Bogaerts et al. (*27*)	Rwanda	1992	Culture	395	115	29.1 (24.8–33.7)
Totten et al. (*28*)	Senegal	1992	PCR	39	22	56.4 (40.9–70.7)
Crewe-Brown et al. (*29*)	South Africa	1981	Culture	100	45	45 (35.5–54.7)
Dangor et al. (*30*)	South Africa	1989	Culture	240	164	68.3 (62.2–73.8)
Chen et al. (*31*)	South Africa	1994	M-PCR	538	171	31.7 (27.9–35.8)
Lai et al. (*32*)	South Africa	1994	M-PCR	160	232	68.9 (62.7–74.5)
	South Africa	1998	M-PCR	94	186	50.5 (43.4–57.6)
Meheus et al. (*33*)	Swaziland	1979	Culture	155	68	43.8 (36.3–51.7)
Ahmed et al. (*34*)	Tanzania	1999	PCR	102	12	11.7 (6.8–19.4)
Le Bacq et al. (*35*)	Zimbabwe	1991	Culture	90	22	24.4 (16.7–34.2)
Asia						
Wang et al. (*36*)	China	1999	M-PCR	96	0	0.0 (0.0–3.8)
Risbud et al. (*37*)	India	1994	M-PCR	302	84	27.8 (23.0–33.1)
Rajan et al. (*38*)	Singapore	1983	Culture	670	56	8·3 (6·4–10·7)
Beyrer et al. (*15*)	Thailand	1996	M-PCR	38	0	0.0 (0.0–9.1)
North America						
Dillon et al. (*39*)	United States	1990	Culture	82	27	32.9 (23.7–43.6)
Mertz et al. (*40*)	United States	1995	M-PCR	143	56	39.1 (231.5–47.3)
Mertz et al. (*41*)	United States	1996	M-PCR	516	16	3.1 (1.9–4.9)
South America						
Sanchez et al. (*42*)	Peru	1995	M-PCR	61	3	4.9 (1.6–13.4)
Caribbean						
Sanchez et al. (*42*)	Dominican Republic	1996	M-PCR	81	21	25.9 (17.6–36.4)
Behets et al. (*43*)	Jamaica	1996	M-PCR	304	72	23·6 (19.2–28.7)
Bauwens et al. (*44*)	Bahamas	1992	PCR	47	7	14·8 (7.4–27.6)
Middle East						
Madani et al. (*45*)	Saudi Arabia	1999	Culture	3,679	78	2.1 (1.7–2.5)
Europe						
Kyriakis et al. (*46*)	Greece	1996	Culture	695	32	4.6 (3.2–6.4)
Bruisten et al. (*47*)	The Netherlands	1996	M-PCR	368	3	0.8 (0.2–2.3)

*GUD, genital ulcer disease; NA, not available; M-PCR, multiplex PCR.
†References *41–47* provided in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/22/1/15-0425-Techapp1.pdf).

Laboratory confirmation of chancroid by PCR or culture was reported in 33 (67%) and 16 (32%) of the 49 studies, respectively. Of 16 studies that used culture, 7 (43%) used Mueller-Hinton agar with a nutritional supplement (e.g., IsoVitalex; Becton Dickinson, Franklin Lakes, NJ, USA), 1% used hemoglobin, and 5 (31%) used chocolate agar–based media; the remaining studies used other culture media. Five (31%) of 16 studies incubated agar plates at low temperatures (33°C–35°C), and 2 (12%) incubated plates at 36°C. Remaining articles did not specify incubating temperature.

Different PCR primer targets were used to amplify DNA sequences, including the 16S rRNA gene, the *groEL* gene, and the hemolysin gene. In addition to herpes simplex virus (HSV) PCR, 23 studies used a multiplex PCR that could simultaneously detect the 3 major causes of GUD (*H. ducreyi*, *Treponema pallidum*, and HSV types 1 and 2) (*9*). Studies encompassed 33 countries: 17 in Africa, 4 in Southeast Asia, 3 in Europe, 2 in the Middle East, 3 in South America, and 2 in the Caribbean, 1 in the United States, and 1 in Australia.

Incidence of Chancroid

Of 49 studies on chancroid analyzed, 35 were published during 1980–1999 (Table 1) and 14 during 2000–2014 (Table 2). In general, data showed a clear decrease in the proportion of chancroid during 1980–2014 in all areas analyzed (Figure 3).

During 1980–1999, the proportion of genital ulcers caused by *H. ducreyi* in these studies ranged from 0.0%

Table 2. Characteristics of 14 studies of genital ulcers caused by *Haemophilus ducreyi*, 2001–2014*

Area, reference†	Country	Year of study	Diagnostic method	No. patients with GUD	No. cases *H. ducreyi* infection	% (95% CI)
Africa						
Paz-Bailey et al. (*16*)	Botswana	2002	PCR	137	1	0.7 (0.1–4.0)
Mehta et al. (*48*)	Kenya	2007	M-PCR	59	0	0.0 (0.0–6.1)
Phiri et al. (*49*)	Malawi	2006	M-PCR	398	60	15.0 (11.8–18.9)
Zimba et al. (*50*)	Mozambique	2005	PCR	79	3	3.8 (1.3–10.9)
Tobias et al. (*51*)	Namibia	2007	PCR	199	0	0.0 (0.0–1.8)
O'Farrell et al. (*52*)	South Africa	2004	M-PCR	162	2	1.2 (0.3–4.6)
Lewis et al. (*53*)	South Africa	2006	M-PCR	613	10	1.6 (0.9–2.9)
Nilsen et al. (*54*)	Tanzania	2001	PCR	232	12	5.1 (2.9–8.8)
Suntoke et al. (*55*)	Uganda	2006	M-PCR	100	2	2.0 (0.5–7.0)
Makasa et al. (*56*)	Zambia	2010	PCR	200	0	0 (0.0–1.8)
South America						
Gomes Naveca et al. (*57*)	Brazil	2009	PCR	434	0	0 (0.0–0.8)
Middle East						
Maan et al. (*58*)	Pakistan	2009	Culture	521	20	3.8 (2.5–5.8)
Europe						
Hope-Rapp et al. (*59*)	France	2005	Culture	278	8	2.8 (1.4–5.5)
Oceania						
Mackay et al. (*60*)	Australia	2002	M-PCR	64	0	0.0 (0.0–5.6)

*GUD, genital ulcer disease; M-PCR, multiplex PCR.
†References *48*–*60* provided in the online Technical Appendix (http://wwwnc.cdc.gov/EID/article/22/1/15-0425-Techapp1.pdf).

in Thailand and China to 68.9% in South Africa (Table 1). Eleven (31.4%) studies reported high proportions (>40%) of cases of infection with *H. ducreyi*. All of these studies were conducted in countries in Africa (CÔte d'Ivoire, Gambia, Kenya, Lesotho, Senegal, South Africa, and Swaziland). Slightly lower proportions (20%–40% of cases) were observed in 15 (42%) studies: 10 in countries in Africa, 2 in the United States during localized outbreaks, 1 in Jamaica, 1 in the Dominican Republic, and 1 in India.

Only a few countries reported low proportions (<10%) of genital ulcers infected with *H. ducreyi*, including Singapore (8.3%), Peru (5%), Greece (4.6%), the Netherlands (0.9%), United States (3.1%), and Saudi Arabia (2.1%). The study in Saudi Arabia was conducted during 1995–1999; a total of 27,490 patients were examined for STIs. Chancroid was diagnosed by culture and was reported as the least common STI during this survey. The only studies that reported no cases of chancroid were conducted in Thailand in 1996 and China in 1999; both studies used multiplex PCR for detection of GUD cases.

During 2000–2014, the proportion of *H. ducreyi* infections was low (<10%) in all studies analyzed, except for 1 study in Malawi (15%) (Table 2). Studies in 5 countries (Kenya, Namibia, Zambia, Brazil, and Australia) did not report any cases of infection with *H. ducreyi*. Other studies reporting proportions of infections <10% were conducted in Botswana, Mozambique, South Africa, Uganda, Pakistan, and France. No reports were found for studies in North America, Southeast Asia, or the Caribbean.

Nongenital Skin Infections with *H. ducreyi*

During 1988–2010, several case reports described 4 children and 4 adults with nonsexually transmitted infections with *H. ducreyi* that manifested as lower leg lesions but no genital lesions. The reported case-patients were travelers who had been to Fiji (reference *55* in online Technical Appendix), Samoa (reference *56* in online Technical Appendix), Vanuatu (reference *57* in online Technical Appendix), or Papua New Guinea (reference

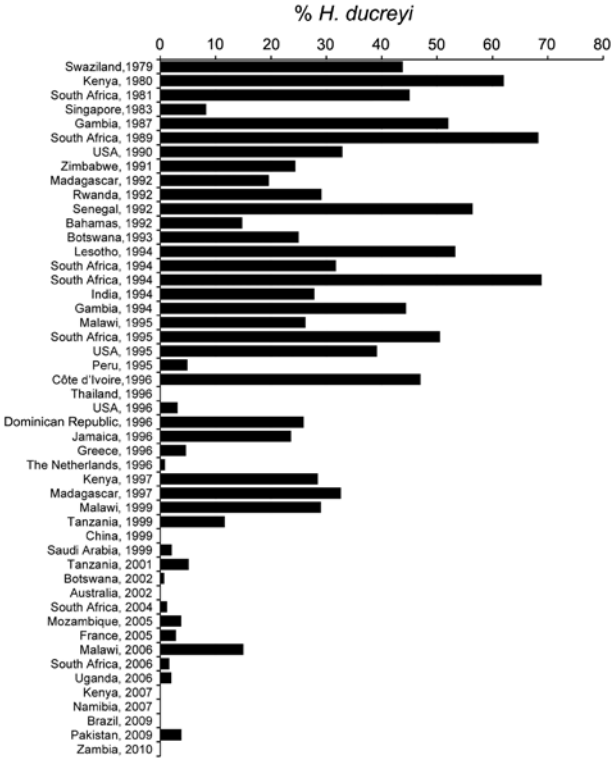

Figure 3. Trend of proportion of genital ulcers caused by infections with *Haemophilus ducreyi,* 1979–2010.

Table 3. Characteristics of 11 studies on skin ulcers caused by *Haemophilus ducreyi*, 1988–2014*

Reference	Country	Year of study	Diagnostic method	No. patients with skin ulcers	No cases *H. ducreyi* infection	% (95% CI)
Marckmann et al. (*7*)	Fiji Islands	1988	Culture	1 man	1	NA
Ussher et al. (*8*)	Samoa	2005	PCR	3 girls <10 y of age	3	NA
McBride et al. (*9*)	Vanuatu	2007	PCR	1 woman	1	NA
Peel et al. (*10*)	Vanuatu and Papua New Guinea	2010	PCR	2 men	2	NA
Humphrey et al. (*11*)	Sudan	2007	PCR	1 boy	1	NA
Mitjà et al. (*3*)	Papua New Guinea	2013	PCR	90	54	60.0 (49.6–69.5)
Mitjà et al. (*6*)	Papua New Guinea	2014	PCR	114	60	60.1 (54.3–65.5)
Marks et al. (*4*)	Solomon Islands	2013	PCR	41	13	31.7 (19.5–46.9)
Chen et al.†	Vanuatu	2013	PCR	176	68	38.6 (31.7–46.0)
Chen et al.†	Ghana	2013	PCR	179	49	27.3 (21.3–34.3)
Ghinai et al. (*5*)	Ghana	2014	PCR	90	8	8.8 (4.5–16.5)

*NA, not applicable.
†Pers. comm.

58 in online Technical Appendix) (Table 3). Outside the south Pacific region, a 5-year-old refugee from Sudan who had lower leg ulceration was also given a diagnosis of infection with *H. ducreyi* (reference *59* in online Technical Appendix).

A cohort study conducted in Papua New Guinea in 2014 showed evidence that *H. ducreyi* is a major cause of chronic skin ulceration; *H. ducreyi* DNA was identified by PCR in 60.0% of skin lesions in children (*3*). Similar studies in other areas reported laboratory-confirmed skin ulcers in children caused by *H. ducreyi* in Papua New Guinea (reference *60* in online Technical Appendix), Solomon Islands (*4*), Vanuatu (C.Y. Chen, pers. comm.), and Ghana (*5*) (Table 3).

Discussion

Our review confirmed 2 major findings. First, reduction in the proportion of genital ulcers caused by *H. ducreyi* has been sustained for the past decade and a half. Second, there is increasing evidence that *H. ducreyi* is a common and newly recognized causative agent of chronic skin ulceration in children from developing countries.

In the 1990s, the global prevalence of chancroid was estimated to be 7 million (*11*). Chancroid was one of the most prevalent GUDs, particularly in resource-poor countries in Africa, Asia, Latin America, and the Caribbean (*1*; reference *45* in online Technical Appendix). Recommendations to introduce syndromic management for treatment of GUD caused by bacteria were published by the World Health Organization in 1991 and fully implemented by 2000 (reference *61* in online Technical Appendix). Since that time, global incidence of GUDs, particularly chancroid, has decreased substantially, and genital herpes viruses (HSV-1 and HSV-2) have become the predominant cause of GUD (reference *47* in online Technical Appendix). Currently in Europe and the United States, chancroid is restricted to rare sporadic cases. Transmission of *H. ducreyi* remains ongoing in only a few countries that have limited access to health services (*2*; reference *60* in online Technical Appendix).

Our data show marked decreases in the proportion of GUD caused by *H. ducreyi* in several countries. Spinola et al. reported similar conclusions obtained from 25 PCR-based studies (reference *62* in online Technical Appendix). For example, in Botswana (*10*), Kenya, (*14*), and South Africa (*23*), the proportion of GUD caused by *H. ducreyi* decreased from 25%–69% to negligible (0.0%–1.2%) levels (*10*; references *42, 46* in online Technical Appendix). Studies in Zambia (reference *50* in online Technical Appendix), Namibia (reference *45* in online Technical Appendix), Brazil (reference *51* in online Technical Appendix), and China (*30*) did not report any cases of chancroid during 2000–2009. A study in Thailand reported elimination of chancroid by introduction of a condom use program in the 1990s (reference *63* in online Technical Appendix). Similar decreases have been reported from Cambodia and Sri Lanka, with rapid elimination of chancroid and congenital syphilis in most settings (reference *63* in online Technical Appendix). However, these findings should be interpreted with caution because, given the short duration of infectivity, even a low prevalence of *H. ducreyi* in a population with GUD implies that a reservoir of infected persons with a high rate of sex partners is present.

Recent research has identified *H. ducreyi* as a previously unrecognized cause of nongenital skin ulcers in tropical areas. In 2013–2015, six studies in Papua New Guinea (*3*; reference *60* in online Technical Appendix), the Solomon Islands (*4*), Vanuatu (C.Y. Chen et al., pers. comm.), and Ghana (5; C.Y. Chen et al., pers. comm.) showed that a high proportion of laboratory-confirmed skin ulcers were caused by *H. ducreyi*. Nearly half of the 690 enrolled patients with ulcers in these 6 studies had *H. ducreyi* detectable by PCR, whereas other bacteria, such as *T. pallidum* subsp. *pertenue*, the causative agent of yaws, were detected in 25% of patients.

These cases of infection with *H. ducreyi* confirmed by molecular analysis suggest that clinicians should be more aware of this newly recognized bacterium in skin ulcers of persons in tropical areas. In the context of new efforts to eradicate yaws, mass treatment with azithromycin in

Papua New Guinea reduced the absolute prevalence of ulcers not caused by yaws, which were mainly caused by *H. ducreyi*, from 2.7% to 0.6% (prevalence ratio 0.23, 95% CI 0.18–0.29) at 12 months after treatment (6). However, persistence of *H. ducreyi* at low levels after mass treatment in Papua New Guinea (*3*) and Ghana (*5*) suggest that 1 round of mass treatment might not be successful in eradicating *H. ducreyi* skin ulcers.

Our review has several limitations. First, the increase in HSV-related GUD as a result of immunosuppression by HIV infection would result in a decrease in the proportion of chancroid among all GUD case-patients. Second, the lack of sequential studies performed in similar clinical settings at multiple time points precludes an optimal interpretation of the apparent decrease. Third, results might be affected by poor-quality data from many developing countries and might be inflated by publication bias. Fourth, PCR is more sensitive than culture. Therefore, increasing diagnostic yield might have partially masked the scale of the decrease in *H. ducreyi* as a cause of GUD.

In summary, we observed a quantitative and sustained reduction in cases of chancroid as a result of antimicrobial drug syndromic management and major social changes. In addition, data from several research groups indicate that *H. ducreyi* can cause nongenital skin lesions in persons residing in different regions. Further studies of this newly described pathogen skin disease association are required, and appropriate policies are needed that include the routine practice of managing tropical skin ulcers.

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Waterborne *Elizabethkingia meningoseptica* in Adult Critical Care[1]

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Elizabethkingia meningoseptica is an infrequent colonizer of the respiratory tract; its pathogenicity is uncertain. In the context of a 22-month outbreak of *E. meningoseptica* acquisition affecting 30 patients in a London, UK, critical care unit (3% attack rate) we derived a measure of attributable morbidity and determined whether *E. meningoseptica* is an emerging nosocomial pathogen. We found monomicrobial *E. meningoseptica* acquisition (n = 13) to have an attributable morbidity rate of 54% (systemic inflammatory response syndrome ≥2, rising C-reactive protein, new radiographic changes), suggesting that *E. meningoseptica* is a pathogen. Epidemiologic and molecular evidence showed acquisition was water-source–associated in critical care but identified numerous other *E. meningoseptica* strains, indicating more widespread distribution than previously considered. Analysis of changes in gram-negative speciation rates across a wider London hospital network suggests this outbreak, and possibly other recently reported outbreaks, might reflect improved diagnostics and that *E. meningoseptica* thus is a pseudo-emerging pathogen.

Elizabethkingia meningoseptica (formerly *Flavobacterium meningosepticum* and, during 1994–2005 *Chryseobacterium meningosepticum*) (*1*) is a gram-negative nonfermenting obligate aerobe. It is widely distributed in the environment (*2*), yet also an acknowledged opportunistic human pathogen. Most frequently associated with neonatal meningitis (*3*,*4*), the organism also has been described in osteomyelitis (*5*) and skin structure infections (*6*,*7*). In addition, *E. meningoseptica* has been associated with colonization of the respiratory tract in ventilated adult patients, but causation of ventilator-associated pneumonia in this cohort is less clear; some studies have attributed pathogenicity (*8*–*10*), but others have found no attributable disease from colonization (*11*,*12*). Outbreaks have been linked to hospital water sources in adult critical care units (*8*,*13*); these outbreaks have been suggested to be attributable to the tolerance exhibited by *Elizabethkingia* species to such environments.

Challenges in the laboratory diagnosis of this organism complicate a true understanding of its role in disease. Difficulties in culture, including variable (strain-dependent) growth on MacConkey agar (*1*) and misidentification on some automated laboratory platforms (*4*,*7*), contribute to diagnostic challenges. Recent changes to clinical laboratory practice, particularly the widespread adoption of matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry, has improved confidence in identification of nonfermenting gram-negative organisms (*14*) and specifically facilitated rapid identification of *E. meningoseptica* from patient samples (*15*). How this advance confounds the reported epidemiology of this organism remains unclear and contributes to the lack of clarity around attributable illness. In the context of recent increased international reporting of *E. meningoseptica* outbreaks among adults, including in the United States (*10*), Brazil (*16*), South Asia (*17*), and Southeast Asia (*8*,*18*), establishing whether *E. meningoseptica* is an emerging pathogenic organism is essential.

We report a retrospective observational study detailing an outbreak of *E. meningoseptica* acquisition in a London teaching hospital adult critical care unit in accordance with the ORION protocol (*19*), analyzing the clinico-physiologic response of patients who acquired *E. meningoseptica*, and deriving a measure of attributable illness. We analyzed case identification in the context of the wider changes to diagnostic laboratory practice to determine whether *E. meningoseptica* is an emerging, or pseudo-emerging (i.e., previously present but unidentified or underidentified), organism (*20*).

Materials and Methods

Setting

The outbreak occurred in a 16-bed critical care unit in a West London teaching hospital that receives acute medicine, acute

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[1]Preliminary findings of this study were presented, in part, at the 27th European Society for Intensive Care Medicine conference, Barcelona, Spain, September 27– October 1, 2014.

surgery, tertiary referral vascular surgery, and major trauma patients from a 400-bed London teaching hospital. The hospital is part of a wider 5-hospital network across West London with overarching institutional policies, including for infection prevention and control and antimicrobial stewardship. The critical care unit comprises 8 en suite single rooms (each with a room sink and a lobby sink) and two 4-bed bays (with 1 clinical sink per bed). Infrared taps are used in all clinical sinks. An off-site sterilization facility processes all endoscopes and procedural equipment. Critical care staffing levels meet mandatory requirements, and a multidisciplinary infection control team provides support with daily critical care antimicrobial rounds. A critical care resistant-organism screening program is in place, and all patients admitted for critical care have cross-infection screening comprising methicillin-resistant *Staphylococcus aureus* sampling (nasal and groin) at admission and then weekly, and resistant gram-negative organism sampling (rectum and throat) once per week.

Microbiological Investigation

A centralized microbiology laboratory processes samples from the 5-hospital network in accordance with standard UK laboratory operating procedures (*21*). Specifically, cross-infection sample processing occurs in line with detection of extended-spectrum β-lactamase/carbapenem-hydrolyzing organism protocols (*21*). Blood cultures are incubated by using a BACTEC system (Becton Dickinson, Franklin Lakes, NJ, USA). Since June 2011, organisms have been identified by Biotyper MALDI-TOF mass spectroscopy (Bruker Daltonik GmbH, Bremen, Germany) with previously described methods used for identifying nonfermenting gram-negative organisms (*14*,*22*); previously, identification was by API (bioMérieux, Marcy l'Etoile, France). Susceptibility testing is by disk diffusion using British Society of Antimicrobial Chemotherapy methods and interpretative criteria (*23*). A representative of the outbreak strain underwent MIC determination by using agar dilution for a broad range of antimicrobial agents at a national reference laboratory (*24*).

Water from all clinical taps in the critical care unit was sampled for bacterial colonization in July 2012, July 2013, and December 2013. A total of 100 mL of water was collected from each tap, filtered by using a 0.45-μ filter membrane, and incubated on MacConkey agar in air at 37°C for 48 hours. Oxidase-positive non–lactose-fermenting colonies were subcultured onto nutrient agar and a 10-μg meropenem disk placed on the inoculum. Organisms displaying meropenem resistance were further identified by using MALDI-TOF mass spectrometry. Clinical and environmental isolates were compared by using pulsed-field gel electrophoresis (PFGE) of XbaI-digested isolate genomic DNA as previously described (*25*), except that switch times of 1–25 seconds were used.

Cases

The index case was identified on January 12, 2012, in a patient from whom *E. meningoseptica* was grown from a respiratory tract sample. This patient and all those in whom *E. meningoseptica* was subsequently isolated from clinical or screening samples were defined as case-patients and are analyzed here. During January 2012–October 2013, we identified 30 cases from among 983 new patients admitted to the critical care unit.

Determination of Attributable Illness

All case-patients had retrospective interrogation of their electronic critical care records to determine clinico-physiologic parameters (pulse rate, oxygen requirements, temperature, C-reactive protein [CRP], leukocyte count, chest radiography); primary outcomes (discharge from critical care, death during admission); and antimicrobial history. The microbiology information management system was interrogated to identify all relevant isolates in the 7 days before or after acquisition, to which any evident clinical infection could otherwise be attributed. After excluding patients in whom multiple organisms were identified, we were able to identify case-patients with monomicrobial *E. meningoseptica* acquisition and, in this subgroup, analyze the trend in clinico-physiologic parameters in the 48 hours before and after acquisition. Three systemic inflammatory response syndrome (SIRS) parameters were investigated (because most patients were ventilated, respiratory rate as a parameter was excluded): new temperature change to <36°C or >38°C, new increase in pulse rate to >90 beats per minute, and new change in leukocyte count to <4 $>12 \times 10^9$ cells/L. We investigated 3 additional criteria: new rise in fraction of inspired oxygen requirement >0.1, new CRP >100 mg/L, and new pulmonary infiltrates on plain chest radiography.

Outbreak Investigation

We undertook spatiotemporal analysis of cases by correlating bed occupancy of confirmed case-patients against each other and possible environmental reservoirs to identify possible routes of cross-transmission or point sources. This analysis was reviewed against sequential interventions to determine effectiveness in outbreak curtailment. Data from serial routine 6-monthly antimicrobial use point-prevalence studies (conducted across the hospital network) were analyzed to identify trends in antimicrobial use. We also analyzed the microbiology information management system to identify any other *E. meningoseptica* in the wider 5-hospital network during the outbreak period and for the 2 preceding years. This analysis enabled identification of any possible out-of-cohort secondary cases and enabled a wider analysis of the epidemiology of *E. meningoseptica* within the hospital

network. Ethical approval was not required for this study; outbreak investigation and analysis was classed as service evaluation by the head of regulatory compliance at the host institute.

Results

We identified 30 patients as acquiring *E. meningoseptica* during the outbreak, yielding an attack rate of 3% for patients admitted to critical care. The median age of *E. meningoseptica* case-patients was 45 years (range 17–83 years); 73% were male (Table 1), compared with a critical care all-admission median age of 55 years (range 8–95 years) and 68% male. Before *E. meningoseptica* acquisition, the median time spent in the critical care unit was 17 days (range 4–35 days), and 26 patients had received broad-spectrum antimicrobial drug regimes (piperacillin/tazobactam or meropenem) in the week preceding acquisition. Of the 30 patients in whom *E. meningoseptica* was identified, 24 had the organism isolated from specimens taken for a clinical indication; for 6, the organism was isolated only through routine screening.

Microbiological Investigation

Identification of isolates from patients and water samples by MALDI-TOF mass spectrometry gave spectra concordant with *E. meningoseptica* for all isolates with relative intensity of matched peak scores ≥2.1. Disk diffusion susceptibility testing demonstrated in vitro resistance to amoxicillin, amoxicillin/clavulanic acid, temocillin, cefuroxime, cefotaxime, ceftazidime, ertapenem, meropenem, gentamicin, tobramycin, amikacin, and colistin but susceptibility to ciprofloxacin, piperacillin/tazobactam, tigecycline, and trimethoprim/sulfamethoxazole. The antibiograms were consistent for isolates from all 30 patients; MICs of selected agents for a representative isolate are shown in Table 2.

Table 1. Clinical and epidemiologic patient characteristics from an *Elizabethkingia meningoseptica* outbreak in an adult critical care unit, West London, UK, 2012–2013*

Patient no.	Age, y/sex	Admission category	Date of *E. m.* acquisition	Hospital day of acquisition	Sample type†	Antimicrobial therapy immediately before *E. m.* acquisition	*E. m.* treatment regimen	Clinical outcome	PFGE designation
1	29/M	Trauma	2012 Jan 12	35	Respiratory	None	None	Discharged	NA
2	45/F	Medical	2012 Feb 27	9	Respiratory	TZP	None	Discharged	EZ1
3	58/M	Medical	2012 Mar 2	22	Respiratory	MEM + CAS	None	Discharged	EZ1
4	34/M	Trauma	2012 Mar 10	18	Respiratory	TZP	None	Discharged	NA
5‡	28/M	Trauma	2012 Mar 20	15	Screening	MEM	TGC	Discharged	EZ2
6	64/M	Surgical	2012 Mar 22	4	Respiratory	None	None	Discharged	NA
7‡	77/M	Medical	2012 Mar 28	10	Screening	MEM + MTZ	None	Discharged	EZ1
8	69/M	Trauma	2012 Apr 18	11	Screening	MEM	None	Discharged	NA
9‡	35/F	Trauma	2012 May 21	19	Screening	TZP + AFG	TMP/SXT	Discharged	EZ2
10‡§	35/F	Surgical	2012 Jul 16	14	Respiratory	MEM	TMP/SXT	Discharged	NA
11‡§	60/F	Medical	2012 Jul 21	22	Respiratory	None	None	Died	EZ1
12	55/M	Surgical	2012 Jul 27	14	Respiratory	TZP + VAN	None	Died	EZ1
13	43/M	Trauma	2012 Sep 13	6	Screening	MEM + MTZ	None	Discharged	NA
14	40/M	Trauma	2012 Dec 27	13	Respiratory	MEM + VAN	None	Discharged	NA
15	40/F	Medical	2013 Jan 3	31	Blood culture	MEM + MTZ	TGC	Died	NA
16‡	23/M	Trauma	2013 Jan 14	13	Respiratory	None	None	Discharged	EZ1
17	57/M	Trauma	2013 Jan 14	13	Respiratory	TZP + FCA	None	Discharged	NA
18‡§	19/M	Trauma	2013 Mar 26	25	Respiratory	TZP + MTZ	None	Discharged	NA
19‡	70/M	Vascular	2013 Apr 8	11	Respiratory	MEM + FCA	TGC	Discharged	Unique
20‡§	61/F	Trauma	2013 Apr 27	11	Respiratory	MEM	TGC	Died	Unique
21‡§	43/M	Surgical	2013 May 1	12	Respiratory	MEM +AFG	None	Discharged	NA
22‡	17/M	Trauma	2013 May 22	28	Screening	MEM + MTZ	None	Discharged	EZ3
23§	60/M	Medical	2013 May 30	13	Respiratory	TZP + FCA	None	Died	NA
24‡§	75/F	Trauma	2013 Jun 21	13	Respiratory	TZP	TGC	Discharged	NA
25	75/M	Trauma	2013 Jun 22	12	Respiratory	MEM + VAN	None	Discharged	NA
26	77/F	Medical	2013 Aug 2	22	Respiratory	TZP	TMP/SXT	Discharged	EZ1
27	31/M	Trauma	2013 Sep 15	26	Respiratory	MEM + VAN	TGC	Discharged	NA
28‡§	83/M	Surgical	2013 Sep 15	28	Respiratory	TZP + FCA	TMP/SXT	Discharged	NA
29‡§	32/M	Trauma	2013 Oct 10	11	Respiratory	TZP + VAN	TMP/SXT	Discharged	NA
30	48/M	Trauma	2013 Oct 29	34	Respiratory	TZP + VAN	None	Discharged	NA
31¶	34/F	Trauma	2014 Apr 12	1	Screening	None	None	Discharged	Unique

*AFG, anidulofungin; CAS, caspofungin; Dis, discharged; *E. m.*, *E. meningoseptica*; FCA, fluconazole; MEM, meropenem; MTZ, metronidazole; NA, isolate unrecoverable for PFGE analysis; PFGE, pulsed-field gel electrophoresis; TGC, tigecycline; TMP/SXT, trimethoprim/sulfamethoxazole; TZP, piperacillin/tazobactam; VAN, vancomycin; TGC, tigecycline; AFG, anidulofungin.
†Respiratory sample types included nondirected bronchoalveolar lavage or endotracheal suction. Cross-infection screens comprise throat, rectum, nose, and groin swab specimens.
‡Patients in whom no other pathogen was identified in the 7 days before or after isolation of *E. meningoseptica*.
§Patients in whom chest radiography demonstrated new-onset signs consistent with a pneumonic process in the 48 hours before and after *E. meningoseptica* isolation.
¶Postoutbreak infection.

Table 2. MICs of selected antimicrobial agents tested against a representative isolate from an *Elizabethkingia meningoseptica* outbreak strain from an adult critical care unit, West London, UK, 2012–2013*

Antimicrobial agent	MIC, mg/L	Interpretation
Ceftazidime	256	Nonsusceptible
Piperacillin/tazobactam	16	Susceptible
Meropenem	>32	Nonsusceptible
Imipenem	64	Nonsusceptible
Aztreonam	>64	Nonsusceptible
Gentamicin	16	Nonsusceptible
Tobramycin	>32	Nonsusceptible
Amikacin	32	Nonsusceptible
Colistin	>32	Nonsusceptible
Ciprofloxacin	1	Intermediate
Minocycline	0.5	Unknown
Trimethoprim/sulfamethoxazole	0.25	Susceptible

*MICs were determined by serial agar dilution by using established methods (*24*). Interpretation of MICs used established British Society of Chemotherapy breakpoints. The intrinsic metallo- and extended-spectrum β-lactamases exhibited by *E. meningoseptica* mean the apparent in vitro susceptibility of the organism to piperacillin/tazobactam should be viewed with caution.

In addition to the isolates derived from patients, 7 *E. meningoseptica* isolates were identified from 5 sinks (1 in July 2012 when 2 additional taps were identified to have *Pseudomonas* spp. colonization; 4 in July 2013 when no further taps had *Pseudomonas* spp. colonization; no organisms were identified in December 2013). Routine analysis of bronchoscope rinse water from decontamination units during the investigation period showed no growth.

PFGE typing (Figure 1) showed that of the 12 patient isolates retrievable, 7 shared a common PFGE pattern (denoted EZ1), 2 shared a different profile (EZ2), 1 had a further identifiable profile (EZ3), and 2 others had unique profiles. Comparative PFGE typing of the 7 environmental isolates demonstrated that 5 were indistinguishable from the EZ1 outbreak strain; the remaining 2 isolates shared a PFGE pattern not identified among patient isolates (EZ4). The 5 EZ1 environmental isolates were isolated from taps from 3 different sink units in the critical care unit.

Attributable Illness

Eleven of the 30 case-patients received antimicrobial drug therapy targeted at *E. meningoseptica*, in all cases for a clinical diagnosis of hospital-acquired pneumonia. Thirteen patients were identified within the outbreak cohort in whom no discernible microbiological evidence of other pathogens was found in the 7 days before or after *E. meningoseptica* acquisition (Figure 2). In the 48 hours before and after *E. meningoseptica* acquisition, in terms of SIRS response, 7 case-patients had new-onset fever, 7 had new tachycardia, and 8 had new leukocyte count change. Additionally, 4 had increasing oxygen requirements, 7 had new increase in CRP, and 8 had new infiltrates on chest radiography. Moreover, targeted *E. meningoseptica* antimicrobial therapy was begun on 8 of these patients by the physicians coordinating care. Therefore, attributable illness (SIRS ≥2) from acquisition of *E. meningoseptica* in this outbreak was 54%.

Five case-patients died, including 2 of those deemed to have monomicrobial *E. meningoseptica* acquisition. However, the cause of death in those 2 patients was not due to infection; that is, no deaths were attributed to *E. meningoseptica* acquisition in this outbreak.

Outbreak Investigation

Analysis of bed occupancy demonstrated that for most of the time the critical care unit had contemporaneous case-patients present. However, 2 notable periods where no cases were identified (October 2012–December 2013 and January–March 2013) suggested a point source was more likely than person-to-person transmission in perpetuating the outbreak. Spatial correlation was observed between all colonized patients and environmental isolates in 1 quadrant of the critical care unit (2 side rooms and 1 bay); environmental sampling implicated 3 clinical sinks as the point source in this quadrant. No ongoing building or plumbing work elsewhere in the contiguous water system was identified.

Analysis of the antimicrobial point-prevalence studies showed that 63%–79% of all patients in the critical care unit were receiving antimicrobial drugs at any 1 time, but no directional trend was exhibited. Antimicrobial drug use in the outbreak unit demonstrated no major difference from that in the other critical care units in the hospital network.

Estimating the Effect of MALDI-TOF Mass Spectrometry Introduction on Identification of *E. meningoseptica*

Interrogation of the microbiology information management system identified 8 other *E. meningoseptica* isolates throughout the wider hospital network: 1 patient in the study hospital in March 2013 for whom no connection to the critical care unit could be established, and 7 patients in the 4 other hospitals in the network during January 2010–October 2013. None of these were from critical care units, and no discernible health care contact was found among the case-patients. Only 2 of these 8 additional cases were detected before MALDI-TOF mass spectrometry was introduced into routine laboratory practice in June 2011, meaning 6 (and all 30 of the outbreak case-patients) were identified after its introduction. Further analysis of the microbiology information management system revealed that throughout the hospital network during January 2010–June 2011, a total of 17% of non–lactose-fermenting gram-negative organisms were not identified to genus/species level; after introduction of the MALDI-TOF mass spectrometry, during July 2011–October 2013, this percentage decreased to 10.9%.

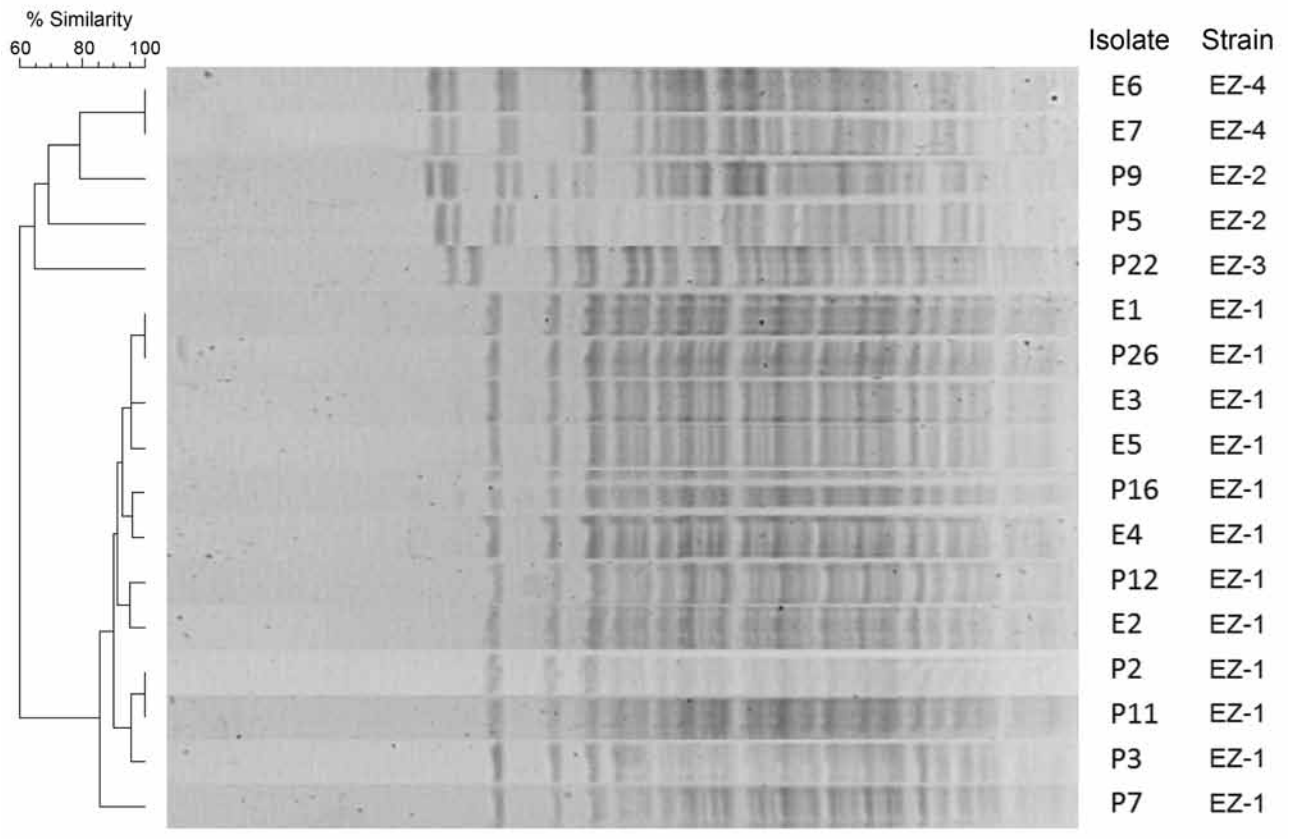

Figure 1. Pulsed-field gel electrophoresis profiles of *XbaI*-digested genomic DNA from patient (P) and environmental (E) *Elizabethkingia meningoseptica* isolates from an outbreak in an adult critical care unit, London, UK, 2012–2013. Two additional isolates from patients demonstrated unique pulsed-field gel electrophoresis profiles and are not shown. Patient numbers (e.g., P9) match those given in Table 1.

Water Reservoirs and Control

Interventions to attempt containment of the outbreak included (sequentially): domestic process review (single cloth per sink; "clean-to-dirty" cleaning protocol) and decluttering of clinical areas (August 2012); instigation of daily sink trap chlorination in all clinical sinks (August 2012); exchange of clinical sink traps (September 2012); and water course remodeling, including removal of flexible tubing segments (September–December 2012). Use of alcohol gel after hand washing was advocated throughout the outbreak. These steps failed to control the outbreak; however, after initiation of 3 times per day automated flushing of all clinical tap units in October 2013, water testing in December 2013 demonstrated an absence of *E. meningoseptica* or *Pseudomonas* species, and no further isolates were identified from patients in the critical care unit from November 2013 onward. The exception was 1 isolate from a cross-infection sample in a patient admitted in April 2014, detected from screening samples taken on the day of admission; typing of this organism showed a unique PFGE profile not related to any of the previously identified isolates.

Discussion

In the context of a prolonged outbreak of *E. meningoseptica* acquisition in an adult critical care unit of a London teaching hospital, we found that acquisition of this organism was associated with clinically significant attributable illness in approximately half of patients, evidence against this organism being a nonpathogenic colonizer. We found clinical and molecular epidemiologic evidence indicating acquisition is associated with water sources in the critical care unit; however, within these water samples we also identified numerous varied strains of *E. meningoseptica*, suggesting more widespread dissemination of this organism than previously thought. From our analysis of microbiology data throughout the hospital network, we found a marked excess of identified *E. meningoseptica* (both outbreak and nonoutbreak) and a contemporaneous decrease in unspeciated nonfermenting gram-negative organisms after MALDI-TOF mass spectrometry was introduced. We propose that wider introduction of this technology across clinical laboratories might be overcoming previous difficulties in identifying *E. meningoseptica*, possibly contributing to the recent increase in reported outbreaks of this emerging pathogen (*8*,*10*,*16*–*18*).

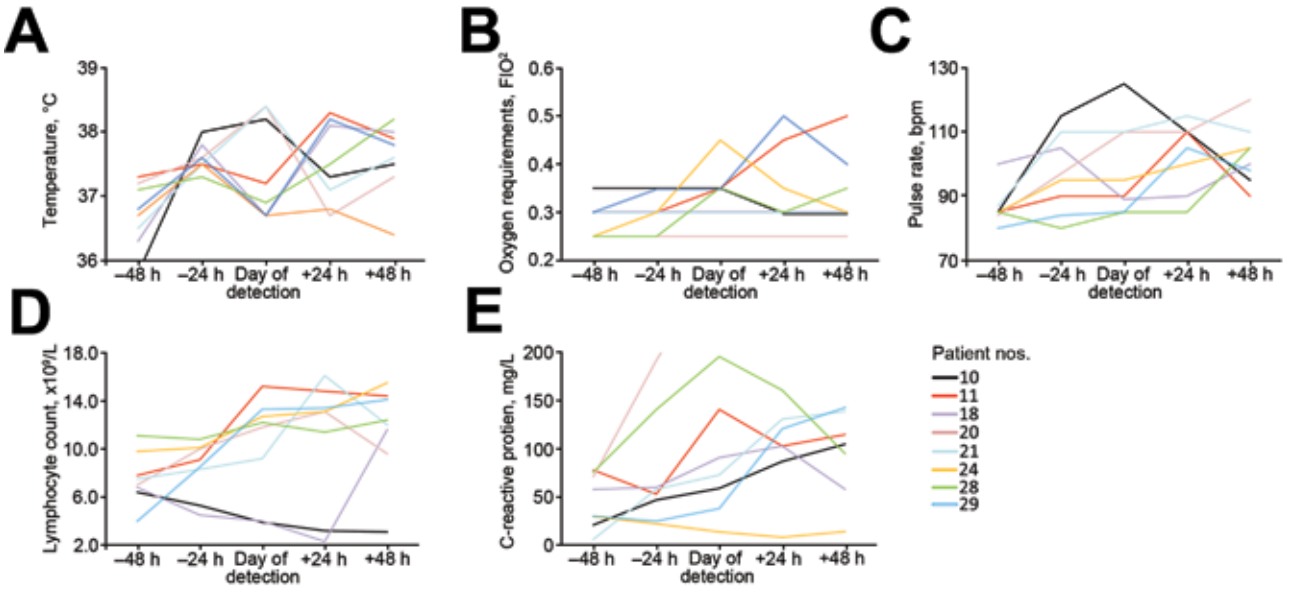

Figure 2. Clinicophysiologic parameters of patients with monomicrobial acquisition of *Elizabethkingia meningoseptica* in an outbreak in an adult critical care unit, London, UK, 2012–2013. Thirteen patients in the outbreak cohort were identified as having monomicrobial *E. meningoseptica* acquisition. Of these, 8 patients demonstrated an increase in 5 clinicophysiologic parameters of inflammation during the 48 hours before and after acquisition of *E. meningoseptica:* A) body temperature; B) oxygen saturation; C) pulse rate; D) lymphocyte count; and D) C-reactive protein. Patient numbers match those given in Table 1.

New-onset rise in temperature, tachycardia, and inflammatory markers occurred in half of the patients who acquired *E. meningoseptica* and culminated in clinical decisions to instigate targeted therapy in the absence of any other organisms. This finding suggests *E. meningoseptica* causes clinical infection and does not just colonize patients in critical care. Furthermore, the high frequency of isolation of *E. meningoseptica* from respiratory samples across the outbreak cohort, combined with new-onset radiographic changes in half of patients with monomicrobial *E. meningoseptica*, suggests that this pathogen is a cause of hospital-acquired pneumonia. Biological plausibility exists, with virulence factors including a propensity for biofilm formation (*26*,*27*), intracellular invasion (*28*), and chromosomal (*29*) and plasmid (*30*) mediated resistance to many antimicrobial drugs, including commonly used β-lactams. This marked antimicrobial drug resistance has been previously documented to include 3 bla_{CME} genes coding for extended-spectrum serine-β-lactamase (Ambler class D) and 2 unrelated metallo-β-lactamases conferring carbapenem resistance: bla_{B} (subclass B1) and bla_{GOB} (subclass B3) (*31*). Acquisition of further resistance elements, including bla_{KPC}, also has been documented (*32*). Phenotypic susceptibility testing on the isolates from this outbreak supports such a marked resistance phenotype, particularly to β-lactam antimicrobial drugs. This high level of antimicrobial resistance may have accounted for the excess appearance of the organism in patients who had a history of broad-spectrum antimicrobial drug therapy; 87% of the patients who acquired *E. meningoseptica* had a history of preceding antimicrobial use (predominantly piperacillin/tazobactam and meropenem), compared with a background of 63%–79% among nonoutbreak critical care patients. Drug resistance also led to a limited armamentarium with which to treat; whereas our treatment strategies were susceptibility testing driven (trimethoprim/sulfamethoxazole and tigecycline), other agents have been advocated, including some typically considered to target gram-positive organisms (*3*).

The noted potential for *E. meningoseptica* to display a strong biofilm biotype might also explain the failure of many of the infection control interventions during this outbreak. The failure of chlorine has been documented (*33*), but use of post–hand washing alcohol gel, previously found effective in terminating outbreaks (*13*,*34*), was not effective in our experience. The apparent success of regular sink flushing in terminating our outbreak might be attributed to the sheer force exerted during this process and is advocated in recent UK guidance for augmented care areas where waterborne pseudomonads are of concern (*35*). The return of the organism in a single patient in April 2014, seven months after the proposed outbreak termination, might be attributable to a failure in the automated flushing protocols but more likely represents contamination from a sink in a nearby area of the hospital (i.e., operating rooms) that does not practice the auto-flushing protocol or from outside the health care environment. Biofilm formation also might

account for the observed predilection for respiratory tract acquisition, and we speculate that, in addition to antimicrobial drug therapy, in those with airway adjuncts repeated device changes might be helpful. Small-molecule disruption of biofilms might in the future provide an alternative therapeutic avenue (*36*).

The identification of numerous strains (albeit with 1 predominating) of *E. meningoseptica* in patients and in water sources suggests a wider issue in the water microbiome. The historical difficulties in identifying *E. meningoseptica* from other nonfermenting gram-negative organisms (including *Pseudomonas* species) in both patient and environmental samples mean that the advent of MALDI-TOF mass spectrometry might simply be helping to describe *E. meningoseptica* epidemiology, and the recent increase in reported outbreaks might indicate ascertainment bias. This possible bias is supported by the wider microbiology data, with few *E. meningoseptica* isolates being identified anywhere in the hospital network before introduction of MALDI-TOF mass spectrometry, after which not only were many more identified, but a concomitant fall in the frequency of nonidentified gram-negative organisms also was observed. Although MALDI-TOF mass spectrometry might therefore be aiding the early phase of outbreak detection through improved organism identification, the extent to which this organism represents an emerging pathogen, as opposed to how much preexisted and is simply newly identified, is unclear. Further work on the utility of MALDI-TOF mass spectrometry in outbreak detection and investigation is warranted, and an additional role in typing might be feasible (*37*–*40*). Integration of this platform into clinical practice, as is happening in many laboratories, must be given due consideration as to such potential unintended consequences.

A failure to subculture many of the isolates from the cohort for PFGE typing is a noted limitation of this study. As described, however, variable growth on commonly used media is a feature of this organism. Moreover, the typing that was conducted was hardly circumstantial and was sufficient to demonstrate a link between isolates from water sources and from patients. A further limitation of this study, in delineating the attributable illness, was the low number of patients for whom clinico-physiologic parameters were analyzed. However, inclusion of cases was purposefully strict, limiting cases to persons from whom no organisms other than *E. meningoseptica* were isolated. This restriction was to enable changes in clinico-pathologic variables to be specifically associated with *E. meningoseptica* rather than any co-cultured organisms; however, the possibility remains that other organisms were present and not cultured.

Transmission of waterborne *E. meningoseptica* to adult critical care patients has an attributable illness rate of 54%. Advances in rapidity and accuracy of microbiology diagnostics, including through adoption of MALDI-TOF mass spectrometry, is leading to increased detection of this organism providing an improved understanding of critical care clinical infections and the waterborne hospital microbiome. Consequently, the recent international increase in *E. meningoseptica* outbreaks in adults, including from the United States, Brazil, and South and Southeast Asia, might indicate a pseudo-emerging, rather than an emerging, nosocomial pathogen. Further work is needed, and network analysis and whole-genome sequencing are likely to facilitate greater understanding of the wider transmission potential of *E. meningoseptica*. Given the attributable illness, the organism's marked antimicrobial resistance profile, and its endurance against standard infection prevention and control procedures, development of robust interventions to combat waterborne outbreaks of this pathogen among critically ill adults is urgently needed.

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etymologia

Elizabethkingia [e-liz″ə-beth-king′e-ə]

Named for Elizabeth O. King, a bacteriologist at the US Centers for Disease Control who studied meningitis in infants, *Elizabethkingia meningoseptica* is a gram-negative, obligate aerobic bacterium in the family *Flavobacteriaceae*. King named the bacterium *Flavobacterium* (from the Latin *flavus*, "yellow") *meningosepticum*, and in 1994 it was reclassified in the genus *Chryseobacterium* (from the Greek *chryseos*, "golden"). In 2005, it was placed in the new genus *Elizabethkingia*.

Six-day-old blood agar growth of *Elizabethkingia meningioseptica* with 5 µg vancomycin (with zone of clearing) and 10 µg colistin disks. Source: Dr. Saptarshi via Wikimedia Commons (https://commons.wikimedia.org/wiki/File:Elizabethkingia_meningoseptica_Blood_agar_plate.JPG).

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Human Papillomavirus Vaccination at a Time of Changing Sexual Behavior

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Human papillomavirus (HPV) prevalence varies widely worldwide. We used a transmission model to show links between age-specific sexual patterns and HPV vaccination effectiveness. We considered rural India and the United States as examples of 2 heterosexual populations with traditional age-specific sexual behavior and gender-similar age-specific sexual behavior, respectively. We simulated these populations by using age-specific rates of sexual activity and age differences between sexual partners and found that transitions from traditional to gender-similar sexual behavior in women <35 years of age can result in increased (2.6-fold in our study) HPV16 prevalence. Our model shows that reductions in HPV16 prevalence are larger if vaccination occurs in populations before transitions in sexual behavior and that increased risk for HPV infection attributable to transition is preventable by early vaccination. Our study highlights the importance of using time-limited opportunities to introduce HPV vaccination in traditional populations before changes in age-specific sexual patterns occur.

Changes in sociocultural norms that regulate sexual behavior have been reshaping the epidemiology of sexually transmitted infections (STIs) in many areas of the world. For example, changes in sexual practices have resulted in an epidemic of syphilis and other STIs in China (*1,2*). Data from surveys of sexual behavior show considerable heterogeneity of age-specific sexual patterns by country. For example, average age difference between spouses or cohabiting partners ranged from 15 years in Burkina Faso to 2 years in Australia (*3*) (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/22/1/15-0791-Techapp.pdf). In addition, marriage at older ages and sexual debut at earlier ages in women have been observed in high-income countries over the past few decades (*4*) and are also now reported in many low- and middle-income countries (*3*). Furthermore, age differences between sexual partners at onset of sexual activity (*5*) and rate of sexual activity in young persons (*2*) have been shown to influence the age-specific distribution of STIs, such as HIV and syphilis, and have been proposed as determinants of international variations in overall and age-specific prevalence of human papillomavirus (HPV) infection (*6*).

We used a transmission-dynamic model to illustrate how changes in age-specific rates of sexual activity and age difference between sexual partners potentially affect HPV prevalence in heterosexual populations. The model also shows how differences in the timing of HPV vaccination relative to changes in age-specific sexual behavior may influence the effectiveness of HPV vaccination programs.

Methods

We adapted a previously described dynamic model of HPV infection (*7*) to simulate transmission and clearance of the infection (online Technical Appendix). We focused on HPV16 infection because type 16 is the most frequent and most carcinogenic HPV type in all world regions (*8*) and is targeted by HPV vaccines.

Sexual Behavior and Study Populations

We adapted a model that used 1) age- and gender-specific rates of sexual activity per year and 2) distribution of age differences between sexual partners to simulate 2 heterosexual populations, one with traditional age-specific sexual behavior and the other with gender-similar age-specific sexual behavior. Traditional sexual behavior indicates a population in which genders have different age-specific sexual activity rates and a wide gap in ages (e.g., an average of 5.6 years, as observed in India) of spouses or cohabitating sexual partners. Gender-similar sexual behavior indicates a population in which genders have similar age-specific sexual activity rates and a narrow gap in ages (e.g., an average of 2.1 years, as observed in the United States) of spouses or cohabitating sexual partners. In the population with traditional sexual behavior, studies suggest that sexual activity (i.e., having new sexual partners) among women occurs mostly at young ages (i.e., at marital age), whereas

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sexual activity among men usually reaches a plateau, typically at postmarital age, and remains consistent throughout their sexually active life (*3,4,9–11*). Conversely, in the population with gender-similar sexual behavior, both men and women concentrate most of their sexual activity at young ages and engage in mainly premarital relationships (*7,12–15*). The number of new sexual partners for this population peaks at ages <25 years.

We then stratified the simulated populations into 2 levels of sexual activity (high or low), according to age-specific rates of sexual activity. The number of new sexual partners per year was obtained by calibration and used as a proxy for sexual activity. To factor in differences in sexual activity rates for the 2 populations, we assumed and imposed on the model a set of age-specific relative rates to represent observed sexual activity patterns for the 2 groups (online Technical Appendix Table 1). We varied the average number of new sexual partners per year from 1 to 2 in the calibration phase, in agreement with values reported in studies that modeled HPV or HIV transmission (i.e., 0.29 and 4.0 partners per year) (*16*).

To represent age differences between sexual partners in the 2 populations with age-different and age-similar sexual patterns, we used age differences between spouses or cohabiting partners reported in India in 2005 (mean 5.6 years; 95% CI 0–13 years) (*17*) and in the United States in 2008 (mean 2.1 years; 95% CI –7 to 11 years) (*18*), respectively. On the basis of available data for age at first intercourse (*3*), sexual activity did not start before 14 years of age for either gender in each simulated population, and all persons were considered susceptible to HPV16 infection when they started sexual activity. We kept constant other demographic, behavioral, and biologic parameters for the 2 populations.

Model Parameterization and Calibration

Using values that we estimated and validated in our previous work with large sets of high-quality data from high-income countries (*7*), we assumed for the model an 80% probability of transmission per sexual partnership for both genders and a 20% probability of developing type-specific immunity after infection clearance (Table). We also assumed that the per-person annual rate of viral clearance decreased 1.3–0.3 person-years for infections that occurred <1 year to >2 years earlier (Table 1). The simulated populations are open stable (i.e., age-specific mortality rates are balanced by the birth rate) and stratified by single-year age groups (ages 10–70 years).

With our model-based projections, we reproduced data from rural India (*19*) and the United States (*20*) as examples of heterosexual populations with gender-different (i.e., traditional) and gender-similar age-specific sexual behaviors (online Technical Appendix Figure 2). Age-standardized HPV16 prevalence (3.6%) in rural India is consistent with prevalence found in traditional populations (*19*) and lower than the corresponding prevalence (5.8%) in the United States (*20*). To obtain adequate matching of model-based projections with the age-specific HPV16 prevalences reported from rural India and the United States, we calibrated the average annual number of new sexual partners (online Technical Appendix Table 2) and the tendency for persons with similar sexual activity to form sexual partnerships (i.e., assortative mixing by sexual activity) (Table) .

Model-Based Analyses

To show the effects of vaccination in populations with traditional and gender-similar sexual behaviors, we simulated the introduction of vaccination against HPV16 for

Table. Model parameters related to HPV16 infection, sexual behavior, and vaccine efficacy and values assigned or calibrated*

Parameter	Value	Source
Probability of transmission per sexual partnership, %	80	Assumed
Fraction of immunity after infection clearance, %	20	Assumed
Rate of clearance by duration since infection, person-year		Assumed
<1 y	1.3	
1–2 y	0.8	
>2 y	0.3	
New sexual partners per year, mean		
Heterosexual population with traditional sexual behavior	2.0	Calibrated
Heterosexual population with gender-similar sexual behavior	1.5	Calibrated
Heterosexual population with gender-similar sexual behavior with increased number of partners	2.0†	SA
Heterosexual population with traditional sexual behavior with decreased number of partners	1.5‡	SA
Mixing between classes of sexual activity§	0.7	Calibrated
	0.3	SA
Vaccination efficacy	95%	Assumed
Duration of vaccine protection	Lifelong	Assumed

*Values have been assumed on the basis of previous research (*7*). We calibrated values by fitting model-based projections to data from rural India (*19*) and the United States (*20*). SA indicates that the value was imposed on the model for univariate sensitivity analysis.
† We increased the average number of partners from 1.5, the calibrated value, to 2.0 in the population with gender-similar sexual behavior.
‡ We decreased the average number of partners from 2.0, the calibrated value, to 1.5 in the population with traditional behavior.
§"Mixing between classes of sexual activity" is a measure of the tendency for persons with similar levels of sexual activity to form sexual partnerships. It is measured on a scale where fully and randomly assortative (i.e., like-with-like) mixing corresponds to values 0 and 1, respectively. For the sensitivity analysis, we changed the value of assortative mixing by level of sexual activity from 0.7, the calibrated value, to 0.3.

11-year-old girls only and for both girls and boys and calculated the percent reduction in HPV16 prevalence attributable to vaccination in the 2 populations with differing age-specific sexual behavior at the postvaccination equilibrium (i.e., 70 years after introduction of the vaccination). We then sought to show how transitioning from traditional to gender-similar age-specific sexual behavior over a 15-year period affects HPV16 prevalence in women 20–34 years of age. The transition from traditional to gender-similar sexual behavior was simulated by assuming a progressive shift towards gender-similar sexual activity rates and reduction of age gap between sexual partners. Finally, we simulated the introduction of HPV vaccination, with and without catch-up vaccination of girls and women >11 years of age, before and after an age-specific sexual behavior transition period. On the basis of previous reports (online Technical Appendix), vaccination coverage was set at 70%, vaccine efficacy against HPV16 was set at 95%, and the duration of protective immunity against HPV16 infection was assumed to be lifelong. To assess the sensitivity of our estimates to the calibrated parameters (i.e., the average number of new sexual partners per year and the assortative mixing by sexual activity), we repeated our analyses and imposed on the model different values than those obtained through model calibration. In particular, we decreased the average number of partners by 0.5 in the population with traditional behavior (i.e., from 2.0, the calibrated value, to 1.5) and increased the average number of partners from 1.5, the calibrated value, to 2.0 in the population with gender-similar sexual behavior. Finally we changed the value of assortative mixing by sexual activity from 0.7, the calibrated value, to 0.3 (on a scale where fully and randomly assortative mixing correspond to values of 0 and 1, respectively; online Technical Appendix Figure 3).

Results

We used the simulations to compare the percent reduction in HPV16 prevalence attributable to vaccination by coverage level after introduction of a vaccination program (for 11-year-old girls only and for both girls and boys) in a traditional sexual-behavior population and in a population with gender-similar sexual behavior (Figure 1). At the postvaccination equilibrium, the estimated percent reduction in HPV16 prevalence attributable to vaccination is larger in the traditional population than in the population with gender-similar sexual behavior, and differences persist until coverage for girls-only vaccination is at 80% and coverage for gender-neutral vaccination is at 60%. These levels of vaccination coverage are sufficient to eliminate HPV16 infection in the population with gender-similar age-specific sexual behavior. In a girls-only vaccination program, the largest projected difference in reduced prevalence attributable to vaccination for the 2 populations is at ≈50% coverage, which enables a reduced HPV16 prevalence in the traditional sexual-behavior population of 85% compared with a 64% reduction in the population with gender-similar sexual behavior. For vaccination programs targeting girls and boys, the largest difference in reduced prevalence attributable to vaccination is at ≈30% coverage: 83% reduction in HPV16 prevalence for the traditional sexual-behavior population versus 58% reduction for the population with gender-similar sexual behavior.

We also simulated changes in HPV16 prevalence among women 20–34 years of age in relation to the timing of the transition from traditional to gender-similar sexual behavior and HPV vaccination introduction (11-year-old girls only, 70% coverage; Figure 2). Our model showed that in a no-vaccination scenario, HPV16 prevalence increases

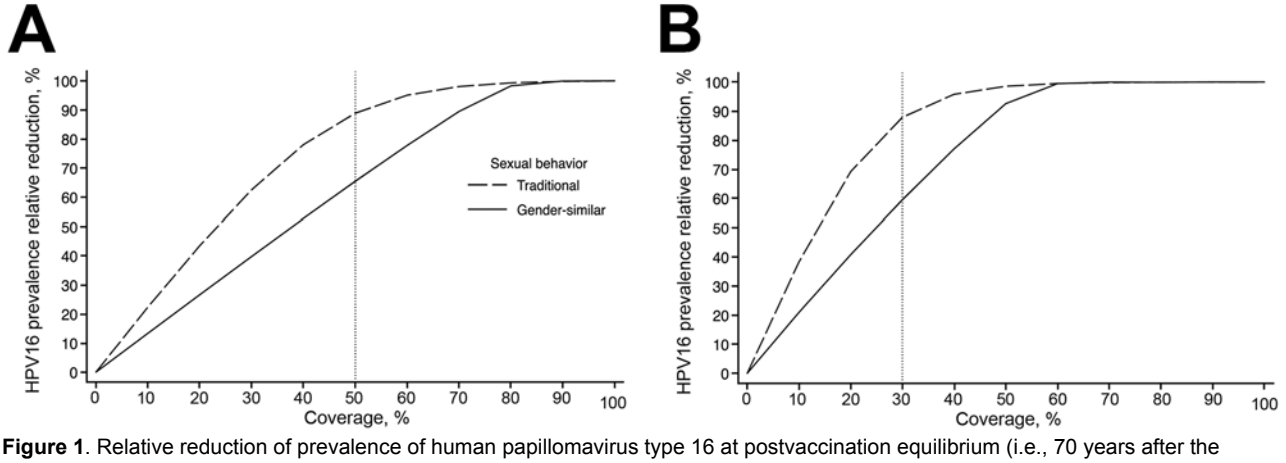

Figure 1. Relative reduction of prevalence of human papillomavirus type 16 at postvaccination equilibrium (i.e., 70 years after the introduction of vaccination) attributable to vaccination among women 20–34 years of age after vaccination of 11-year-old girls or 11-year-old girls and boys, by coverage and a population's age-related sexual behavior. A) 30% vaccine coverage; B) 50% vaccine coverage. Traditional sexual behavior indicates a population in which genders have different age-specific sexual activity rates and a wide gap in ages (e.g., an average of 5.6 years, as observed in India) of spouses or cohabitating sexual partners. Gender-similar sexual behavior indicates a population in which genders have similar age-specific sexual activity rates and a narrow gap in ages (e.g., an average of 2.1 years, as observed in the United States) of spouses or cohabitating sexual partners.

from 3% to 8% with transition to gender-similar sexual behavior. The introduction of HPV vaccination before transition to gender-similar sexual behavior halts this increase in ≈10 years and induces a decrease in prevalence to ≈1.5% in 20 years. In 30 years, vaccination reduces HPV16 prevalence to ≈1% at equilibrium. In contrast, if vaccination is introduced after transition to gender-similar sexual behavior, HPV16 prevalence will not reach 1% equilibrium for 40 years after vaccination introduction. Advantages of vaccination in populations before age-specific sexual-behavior transitions occur are reduced potential increases of HPV16 prevalence and earlier effects of vaccination.

To assess the sensitivity of our estimates to the assumed average number of new sexual partners per year, we repeated our simulations by imposing the same average number (1.5 and 2.0) of partners on the population with age-similar sexual partners and on the traditional population with age-different sexual partners (Table; online Technical Appendix Figure 4, panels A, B). Decreasing the average number of partners by 0.5 in the population with traditional sexual behavior (i.e., from 2.0 to 1.5) and increasing the average number of partners from 1.5 to 2.0 in the population with gender-similar sexual behavior modified the HPV16 prevalence among women 20–34 years of age by similar magnitudes in both populations: 2.4% (from 3.1% to 0.7%) for the population with traditional sexual behavior and 2.8% (from 8.2% to 11.0%) in the population with gender-similar sexual behavior. Despite the sensitivity of HPV16 prevalence to the average number of new sexual partners per year, the benefit of introducing HPV vaccination before transition was confirmed (online Technical Appendix Figure 4). In addition, our findings were robust to the assumption of a more assortative mixing between classes of sexual activity, with prevalence increasing from 3.4% to 7.0% with transition in age-specific sexual behavior (online Technical Appendix Figure 3).

Discussion

We show that the effects of a vaccination program are influenced by a population's age-specific sexual behavior (i.e., traditional or gender-similar). We also highlight the benefits of implementing HPV vaccination in traditional populations before transition to gender-similar sexual behavior occurs. The earlier that vaccination is established in a population undergoing sexual-behavior transition, the more likely it is that vaccination will be highly effective, even if initial coverage is suboptimal. Sensitivity analyses show that our findings are robust to uncertainties about average number of partners and assortative mixing by sexual activity in the 2 types of sexual behavior patterns.

In our simulated traditional population, the transition to gender-similar sexual behavior entails a 2.6-fold increase, from 3% to 8%, in HPV16 prevalence in women 20–34 years of age. In populations with gender-similar sexual behavior, sexual activity of both men and women peaks at young ages (<30 years of age), and age difference between partners is narrow. As a result, the corresponding peaking sexual activity of young women and men is more likely to enable an efficient and rapid spread of HPV infection among susceptible young persons with multiple new sexual partners per year. Nonoverlapping age-specific peaks of sexual activity and larger age differences between sexual partners, as observed in traditional populations, can decrease the basic viral reproductive number and the spread of HPV infection and increase the herd immunity effect of vaccination programs (*21*). Consequently, vaccination has stronger effects in populations with traditional rather than gender-similar sexual behavior. According to our model, the largest difference in percent reduction in HPV16 prevalence attributable to vaccination is seen if coverage is 50% (for girls-only vaccination) or 30% (for girls-and-boys vaccination). With this level of coverage, the reduction in HPV16 prevalence achievable in women 20–34 years of age is estimated to be ≈80%, compared with ≈60% if vaccination is introduced after a transition to gender-similar sexual behavior. Similarly, according to our projections (online Technical Appendix Table 3), a 1-time catch-up campaign is more efficient in a population with traditional sexual behavior

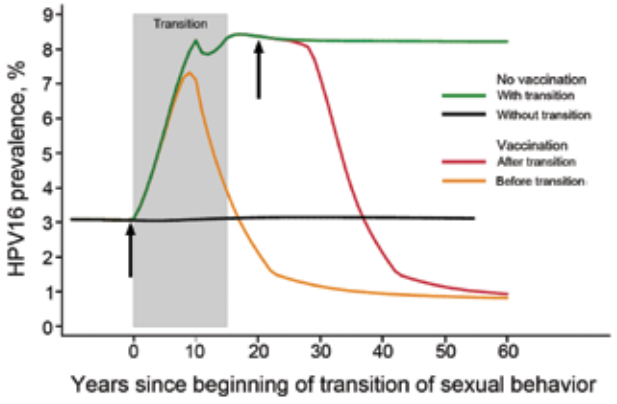

Figure 2. Changes in prevalence of human papillomavirus type 16 among women 20–34 years of age in relation to the number of years since the beginning of a population's transition from traditional to gender-similar age-related sexual behavior and the introduction of vaccination among 11-year-old girls (with assumption of 70% coverage) before and after transition. Shaded area shows an assumption of a 15-year transition period. Arrows show approximate timing of vaccination occurring before or after a transition has occurred. Traditional sexual behavior indicates a population in which genders have different age-specific sexual activity rates and a wide gap in ages (e.g., an average of 5.6 years, as observed in India) of spouses or cohabitating sexual partners. Gender-similar sexual behavior indicates a population in which genders have similar age-specific sexual activity rates and a narrow gap in ages (e.g., an average of 2.1 years, as observed in the United States) of spouses or cohabitating sexual partners.

than in a population that has transitioned to gender-similar sexual behavior. Ambitious catch-up (i.e., up to age 18 or 25 years) would confer protection on women for whom high-quality cervical cancer screening may not be available (*22*).

Our model shows that early implementation of HPV vaccination attenuates increased risk of HPV infection that accompanies transition to gender-similar sexual behavior. This finding affects the interpretation of studies on the surveillance of HPV vaccination. For example, in the absence of reliable data regarding the sexual behavior of a population and on vaccination coverage, increased HPV prevalence might be erroneously interpreted as a lack of vaccine effectiveness.

Our study has strengths and limitations. One strength is the use of a validated transmission model to represent changes in HPV16 prevalence. Transmission models can capture the dynamics of infection circulation (*23*) in a population and have the distinct advantage of including the effect of herd-immunity attributable to vaccination (*24,25*). We derived estimates for the parameters governing the natural history of HPV16 infections from a large cervical cancer screening study conducted in Italy (*26*) and validated the estimates by comparing them with data from a large dataset from Sweden (*27*).

Although cervical cancer reduction is the ultimate aim of vaccination, we chose a viral endpoint rather than cervical disease endpoints to avoid the inclusion of additional uncertainties that would be introduced by other parameters that regulate the progression or regression of HPV infection into precancerous cervical lesions and cancer. Viral endpoints are also the earliest to manifest and offer the opportunity to monitor vaccination programs and the adequacy of our model. We also chose to focus on HPV16 only because information about the natural history of types other than HPV16 and about vaccine efficacy against these types is limited, but data are generally consistent for HPV16. However, use of viral endpoints does not eliminate uncertainties related to the acquisition and clearance of HPV16 and subsequent immunity to the virus (*21*).

To keep our model simple, we accounted for heterogeneity in sexual behavior by stratifying the simulated heterosexual populations into 2 classes of sexual activity and did not account for same-sex or concurrent sexual partnerships (*23*). Ideally, an exhaustive description of exposure to HPV infections should consider the representation of the entire sexual network in which HPV infections are transmitted, but such information is rarely available (*28,29*). The method we adopted to represent sexual activity has been extensively used to investigate the epidemiology of STI other than HPV (*23*), and the sexual activity rates we used to account for country- and age-specific HPV curves are consistent with those observed in high- and low-income countries (*7,9–14,16,30*).

To calibrate our HPV transmission model, we chose to use data from rural India and the United States as examples of populations with traditional and gender-similar sexual behavior, respectively. Obviously, the classification of sexual behavior into traditional or gender-similar behaviors is an oversimplification, as is our assumption that the age-specific profile of HPV16 prevalence could be sufficiently explained by differences in the number of new sexual partners and age difference between heterosexual partners. Other authors have evoked HPV reactivation as a cause of high HPV prevalence in older women (*31*). In the absence of specific information, we assumed that the increase in premarital sex that characterizes the transition to gender-similar sexual behavior (*3,4*) can be accounted for by modulating the number of new sexual partners as a function of age. Likewise, we have assumed that recorded age differences between cohabiting partners or spouses in India and in the United States are representative of age differences between sexual partners in general.

On the basis of results of our model, we find that traditional or gender-similar age-specific sexual behavior can shape age-specific HPV prevalence curves and that a particularly favorable, time-limited window currently exists for the introduction of HPV vaccination in traditional populations in low- and middle-income countries. National and international agencies should seize this opportunity with adequate political commitment, planning, and funding.

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Multiorgan WU Polyomavirus Infection in Bone Marrow Transplant Recipient

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WU polyomavirus (WUPyV) was detected in a bone marrow transplant recipient with severe acute respiratory distress syndrome who died in 2001. Crystalline lattices of polyomavirus-like particles were observed in the patient's lung by electron microscopy. WUPyV was detected in the lung and other tissues by real-time quantitative PCR and identified in the lung and trachea by immunohistochemistry. A subset of WUPyV-positive cells in the lung had morphologic features of macrophages. Although the role of WUPyV as a human pathogen remains unclear, these results clearly demonstrate evidence for infection of respiratory tract tissues in this patient.

Polyomaviruses are small, circular, double-stranded DNA viruses. Infection with BK polyomavirus (BKPyV) or JC polyomavirus causes substantial illness and death in immunocompromised populations, including transplant recipients and HIV patients (*1*). Infection with either virus is typically asymptomatic unless the host is immunocompromised. Since 2007, a total of 11 additional human polyomaviruses have been discovered. Two of these viruses (Merkel cell carcinoma polyomavirus and trichodysplasia spinulosa–associated polyomavirus) have also been implicated as human pathogens in the context of immunosuppression (*2*,*3*); the former causes Merkel cell carcinoma, a rare but aggressive skin cancer (*3*), and the latter is associated with trichodysplasia spinulosa, a rare skin disease seen in transplant recipients (*2*). Several of the other new human polyomaviruses (e.g., human polyomavirus 9 and New Jersey polyomavirus) were also initially identified in immunocompromised patients (*4*,*5*).

In 2007, WU polyomavirus (WUPyV) was discovered in a child in Australia with pneumonia (*6*). Although the virus has yet to be implicated in human disease, epidemiologic studies have shown that 69%–80% of persons (*7*–*9*) are seropositive for this virus; infection probably occurs during early childhood. In addition, viral DNA has been detected in blood, feces, respiratory tract secretions, tonsils, and cerebrospinal fluid (*10*). Although these studies have contributed to a better understanding of WUPyV, only 1 has explored the in vivo tropism of the virus or described the detection of viral antigen in tissue (*11*). That study determined that WUPyV was present in epithelial cells from a bronchoalveolar lavage from a lung transplant recipient with Job syndrome. A complete understanding of the types of cells and tissues in which the virus replicates is critical for identifying potential diseases with which it may be associated. However, the propensity for viruses of the family *Polyomaviridae* to cause disease only within the context of immunosuppression makes disease association particularly challenging. We describe molecular characterization and immunohistologic and microscopic localization of WUPyV in tissues from a deceased patient who had had viral pneumonitis.

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Materials and Methods

The Case

In January 2001, a 27-month-old girl was admitted to an upstate New York area hospital for a 5/6 human leukocyte antigen–matched cord blood transplant from an unrelated donor. The patient had been born by normal vaginal delivery after 40 weeks of gestation. Her medical history included leukocytosis at 3 months and splenomegaly at 6 months of age. Refractory juvenile myelomonocytic leukemia was diagnosed when she was 16 months of age, and she underwent splenectomy in September 2000. She had multiple infections before 2 years of age, including otitis media, a central vein catheter infection, and a urinary tract infection. She also demonstrated failure to thrive, developmental delay, mild pulmonic stenosis, and gastroesophageal reflux.

Three weeks after the bone marrow transplant, the child experienced fever; diarrhea; hepatomegaly; and erythema on her face, palms, and soles. She was evaluated for graft versus host disease, viral exanthema, and drug eruption. The results of skin biopsies performed at 4 weeks after transplantation ruled out graft versus host disease and drug eruption. A rectosigmoid biopsy performed at the same time as the skin biopsies showed mild stromal edema but was negative

for adenovirus and cytomegalovirus by immunohistochemistry (IHC) staining. Throughout the course of the patient's hospitalization, adenovirus was intermittently isolated from her feces and urine and influenza B virus was detected in her nose and throat. PCR testing of the blood for cytomegalovirus was consistently negative. Treatment included cyclosporine; Solu-medrol (Pharmacia & Upjohn LLC, New York, NY, USA); intravenous immunoglobulin; ribavirin; Zosyn (Pfizer Inc., New York, NY, USA); Flagyl (Pfizer Inc.); Flutamine (Schering-Plough, Kenilworth, NJ, USA); Tamiflu (Roche Pharmaceuticals, Nutley, NJ, USA); Demerol (Sanofi-Aventis U.S. LLC, Bridgewater, NJ, USA); Zofran (GlaxoSmithKline, Philadelphia, PA, USA); Phenergan (Wyeth, Madison, NJ, USA); Tylenol (Johnson & Johnson, New Brunswick, NJ, USA); albuterol; isradipine; Spironolactone (Mylan Pharmaceuticals, Morgantown, WV, USA); hemotransfusion; and platelet transfusion. Despite this aggressive therapy, the patient's condition continued to deteriorate. On March 1, 2001, the patient was transferred to the pediatric intensive care unit because of respiratory failure. Chest radiographs revealed pulmonary edema. Her condition was stabilized 2 days later, but severe acute respiratory distress syndrome and distended abdomen developed on April 1. Treatment was continued and mechanical ventilation was added. A radiograph taken on April 15 (≈11 weeks after transplantation) revealed free air in the abdominal cavity, but the source was not identified. The patient died later that day; the probable cause of death was viral pneumonitis. An autopsy was performed.

Electron Microscopy

Lung tissues were fixed in formalin and then postfixed in 2.5% glutaraldehyde/0.1 mol/L Millonig phosphate buffer before processing into epoxy resin for sectioning and film photography. Microscopic examination was performed with a Hitachi 7100 transmission electron microscope (Hitachi High-Technologies Science America Inc., Northridge, CA, USA).

IHC

IHC was performed as described previously (*11*). In brief, formalin-fixed paraffin-embedded blocks of tissue were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide. Antigen was retrieved, and samples were blocked in 1.5% normal horse serum. A mouse monoclonal antibody against viral protein (VP) 1 from WUPyV (WU-VP1) (NN-Ab06) or an isotype-matched control antibody (mouse IgG2b; BD Biosciences, San Jose, CA, USA; no. 557351) was incubated overnight. After incubation with the biotinylated antimouse IgG secondary antibody (Vector BA-2000; Vector Laboratories, Inc., Burlingame, CA, USA), slides were developed by using the Vectastain ABC kit (Vector Laboratories, Inc.; no. PK-6100) and DAB (Vector Laboratories, Inc.; no. SK-4100), counterstained with hematoxylin, dehydrated, cleared, and mounted.

Double IHC

The double IHC (dIHC) staining protocol was similar to the regular IHC protocol with the addition of several steps. After the blocking step, slides were incubated with NN-Ab06 and then the secondary antibody. Development was accomplished by using the ABC kit and ImmPACT SG (Vector Laboratories, Inc.; no. SK-4705). Tissues were blocked with avidin and biotin (Vector Laboratories, Inc.; no. SP-2001) then with 1.5% normal horse serum. Slides were incubated in the second primary antibody against CD68 (Dako, Glostrup, Denmark; no. M081401) and then in biotinylated antimouse IgG secondary antibody. The second set of staining was developed by using the ABC kit and 3,3′-diaminobenzidine, followed by dehydration. For the MUC5AC dIHC assay, staining was first performed with the monoclonal antibody against MUC5AC (Thermo Fischer, Rockford, IL, USA; no. MA1–38223), which was developed with 3,3′-diaminobenzidine. Staining with NN-Ab06 and development with SG substrate (Vector Laboratories, Inc.) followed the blocking steps. Tissues stained by using the dIHC protocol were not counterstained. Control staining with an IgG2b isotype antibody (for NN-Ab06) and an isotype antibody against IgG1 (for the CD68 and MUC5AC antibodies) was also performed.

Nucleic Acid Extraction and PCR

DNA was extracted from formalin-fixed paraffin-embedded samples by using the QIAGEN BioRobot M48 workstation and MagAttract DNA Mini Kit (QIAGEN, Valencia, CA, USA). A quantitative real-time PCR (qPCR) assay for detection and viral load estimation of WUPyV/KIPyV was developed and used to screen the extracted DNA. Primer and probe sequences for the qPCR assay were forward 5′-GTAGCTGGAGGAGCAGAGGC-3′; 5′-CACCAAGRGCAGCTAARCCTTC-3′; and probe 5′-CTGGWTCTGGAGCTGCMATAGCWACTGGT-3′. qPCRs were performed by using iQ Supermix reagents (Bio-Rad, Hercules, CA, USA); each 25-μL reaction mixture contained 0.6 μmol/L forward primer, 0.3 μmol/L reverse primer, 0.1 μmol/L probe, and 5 mL nucleic acid extract. Amplification was conducted on an iCycler iQ Real-time Detection System (Bio-Rad). Thermocycling conditions consisted of 3 min at 95°C for activation of the iTaq DNA polymerase and 45 cycles of 15 s at 95°C and 1 min at 60°C. Extracts were also tested for human bocavirus (HBoV) by using a previously described qPCR assay (*12*). For distinguishing between WUPyV and KIPyV, conventional PCR and sequencing was performed by using primers forward 5′-GGAGCTGTAYAGAATGGAAAC-3′ and reverse 5′-TTCATCCAAYAGTGGAATTG-3′.

Complete Genome Sequencing

Multiple segments of the WUPyV genome were amplified and sequenced by using overlapping primer sets (Table 1) designed from reference strain WU/Wuerzburg/02/07 (GenBank accession no. EU711057.1). Amplicons were directly sequenced by using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, version 3.1, on an ABI 3130 XL DNA Sequencer (both from Applied Biosystems, Carlsbad, CA, USA). The complete genome sequence for isolate Rochester-7029 is available through GenBank under accession no. FJ794068.

Results

The most remarkable gross autopsy findings were bilateral pulmonary consolidation, acute tracheobronchitis, hepatomegaly with cholestasis, deep mucosal ulcerations throughout the small bowel, and prominent generalized lymphadenopathy. The most notable pathologic finding was heavy hemorrhagic foci in the lungs. Microscopic examination of the lungs confirmed the presence of interstitial emphysema with profound hemorrhage in the right upper, right middle, left upper, and left lower lobes. Multiple smudge cells and cells with Cowdry type A nuclear inclusions were identified inside reactive bronchial epithelium from both lungs. Similar inclusions were seen in epithelium of the trachea, bile duct, renal tubules, and urinary bladder. Small bowel mucosa revealed multifocal ulcerations and scattered inclusion-bearing cells in the epithelium. Findings after immunoperoxidase staining were negative for cytomegalovirus, adenovirus, influenza virus, human papillomavirus, respiratory syncytial virus, and simian virus 40, and in situ hybridization was negative for Epstein-Barr virus. Attempts to culture virus from the lung, liver, gastrointestinal tract, and lymph node tissues were unsuccessful. Gram-stained sections of lung, liver, and lymph node showed >25 neutrophils per low-power field without presence of organisms. Coagulase-negative *Staphylococcus* spp. grew from a blood culture (<30 CFU/mL) and culture of the gastrointestinal tract. No strictly anaerobic growth was observed.

Electron micrographs of the lungs showed viral particles in the nuclei, many in para-crystalline arrays (Figure 1). The particles were 30.4–34.7 nm (mean 32.1 nm) in diameter. The diameter was less than the conventional diameter for polyomaviruses (45 nm), but the size of viral particles can vary according to the method of fixation and embedding (*14*). In addition, previously published electron microscopy findings for BKPyV particles indicated measurements of 30–50 nm (*15*). Despite the presence of viral particles indicative of polyomavirus, IHC on lung tissue with a primary antibody against simian virus 40, which is known to cross-react with BK and JC polyomaviruses, was negative. IHC for adenovirus and respiratory syncytial virus was also negative. Although electron microscopy indicated a probable viral infection in the patient's lungs, because of the negative IHC results, no further testing was performed at that time.

Table 1. Primers used to sequence WU polyomavirus in lung tissue from a child with acute respiratory illness

Oligonucleotide	Sequence, 5′ → 3′ (reference, if applicable)
WUPYV-F1	GTAGCTGGAGGAGCAGAGGC
WUPYV-R1	CACCAAGAGCAGCTAAACCTTC
WUPYV-F2	CCACGCCCCCTACCCAG
WUPYV-R2	AATATGATGTCCAGATTCCATAGGC
WUPYV-F3	CCAAGGAGGTGGACTTAATATCCA
WUPYV-R3	ACCTGCCAGTGCCATTCC
WUPYV-F4	CGTTGGATATAAAGGGTCACCA
WUPYV-R4	GCCTCTGCTCCTCCAGCTA
WU seq F1	AGCTAAGCATGATTGACAGTGTG
WU seq R1	CAGACTCAACGGAGATGTCACA
WU seq F2	TCACTGTTATGTGCAGGAATGT
WU seq R2	ACAGCAAGCAATATGCCCATC
WU seq F3	TATTGGTGCTACCGTCTCGAAC
WU seq R3	GTGGATGGACTGGATATTAAGTC
WU seq F4	ATATATACAGCTTTAGCAGCAGATC
WU seq R4	CTTACTTGTTCAACTATAGCATTTACTG
WU seq F5	CAGTAGTTAATAGAGCAGTTAGTGAAGA
WU seq R5	TAGAAATGTCACTGTTTAGCTCTTC
WU seq F6	GATGGCTTTAATGCACTTAGTGATG
WU seq R6	GTAGCACACAGTAGTATCAGCATCAG
WU seq F7	ATTAGTAGCCCACTTAAAACTGCTG
WU seq R7	TCTGCCACCCATGATTCAATG
WU seq F8	GTTTATTCCAGTTCTGAAACACC
WU seq R8	GCAAATGAGACAAATTACTGGTTG
WU seq F9	CTTTATAAGCAGGTGTTTAATAAGC
WU seq R9	TAAAAGAAAGTCTGGATAAAACTCC
WU seq F10	TTCTTTCCAATACACAACTTTAGC
WU seq R10	GGTAAAACAACTGTTGCTGCT
WU seq F11	CTCCTACTTGACCTTTTACATCTTC
WU seq R11	CAACTCATAATAGACTTCATATGGAAC
WU seq F12	TCTTCTAGCTAATAAATCTTCTCTGG
WU seq R12	GTAATACATACCACCAAAGAAAAGG
WU seq F14	CAGCACTAACTCTATGTCTAAAAGG
WU seq R14	GGTGCTATAGAGAGTGGTTTGG
WU seq F15	CTCATTACATCTTAGTTCTTCTTCC
WU seq R15	AAGAATTTCATCCTGACAAAGG
WU seq F16	TCTACCTGTGAAGAGCTCCACAC
WU seq R16	CACATTCCTGCACATAACAGTG
WU seq F17	CTAAGCATGATTGACAGTGTGG
WU seq R17	TGATAGTGCCTCTGCTCCTC
AG0058	GCTCCACCTTGTGGCTGCTA (*6*)
AG0036	GCATTTACTGGGTCAGATTCC (*6*)
AG0035	TGCATTCTACCTGTGAAGAGC (*6*)
WU seq F6.5	GTACCACTGTCAGAAGAAACAGAG
WU seq F7.5	GATGTGCTAGGACTTGCTCC
WU seq R9.5	CCTCCAGGTATTGTAACAATGAATG
WU-C-4824-F	GGCACGGCGCCAACT (*13*)
WU-C-4898-R	CCTGTTGTAGGCCTTACTTACCTGTA (*13*)
WU 4422R	GAAATGCCTAAATCTCCTGGAG
WU 4341F	GTGTTGCCTGTGAACATTGTG
WU 4810R	AGACTGGGACATATGCTTAAAGG
WU 4571F	GCTTACCTGGTTAAGCCAAC
WU 4945R	GTGAAGTAGAAGAAGAAGTAAATCA
WU 5225R	AAAGCCTCAACTTTCTGAACTA
WU 783R	AAGCTCAGGTACTTTTGTTAGTACAG
PyVseq 844F	GGAGCTGTAYAGAATGGAAAC
PyVseq 2419R	TTCATCCAAYAGTGGAATTG

Table 2. Summary of virus findings in tissue samples from child with acute respiratory illness*

Study	Lung	Liver	Kidney	Gastrointestinal
Intranuclear inclusions	Positive	Positive	Positive	Positive
Electron microscopy	Positive	Not performed	Not performed	Not performed
WUPyV/KIPyV qPCR	Positive (16.6)	Positive (30.8)	Positive (30.4)	Positive (30.2)
WUPyV PCR and sequencing	Positive	Positive	Positive	Positive
KIPyV PCR and sequencing	Negative	Negative	Negative	Negative
WUPyV IHC	Positive	Negative	Negative	Negative
HBoV qPCR	Negative	Negative	Negative	Negative

*Numbers in parentheses indicate cycle thresholds from qPCR. HBoV, human bocavirus; IHC, immunohistochemistry; KIPyV, KI polyomavirus; qPCR, real-time quantitative PCR; WUPyV, WU polyomavirus.

WUPyV in the Lungs

The recent discoveries of HBoV, another small, circular DNA virus, in 2005 and 2 new polyomaviruses, WU and KI (KIPyV), in 2007, in the respiratory tracts of children with acute respiratory illness prompted us to investigate the involvement of these viruses in this case. DNA extracted from formalin-fixed paraffin-embedded lung, liver, kidney, and gastrointestinal tissues was tested by qPCR for WUPyV, KIPyV, and HBoV. All 4 tissues were positive for WUPyV, whereas KIPyV and HBoV were not detected in any of the tissues. Virus loads estimated by qPCR were substantially higher in samples from the lung (cycle threshold 16.6) than in samples from the liver, kidney, and gastrointestinal tract (cycle threshold 30.2–30.8; Table 2). The entire WUPyV genome (designated Rochester-7029, GenBank accession no. FJ794068) was subsequently sequenced from lung tissue to 4× coverage (each base sequenced independently 4 times) by using multiple primer sets and found to be 5,306 bp. Compared with 79 complete WUPyV genome sequences available in GenBank, nucleotide identity scores for Rochester-7029 ranged from 0.970 to 0.985. Compared with the reference sequence, Rochester-7029 had 8 single-nucleotide polymorphisms, 5 of which were in coding regions. Two of these 5 were synonymous mutations. We predicted an amino acid change in VP2 and VP3 from glutamic acid to glutamine at positions 250 and 107, respectively. We also predicted amino acid changes in large T-antigen: glutamine to glutamic acid at position 134 and isoleucine to leucine at position 594. Of note, the Rochester-7029 genome contained a 77-bp terminal duplication in the large T-antigen as compared with the reference WUPyV genome, which was not predicted to have any effect on the size or sequence of the translated protein because it was located 3′ to the T-antigen stop codon.

After detection of WUPyV in the patient's tissues by real-time qPCR, WUPyV-specific IHC with a previously described assay (*11*) was performed on available tissues (lung, liver, kidney, and gastrointestinal tract) to determine whether WU-VP1 antigen was also present (Figure 2). Liver, kidney, and gastrointestinal tissues were all negative (Table 2). Staining was observed in the lung (Figure 2, panels A, C) and the trachea (Figure 2, panel E), but no staining was observed in serial sections stained with an isotype control antibody (Figure 2, panels B, D, F). Serial sections stained with no primary or secondary antibodies were also negative (not shown). Overall, we saw 3 patterns of staining in the lung. In some cells, WU-VP1 staining was primarily in the nucleus. In others, the perimeter of the nucleus was strongly positive. And in others, the staining was diffuse, making it difficult to discern its position within cells. Of note, the tracheal staining was within a submucosal gland, where WUPyV tropism has not been previously described.

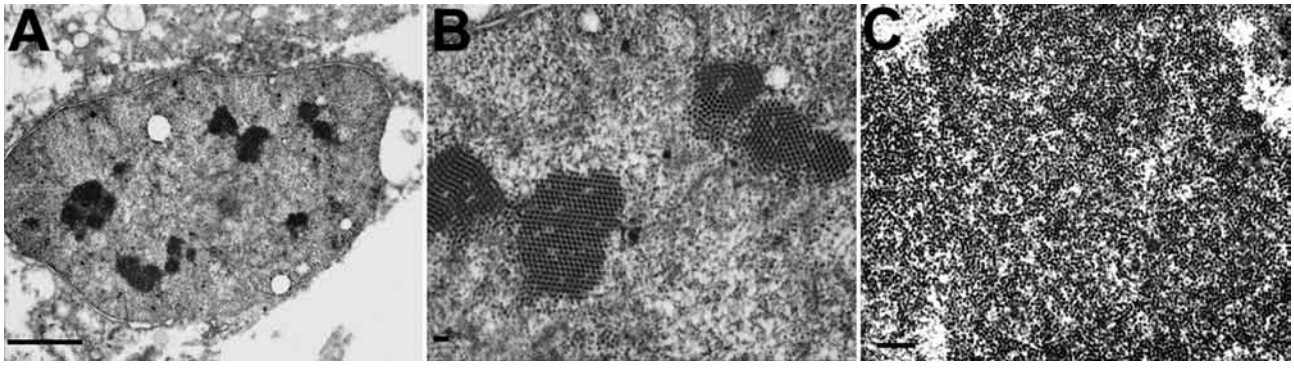

Figure 1. Electron micrographs of polyomavirus-like particles in lung from a child with fatal acute respiratory illness. A) Low-power view of a nucleus displaying multiple electron dense crystalline arrays. Scale bar indicates 0.5 μm; original magnification ×10,000. B) Higher-power magnification of nucleus in panel A. Scale bar indicates 100 nm; original magnification ×30,000. C) Large cluster of putative polyomavirus virions. Scale bar indicates 250 nm; original magnification ×20,000.

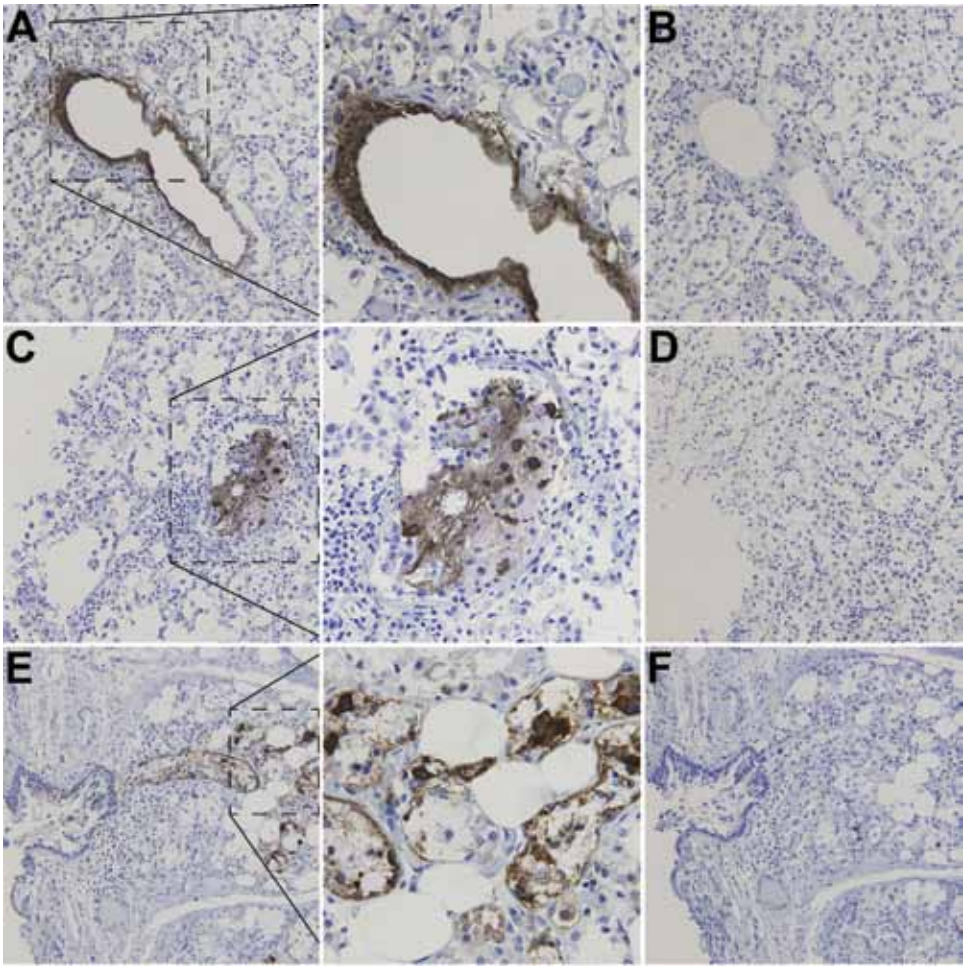

Figure 2. Immunohistochemical detection of WU polyomavirus viral protein 1 in respiratory tract of a child with fatal acute respiratory illness. Human lung tissue at original magnification of ×200, stained with a monoclonal antibody against WU polyomavirus viral protein 1 (designated NN-Ab06) (A, C) or an isotype control antibody (B, D). Human tracheal tissue at original magnification of ×200, stained with NN-Ab06 (E) or an isotype control antibody (F). The middle panels show insets from panels A, C, and E (dotted boxes) at higher original magnifications (×600).

WU-VP1 in CD68-Positive Cells

A recent article described detection of WU-VP1 in epithelial cells obtained from a bronchoalveolar lavage of a lung transplant recipient with Job syndrome (*11*). We anticipated that some WUPyV-positive cells in lung tissues of the patient reported here were also epithelial cells, but we did not explicitly confirm this suspicion because of the limited amount of lung tissue available. Instead we chose to explore additional hypotheses regarding potential tropisms of WUPyV because of our recent detection of KIPyV in CD68-positive cells (*16*). CD68 is a glycoprotein present on monocytes and macrophages. We performed dIHC testing by using the monoclonal antibody against WU-VP1 (NN-Ab06) and a monoclonal antibody against CD68, which primarily labels macrophages and monocytes. Cells positive for both WU-VP1 and CD68 were detected within the patient's lung tissue (Figure 3). In addition, the cell shown in Figure 3, panel B (arrow) is morphologically consistent with a foamy macrophage, a specific morphotype of macrophage that is laden with lipid droplets in the cytoplasm (*17*). A serial section stained with isotype-matched antibodies (IgG2b for NN-Ab06 and IgG1 for the anti-CD68 antibody) was negative (not shown). In a recent study, KIPyV was also detected in a foamy macrophage (*16*).

WU-VP1 Antigen in Association with Mucin-Producing Cells

The initial IHC staining of tracheal tissue revealed positive cells within a submucosal gland. MUC5AC is the principal mucin produced by goblet cells, and MUC5B is produced by submucosal glands (*18*). We developed 2 dIHC assays: NN-Ab06 and a monoclonal antibody against MUC5AC and NN-Ab06 and a polyclonal antibody against MUC5B. Each assay was performed on control cell pellets (not shown). The MUC5AC dIHC assay yielded clearer staining, so we chose to apply this assay to the tracheal tissue from the patient. We detected WU-VP1–positive cells in association with a cluster of cells showing MUC5-AC positivity (Figure 4). It is unclear whether the 2 antigens colocalize to the same cell. WU-VP1–positive cells were also seen separate from MUC5AC-positive cells, suggesting that a subset of virus-positive cells do not produce mucin. We found 2 such areas in the tracheal tissue.

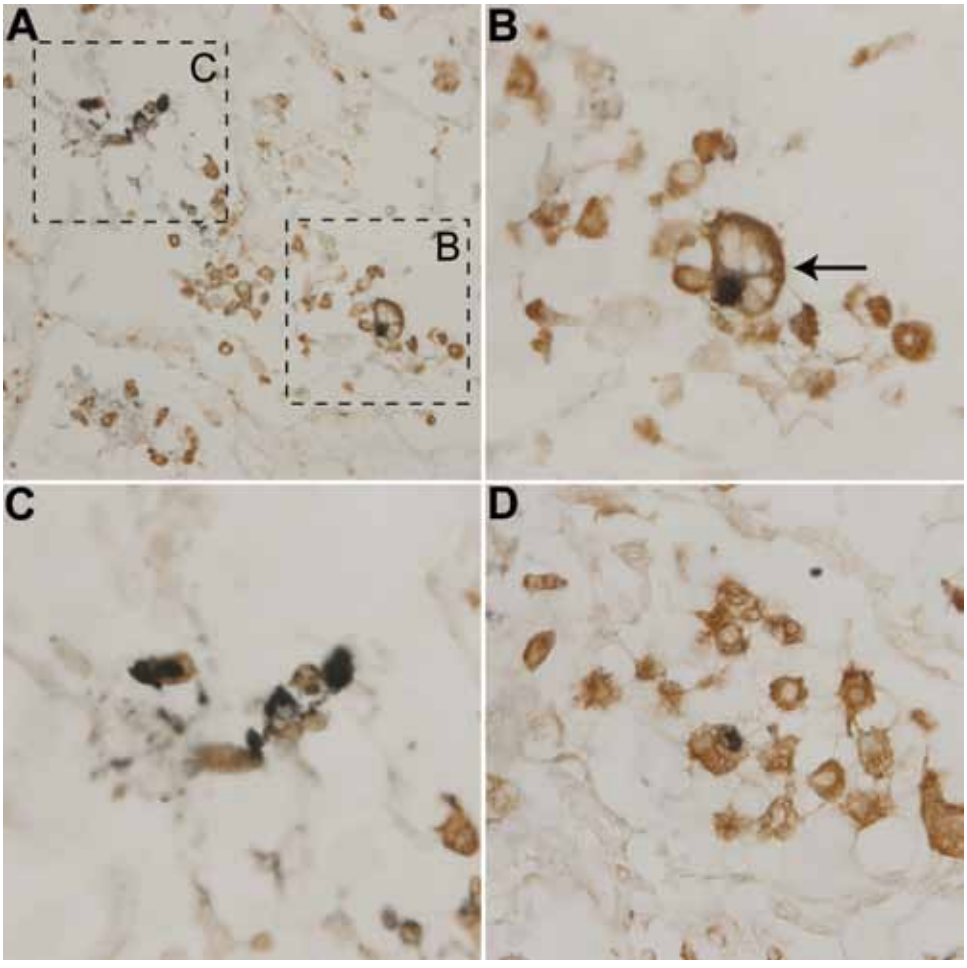

Figure 3. Detection of WU polyomavirus viral protein 1 in CD68-positive cells from a child with fatal acute respiratory illness. Lung tissue stained with NN-Ab06 (blue) and a monoclonal antibody against CD68 (brown). A) Tissue at original magnification of ×400. B) Closer view of cell from panel A consistent with a foamy macrophage (arrow). Original magnification ×1,000. C) Closer view of cells from panel A. Original magnification ×1,000. D) Different field of the tissue section with another double-positive cell. Original magnification ×1,000.

Discussion

We describe a case of viral pneumonitis in a bone marrow transplant recipient who died in 2001. Before her death, influenza and adenovirus were identified from the patient. Although samples from the patient were initially tested for several viruses by IHC and culture, no agent was identified. Subsequent work since 2007 showed that multiple tissues from this patient were positive for WUPyV by real-time PCR and IHC, but the same tissues were negative for KIPyV and HBoV by PCR. To date, WUPyV has been the only virus detected in tissue samples from this patient. Crystalline lattices of polyomavirus-like particles were seen in the lung, which substantiated the high virus titers measured by qPCR. Collectively, these observations suggest a potential pathogenic role for WUPyV infection in this case. However, we cannot rule out the possibility that WUPyV infection was simply opportunistic in this severely immunocompromised patient.

Analysis of samples from this patient provided novel insights into fundamental properties of WUPyV infection in vivo. In the lungs, we detected WUPyV antigen in CD68-positive cells (probably of the macrophage/monocyte lineage) by immunohistochemistry. WUPyV is most closely related to KIPyV, and the viruses share many similarities, including an apparent tropism for CD68-positive cells (*16*). Other polyomaviruses have also been detected in cells of the monocytic lineage (*16*). We do not believe that this detection represents phagocytosis of other WUPyV-infected cell types because WU-VP1, a late-expressed protein, was detected in the nucleus of CD68-positive cells, suggesting an infection in this patient. Granted, we did not prove that infectious particles were produced.

WU-VP1 was also detected in close proximity to MUC5AC-positive cells in tracheal tissue. The detection of WUPyV in tracheal tissue was unexpected and expands the known tissue tropism of the virus. As previously mentioned, MUC5AC is a mucin primarily produced by goblet cells in the airway. Several viruses in glandular cells in the trachea have been described: adenovirus has grown in primary cultured peribronchial submucosal gland cells (*19*); rhinovirus has grown in human respiratory submucosal gland cells (*20*); severe acute respiratory syndrome coronavirus antigen and RNA have been detected in tracheal/bronchial serous gland epithelium (*21*); and BKPyV has been shown to replicate in salivary gland cells (*22,23*).

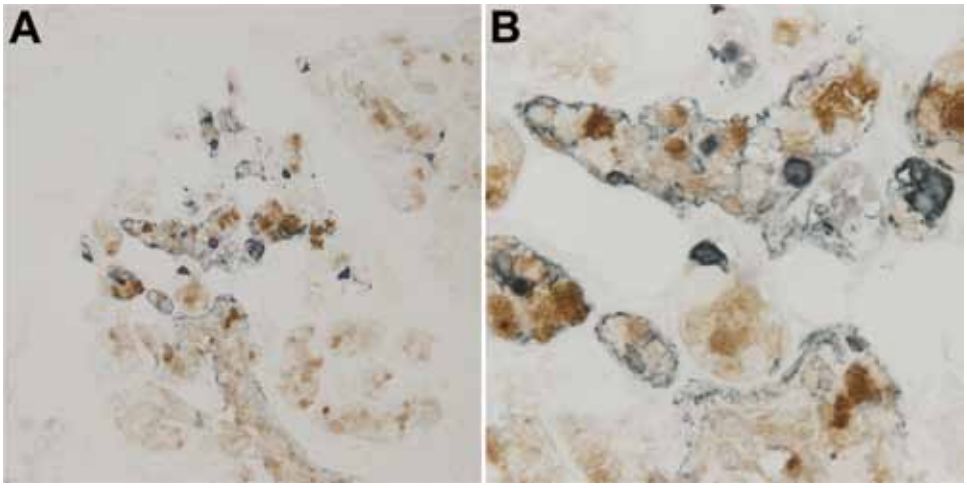

Figure 4. Detection of WU polyomavirus viral protein 1 in close proximity to MUC5AC-positive cells in the trachea of a child with fatal acute respiratory illness. Tracheal tissue stained with NN-Ab06 (blue) and a monoclonal antibody against MUC5AC (brown). A) Tissue at original magnification of ×200. B) Tissue at original magnification of ×600.

In conclusion, WUPyV was detected by multiple methods in the lung of a bone marrow transplant recipient who had viral pneumonitis at the time of death. Tracheal tissue from this patient was also positive for WU-VP1. Viral antigen was specifically detected in CD68-positive cells and in close association with MUC5AC-positive cells within a tracheal submucosal gland. The role of WUPyV as a human pathogen remains unclear, although the evidence for an infection of the respiratory tract in this patient is strong. This study expands our understanding of WUPyV biology and tropism beyond detection of virus in body fluids.

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Multifacility Outbreak of Middle East Respiratory Syndrome in Taif, Saudi Arabia

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Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) is a novel respiratory pathogen first reported in 2012. During September 2014–January 2015, an outbreak of 38 cases of MERS was reported from 4 healthcare facilities in Taif, Saudi Arabia; 21 of the 38 case-patients died. Clinical and public health records showed that 13 patients were healthcare personnel (HCP). Fifteen patients, including 4 HCP, were associated with 1 dialysis unit. Three additional HCP in this dialysis unit had serologic evidence of MERS-CoV infection. Viral RNA was amplified from acute-phase serum specimens of 15 patients, and full spike gene-coding sequencing was obtained from 10 patients who formed a discrete cluster; sequences from specimens of 9 patients were closely related. Similar gene sequences among patients unlinked by time or location suggest unrecognized viral transmission. Circulation persisted in multiple healthcare settings over an extended period, underscoring the importance of strengthening MERS-CoV surveillance and infection-control practices.

Middle East respiratory syndrome (MERS) coronavirus (MERS-CoV) is a novel betacoronavirus associated with a broad spectrum of respiratory illness; infection results in death in ≈35%–40% of cases (*1*). Since the virus was first identified 2012, more than 85% of cases have occurred in Saudi Arabia (*1*). Although risk factors for transmission have not been well described, camels (*Camelus dromedarius*) are suspected reservoirs, as suggested by case investigations (*2,3*), serologic studies (*4,5*), and isolation of live infectious MERS-CoV (*2,3,6*). Limited human-to-human transmission has been documented in households (*7*) and healthcare facilities (*8–10*), but no sustained community transmission has been documented (*1*). In Jeddah in 2014, secondary transmission (i.e., from infected to noninfected persons) accounted for 97% of assessed cases (*9*).

Although detection of MERS-CoV RNA from persons with mild symptoms, typically in healthcare personnel (HCP), is well-documented (*11*), the potential role that mild cases play in transmission is not well defined (*12*). In healthcare facilities, extensive transmission of MERS-CoV in dialysis units has been documented (*8,9*); in those events, strengthening infection-control precautions preceded decreased numbers of reported cases. Currently, the surveillance case definition for MERS in Saudi Arabia requires the presence of symptoms (*13*), and testing is reserved primarily for symptomatic patients, often with severe illness.

MERS cases were first reported from Taif Governorate (population 1.1 million) in the Makkah Region of Saudi Arabia in June 2013, and 15 cases were reported during June 2013–June 2014. Beginning in September 2014, additional cases of MERS were reported from multiple healthcare facilities in Taif, including a cluster associated with a dialysis unit. The Saudi Arabia Ministry of Health (MoH), assisted by the US Centers for Disease Control and Prevention (CDC), began an investigation to determine the cause and scope of the outbreak, epidemiologic links between patients, and epidemiologic and clinical features of patients.

Methods

Setting

Hospital A is a 368-bed tertiary acute-care facility and serves military staff and their families. Hospital B is a 500-bed tertiary MoH hospital with an associated but physically separate outpatient renal dialysis unit. Hospital C is a 250-bed MoH facility and is the MERS-CoV designated referral hospital for Taif. Hospital D is a private hospital.

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Epidemiologic Investigation

We defined a case-patient as any patient from Taif Governorate who was reported with laboratory-confirmed MERS-CoV infection during August 1, 2014–February 1, 2015. In Saudi Arabia, reporting is required for all patients with clinical or radiologic evidence compatible with MERS-CoV disease and with a positive real-time reverse transcription PCR (rRT-PCR) on 2 specific gene targets: the region upstream of the E gene and open reading frame 1a (*13*). We reviewed available medical and public health records for all reported case-patients during the study period and conducted interviews with available hospital staff. We collected available demographic information, medical history, symptoms at onset, clinical course, preillness exposures, and evaluation and treatment locations. We grouped together case-patients whose illness onset occurred within 2–14 days of exposure (work- or treatment-related) to the same facility. Available residual patient specimens were analyzed at CDC.

Laboratory Investigation

Molecular Detection and Gene Sequencing

At the MoH Regional Laboratory at Makkah, rRT-PCR testing for MERS-CoV RNA was performed on nasopharyngeal (NP) specimens. Serum specimens collected from laboratory-confirmed case patients were sent to CDC for MERS-CoV serology and were tested for viremia by rRT-PCR (*14*). Positive respiratory specimens were not retained and thus unavailable for confirmatory rRT-PCR testing or sequencing at CDC.

Sequencing of the coding region of the spike protein gene (4,062 nt) was performed by using a 3130xl Genetic Analyzer (Applied Biosystems, Grand Island, NY, USA); analysis was performed by using Sequencher 4.8 (Gene Codes, Ann Arbor, MI, USA) for sequence assembly and editing. Sequence alignments were prepared by using ClustalX 1.83 (http://www.clustal.org/) and implemented in BioEdit 7.2.5 (http://www.mbio.ncsu.edu/BioEdit/page2.html). Phylogenetic analyses were performed by using MEGA 6.06 (http://www.megasoftware.net). The neighbor-joining method (tree algorithm inferred with the Kimura 2-parameter substitution model of sequence evolution) was used to construct phylogenetic trees, and bootstrap resampling analysis was performed (1,000 replicates) to test tree-branching significance.

Serologic Assessment

MERS-CoV antibody positivity was defined as a positive result from screenings of MERS-CoV nucleocapsid ELISA and confirmatory positive results by immunofluorescence and microneutralization assays, as described (*15*). A serosurvey of HCP who were exposed to confirmed MERS-CoV patients in the dialysis unit of hospital B was conducted 3 weeks after the period of suspected transmission (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-1370-Techapp.pdf).

Statistical Analysis

For reported demographic and clinical characteristics, differences were assessed for significance ($p = 0.05$) by using χ^2 test, Fisher exact test, and *t*-test, as appropriate. All data were analyzed by using SAS 9.3 (SAS Institute, Cary, NC, USA).

Results

Epidemiologic Investigation

During August 1, 2014–February 1, 2015, the MoH received reports of 38 patients with laboratory-confirmed MERS-CoV (Figure 1). Twenty-eight (74%) were men, 22 (58%) were of Saudi nationality, and median age was 51 (range 17–84) years (Table 1). Thirteen (34%) patients were HCP: 7 nurses, 2 physicians, 2 cleaning personnel, 1 administrative professional, and 1 clerk. The most common underlying medical conditions were diabetes, reported by 16 (47%), and renal failure requiring dialysis, reported by 12 (33%). At illness onset, 35 (92%) patients reported ≥1 respiratory symptom. Two patients, both HCP identified through routine testing of contacts of previously identified patients, reported no symptoms (Table 1).

Twenty-one (55%) of the 38 patients died, all in the hospital. Deceased patients were significantly older than survivors (median age 60.4 vs. 39.4 years; $p = 0.001$) and were more likely to be men (90% vs. 53%; $p = 0.023$) and Saudi nationals (76% vs. 35%; $p = 0.020$). Median time from onset to death or discharge was 17 (range 1.0–84.0) days. HCP patients were more likely than non-HCP patients to be women (54% vs. 12%, $p = 0.016$), non-Saudi (92% vs. 16%, $p<0.001$), and younger (median age 37 vs. 65 years; $p<0.001$); they were also more likely to survive (85% vs. 24%, $p<0.001$). Two of the 13 HCP patients died. Both were non-Saudi men: a 40-year-old physician with no underlying medical conditions and a 46-year-old information technologist with a history of smoking and hypertension.

Of the 38 MERS-CoV patients reported and investigated during the outbreak period, 33 were associated with 4 facilities (Figure 1). We were unable to link 5 patients epidemiologically to other patients (Table 2).

Hospital A

The first patients in this outbreak were reported from hospital A. Of 10 patients associated with this hospital, 6 had illness onset during September 5–October 2, and 4 had onset during November 20–December 10; patients were tightly clustered in time during these 2 periods (Table 2). The initial patient reported was a 45-year-old male military

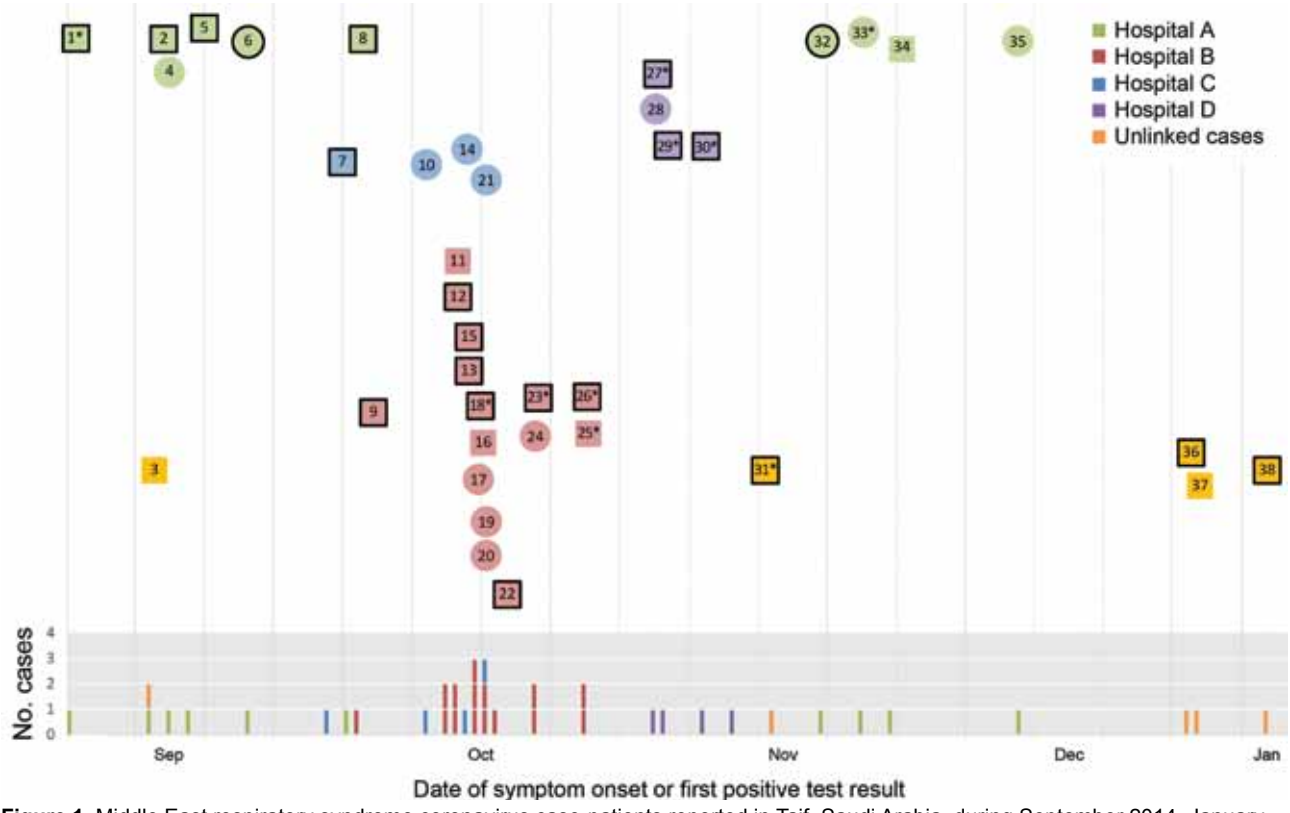

Figure 1. Middle East respiratory syndrome coronavirus case-patients reported in Taif, Saudi Arabia, during September 2014–January 2015. Cases are indicated on the baseline on the basis of time of symptom onset or first positive laboratory testing. Healthcare setting where transmission likely occurred is indicated. Circles indicate healthcare personnel (HCP), squares non-HCP; heavy black outlines indicate that patient died. Asterisks (*) indicate that sequencing was performed on the patient's serum sample.

employee with an unspecified exposure to an outlying farm. Onset of cough, shortness of breath, and fever began on September 5, 2014; he was admitted with respiratory compromise to hospital A on September 10. During September 13–October 2, five additional patients were reported, including 2 HCP employed by the hospital and 2 long-term care inpatients with no community exposures. During November 20–December 10, four additional patients were reported; 3 were HCP. One long-term care patient was admitted to the hospital on June 30, had MERS symptom onset on October 3, and died on December 15, 2014; his hospital course spanned both periods of clustered patients at this facility. Five HCP were among this hospital's clusters: a 37-year-old male clerk who was married to an intensive-care nurse; a 40-year-old male cleaner; a 29-year-old female nurse; and a 40-year-old male physician and a 46-year-old male working in information technology, both of whom died. Of this hospital's 10 reported patients, only the 29-year-old nurse had recognized contact with a known MERS-CoV patient before her illness onset. Six (60%) of the 10 patients died during their hospital course: 5 (83%) of 6 patients during the first transmission period and 1 (25%) of 4 patients during the second period.

Hospital B Dialysis Unit

Hospital B reported 15 patients from its outpatient renal dialysis unit, which was located in a building separate from the acute-care facility. When the outbreak occurred, the dialysis unit had 58 dialysis machines in 8 common rooms, 71 nursing staff, and 377 registered patients receiving periodic hemodialysis. For the 15 patients reported in this cluster, onsets occurred during October 4–27, 2014. Eleven were dialysis patients, and 4 were dialysis unit HCP. The first recognized patient associated with this setting was a 53-year-old man with onset of MERS-related symptoms on October 4, 2015. He underwent dialysis on October 4, 6, 8, and 9 in a 9-bed common room while he was symptomatic. During October 13–28, ten dialysis patients and 3 HCP became ill, and their NP specimens tested positive for MERS-CoV. A fourth HCP reported no symptoms, but his NP specimen was confirmed by rRT-PCR to be MERS-CoV positive on October 25, after RT-PCR screening of NP specimens from identified HCP contacts. Of the 4 MERS-CoV–confirmed HCP, 2 reported working in the dialysis unit while symptomatic on October 18, 20, and 27, just before their MERS-CoV confirmatory testing.

Table 1. Demographic and clinical characteristics of patients with laboratory-confirmed Middle East respiratory syndrome coronavirus infection, Taif, Saudi Arabia, August 2014–February 2015*

Characteristic	Patients, n = 38	Survived, n = 17	Died, n = 21	p value
Male sex	28/38 (74)	9/17 (53)	19/21 (90)	0.023
Nationality				
Saudi	22/38 (58)	6/17 (35)	16/21 (76)	0.020
Non-Saudi	16/38 (42)	11/17 (65)	5/21 (24)	
Occupation				
Healthcare personnel	13/38 (34)	11/17 (65)	2/21 (10)	<0.001
Non–healthcare personnel	25/38 (66)	6/17 (35)	19/21 (90)	
Underlying medical conditions or behaviors				
Renal failure requiring dialysis, n = 36	12/36 (33)	3/16 (19)	9/20 (45)	0.157
Diabetes, n = 34	16/34 (47)	5/16 (31)	11/18 (61)	0.082
Heart disease, n = 30	9/30 (30)	1/14 (7)	8/16 (50)	0.017
Smoker, n = 27	6/27 (22)	1/16 (6)	5/11 (45)	0.027
Any above underlying conditions or behaviors, n = 38	26/38 (72)	7/15 (47)	19/21 (90)	0.007
Symptoms at onset				
Cough, n = 35	27/35 (77)	14/16 (89)	13/19 (68)	0.244
Fever, n = 38	35/38 (92)	15/17 (88)	20/21 (95)	0.577
Shortness of breath, n = 36	21/36 (58)	8/15 (53)	13/21 (62)	0.607
Any respiratory symptoms at onset, n = 38	35/38 (92)	15/17 (88)	21/21 (100)	0.194
Diarrhea, n = 32	2/32 (6)	1/15 (7)	1/17 (6)	1.000
Clinical course				
Pneumonia, n = 36	30/36 (83)	11/17 (65)	19/19 (100)	0.006
Intubation, n = 32	18/32 (56)	3/15 (20)	15/17 (88)	<0.001
Intensive care, n = 35	23/35 (66)	5/17 (29)	18/18 (100)	<0.001
Age, y	51 (17–84)	39 (17–75)	60 (22–84)	0.001
Onset to hospitalization, d	3 (0–10)	4 (0–10)	2 (0–7)	0.060
Onset to death or discharge, d	17 (1–75)	18 (12–42)	14 (1–75)	0.762

*Values are no./total (%) or median (range). Denominators (total number of patients and total numbers of patients who survived and died) vary by characteristic because information was sometimes unavailable in medical charts.

On October 22, infection-control practices were changed on the basis of an onsite assessment by MoH Infection Prevention and Control staff. The changes included screening patients for fever and respiratory symptoms before admission to the dialysis unit; eliminating waiting and prayer areas; discouraging early arrival for dialysis; enforcing a no-visitation policy; increasing distance between patients undergoing dialysis (by reducing number of beds from up to 9 to 6 per room); establishing isolation of dialysis patients with respiratory symptoms; and providing additional infection-control training for staff.

The 15th patient reported from this cluster had illness onset on October 27, after changes were implemented. Of patients in this cluster, 8 (72.7%) of 11 non-HCP died; the 4 HCP survived. Besides the 15 patients reported from this facility, a 17-year-old man who underwent dialysis at this facility on October 4, 6, 8, 11, and 13 reported symptom onset on October 14; his NP specimen was confirmed positive on October 18, after he traveled to Riyadh and was admitted to a hospital there.

Hospital C

On October 3, a 60-year-old man was transferred to hospital C from an outlying hospital in Taif Governorate after respiratory symptoms developed on October 1 and laboratory testing of his NP specimen confirmed MERS-CoV on October 3. He was transferred to a hospital in Jeddah on October 5 and died there on December 25. On October 11, 15, and 17, three HCP (2 nurses and 1 physician) became ill and were hospitalized at hospital C. Each eventually recovered and was discharged, and no further cases were reported from hospital C.

Hospital D

On November 1, a 75-year-old woman was transferred to hospital D and admitted to the intensive care unit. She had been evaluated at hospital C on October 22 and November 1 for respiratory complaints and fever. Laboratory testing at hospital D confirmed her NP specimen as MERS-CoV positive on November 3; she was transferred back to Hospital C on November 4 for MERS-CoV treatment and died there on November 9. During HCP contact screening on November 4, an NP specimen from the cleaner of her room at hospital D on November 1–4 was confirmed as positive for MERS-CoV by RT-PCR. He denied symptoms consistent with MERS. On November 8, respiratory symptoms developed in the patient's 22-year-old grandson; his NP specimen tested positive for MERS-COV on November 11, and he died on December 14. On November 11, an 81-year-old inpatient staying on the same floor where the initial patient received care had onset of respiratory symptoms, and her NP specimen tested positive for MERS-CoV. She died on November 20.

Additional Cases

Five cases were unlinked to cases reported from the 4 hospitals. The first case-patient was a 65-year-old male retiree

with a history of diabetes, heart disease, smoking, and hypertension. After shortness of breath and fever developed on September 13, he sought care at a private hospital on September 17. His NP specimen tested positive for MERS-CoV, and he was referred to hospital C the same day. He was discharged on October 8.

The second case-patient was a 72-year-old male taxi driver with a history of smoking. On November 15, fever developed, followed by sore throat, vomiting, and respiratory failure 2 days later. On November 19, he was admitted to hospital C, where pneumonia was diagnosed, and his NP specimen tested positive for MERS-CoV. He died on December 8.

The third case-patient was a 76-year-old male farmer with a history of diabetes, heart disease, and hypertension. Fever and respiratory symptoms developed on December 27, and he was admitted to hospital C on December 31. MERS-CoV was confirmed by laboratory testing on January 1, and he died on March 3, 2015.

The fourth case-patient was a 33-year-old man who had a history of diabetes and worked as a security guard for a private home. Cough, fever, and headache developed on December 28; on January 7, 2015, he was admitted to hospital C, where pneumonia was diagnosed and his laboratory specimen was MERS-CoV positive. He was discharged on January 19.

The fifth case was a 73-year-old male retiree with diabetes and hypertension. Fever, shortness of breath, nausea, vomiting, and gum bleeding developed on January 4, 2015, and he was admitted to hospital D on January 9 and transferred to hospital C on January 13, 2015. NP specimens collected on January 11 and 19 were positive for MERS-CoV. He died on January 20.

Table 2. Selected characteristics of patients with laboratory-confirmed Middle East respiratory syndrome coronavirus infection, Taif, Saudi Arabia, August 2014–February 2015

Patient no.	Cluster	Healthcare personnel	Date of symptom onset	Date of hospital admission	Date of first positive specimen	Date of death or discharge	Outcome
1	Hospital A	No	2014 Sep 5	2014 Sep 10	2014 Sep 10	2014 Sep 29	Died
2*	Hospital A	No	2014 Sep 13	2008 Jan 1	2014 Sep 13	2014 Sep 20	Died
3	Unlinked	No	2014 Sep 13	2014 Sep 17	2014 Sep 17	2014 Oct 8	Discharged
4	Hospital A	Yes	2014 Sep 15	2014 Sep 21	2014 Sep 21	2014 Oct 27	Discharged
5	Hospital A	No	2014 Sep 17	2014 Sep 24	2014 Oct 5	2014 Oct 17	Died
6	Hospital A	Yes	2014 Sep 23	2014 Sep 23	2014 Sep 23	2014 Oct 3	Died
7	Hospital C	No	2014 Oct 1	2014 Oct 2	2014 Oct 3	2014 Dec 25	Died
8*	Hospital A	No	2014 Oct 3	2014 Jun 30	2014 Oct 6	2014 Dec 15	Died
9	Hospital B	No	2014 Oct 4	2014 Oct 9	2014 Oct 10	2014 Oct 28	Died
10	Hospital C	Yes	2014 Oct 11	2014 Oct 14	2014 Oct 15	2014 Nov 6	Discharged
11†	Hospital B	No	2014 Oct 13	Unknown	2014 Oct 15	2014 Nov 11	Discharged
12	Hospital B	No	2014 Oct 13	2014 Oct 16	2014 Oct 16	2014 Oct 19	Died
13	Hospital B	No	2014 Oct 14	2014 Oct 14	2014 Oct 14	2014 Oct 15	Died
14	Hospital C	Yes	2014 Oct 15	2014 Oct 18	2014 Oct 18	2014 Oct 23	Discharged
15	Hospital B	No	2014 Oct 16	2014 Oct 17	2014 Oct 18	2014 Oct 22	Died
16	Hospital B	No	2014 Oct 16	2014 Oct 23	2014 Oct 23	2014 Oct 30	Discharged
17‡	Hospital B	Yes	–	2014 Oct 25	2014 Oct 25	2014 Oct 30	Discharged
18	Hospital B	No	2014 Oct 16	2014 Oct 18	2014 Oct 27	2014 Oct 27	Died
19	Hospital B	Yes	2014 Oct 17	2014 Oct 27	2014 Oct 27	2014 Nov 2	Discharged
20	Hospital B	Yes	2014 Oct 17	2014 Oct 17	2014 Oct 26	2014 Nov 2	Discharged
21	Hospital C	Yes	2014 Oct 17	2014 Oct 21	2014 Oct 27	2014 Nov 3	Discharged
22	Hospital B	No	2014 Oct 18	2014 Oct 20	2014 Oct 19	2014 Oct 25	Died
23	Hospital B	No	2014 Oct 22	2014 Oct 22	2014 Oct 23	2014 Nov 4	Died
24	Hospital B	Yes	2014 Oct 22	2014 Oct 26	2014 Oct 26	2014 Nov 9	Discharged
25	Hospital B	No	2014 Oct 27	2014 Oct 27	2014 Oct 29	2014 Nov 12	Discharged
26	Hospital B	No	2014 Oct 27	2014 Oct 28	2014 Oct 28	2014 Nov 10	Died
27	Hospital D	No	2014 Nov 3	2014 Nov 1	2014 Nov 3	2014 Nov 10	Died
28‡	Hospital D	Yes	–	2014 Nov 5	2014 Nov 4	2014 Nov 11	Discharged
29	Hospital D	No	2014 Nov 8	2014 Nov 10	2014 Nov 11	2014 Dec 14	Died
30	Hospital D	No	2014 Nov 11	2014 Oct 21	2014 Nov 11	2014 Nov 20	Died
31	Unlinked	No	2014 Nov 15	2014 Nov 19	2014 Nov 20	2014 Dec 8	Died
32	Hospital A	Yes	2014 Nov 20	2014 Nov 20	2014 Nov 22	2014 Nov 27	Died
33	Hospital A	Yes	2014 Nov 24	2014 Nov 27	2014 Nov 27	2014 Dec 11	Discharged
34	Hospital A	No	2014 Nov 27	2014 Dec 2	2014 Dec 4	2014 Dec 25	Discharged
35	Hospital A	Yes	2014 Dec 10	2014 Dec 15	2014 Dec 15	2014 Dec 22	Discharged
36	Unlinked	No	2014 Dec 27	2014 Dec 31	2015 Jan 1	2015 Mar 3	Died
37	Unlinked	No	2014 Dec 28	2015 Jan 7	2015 Jan 6	2015 Jan 19	Discharged
38	Unlinked	No	2015 Jan 4	2015 Jan 9	2015 Jan 11	2015 Jan 20	Died

*Long-term care patient.
†Hospitalized only in Riyadh Governorate.
‡Patients had no reported symptoms so no date of onset; they were identified through routine testing of contacts of known patients.

Laboratory Investigation

Molecular Detection and Spike Gene Sequencing

CDC performed laboratory confirmation of MERS-CoV by rRT-PCR on acute-phase serum samples from 17 patients whose NP specimens had been previously confirmed positive for MERS-CoV by RT-PCR (online Technical Appendix Table). Median number of days from symptom onset to serum collection was 3.5 (range 0–18 for 16 patients). Serum samples from 15 (88.2%) patients, including 4 samples collected 9–18 days after symptom onset, were confirmed to be positive by rRT-PCR by at least 2 independent assays. The mean rRT-PCR cycle threshold (C_t) value for a region upstream of the E gene from 9 respiratory specimens was 26.4 (range 17.5–37.9), compared with 34.8 (range 31.1–38.1) for acute serum samples collected on the same day. In general, patients with low C_t values (proxies for virus load) in respiratory specimens also had low C_t values in serum samples.

Because of limited available serum volume and generally low virus loads, we focused sequencing efforts on the MERS-CoV spike gene, which has been shown to be a reliable proxy for virus genotyping (*16*) and encodes the receptor-binding domain responsible for attachment to host cells. Sequencing of the spike gene coding region was attempted on all rRT-PCR–positive specimens; complete sequences were obtained from serum samples of 10 patients. The mean N2 rRT-PCR C_t value of serum samples that were successfully sequenced was 32.8 (range 31.1–35.6), compared with 37.8 (range 35.6–40.6) for samples with failed sequencing. Phylogenetic analysis of the 10 Taif spike sequences showed that the viruses formed a single, discrete cluster located within the Hafr-Al-Batin clade (*17*) and were most closely related to MERS-CoV viruses circulating in Riyadh during 2013 and 2014 (Figure 2). Sequences from 6 patients (1 from hospital A, 4 from hospital B's dialysis unit, and 1 from hospital D) were identical, and all 10 sequences possessed 2 defining base substitutions at positions 3,670 (G>A) and 3,840 (C>T) (online Technical Appendix Table). Sequences from 2 epidemiologically linked cases (patients 27 and 30 in the online Technical Appendix Table) associated with hospital D formed a subcluster among the Taif viruses on the basis of 2 defining base substitutions at positions 1,679 (C>T) and 3,496 (G>A).

Five unique nucleotide substitutions conferring predicted amino acid changes were identified among the 10 sequences, of which 3 (E536K, D537E, T560I) were located in the spike protein receptor-binding subdomain that directly interacts with the dipeptidyl peptidase 4 receptor (*18*) (online Technical Appendix Table). Random coding changes in the MERS-CoV spike protein may be functionally inconsequential or may confer selective advantage by enabling greater adaptation to the host and possibly enhanced virus transmission (*18*).

Serologic Testing

In addition to conducting rRT-PCR on serum samples of 17 patients, CDC performed MERS-CoV serologic assays on these specimens. Specimens from 4 (23.5%) of the 17 patients were considered positive by serologic testing for MERS-CoV antibodies (online Technical Appendix Table); 3 of the 4 were positive by ELISA (titers of 6,400), immunofluourescence, and microneutralization (titers of 320). The fourth patient was positive by ELISA (titer of 1,600) and was confirmed positive by microneutralization (titer of 20). Four of 62 HCP exposed to MERS-CoV patients in hospital B's dialysis unit were positive for antibodies to MERS-CoV, including 1 patient whose specimen was previously confirmed positive by rRT-PCR (online Technical Appendix).

Discussion

Although initial epidemiologic investigation indicated separate transmission events within hospitals A, B, C, and D in Taif Governorate, Saudi Arabia, during September 5–December 15, 2014, sequencing the spike gene coding regions from samples of residual serum from 10 patients indicated a single, discrete cluster. Of the 10 spike sequences from samples collected during September 5–November 9 from patients at 3 facilities, 6 were identical: 1 collected from hospital A, 4 from hospital B's dialysis unit, and 1 from hospital D. This finding suggests linked transmission among these facilities during this 2-month period. However, the presence of sequences that were not identical to the others may indicate >1 initiating event, even in the same hospital (e.g., patients 1 and 33 in hospital A; Table 2). Despite an exhaustive review of medical charts and interviews with HCP, we could establish no clear epidemiologic links among these facilities, suggesting that unrecognized cases of MERS-CoV infection might not have been captured by the existing surveillance system.

Results from serologic testing of 17 patients showed that 4 were seropositive, despite the relatively short interval between reported onset of illness and collection of serum samples (range 5–7 days). These patients could not be interviewed to confirm exact symptom onset, which may be nonspecific in early MERS-CoV illness. Also, 1 of the 4 seropositive specimens could not be confirmed by rRT-PCR at CDC but was found to be rRT-PCR positive in Saudi Arabia.

These findings highlight the challenges and limitations of epidemiologic investigations of MERS and show the value of molecular techniques. In addition to standardized data collection, viral sequencing should be attempted when possible to enable better understanding of transmission events. Our investigation shows the highly infectious nature of MERS-CoV, including high rates of illness and death from within dialysis settings, as previously noted (*8,9*). In hospital B's dialysis unit, 15 persons had MERS-CoV

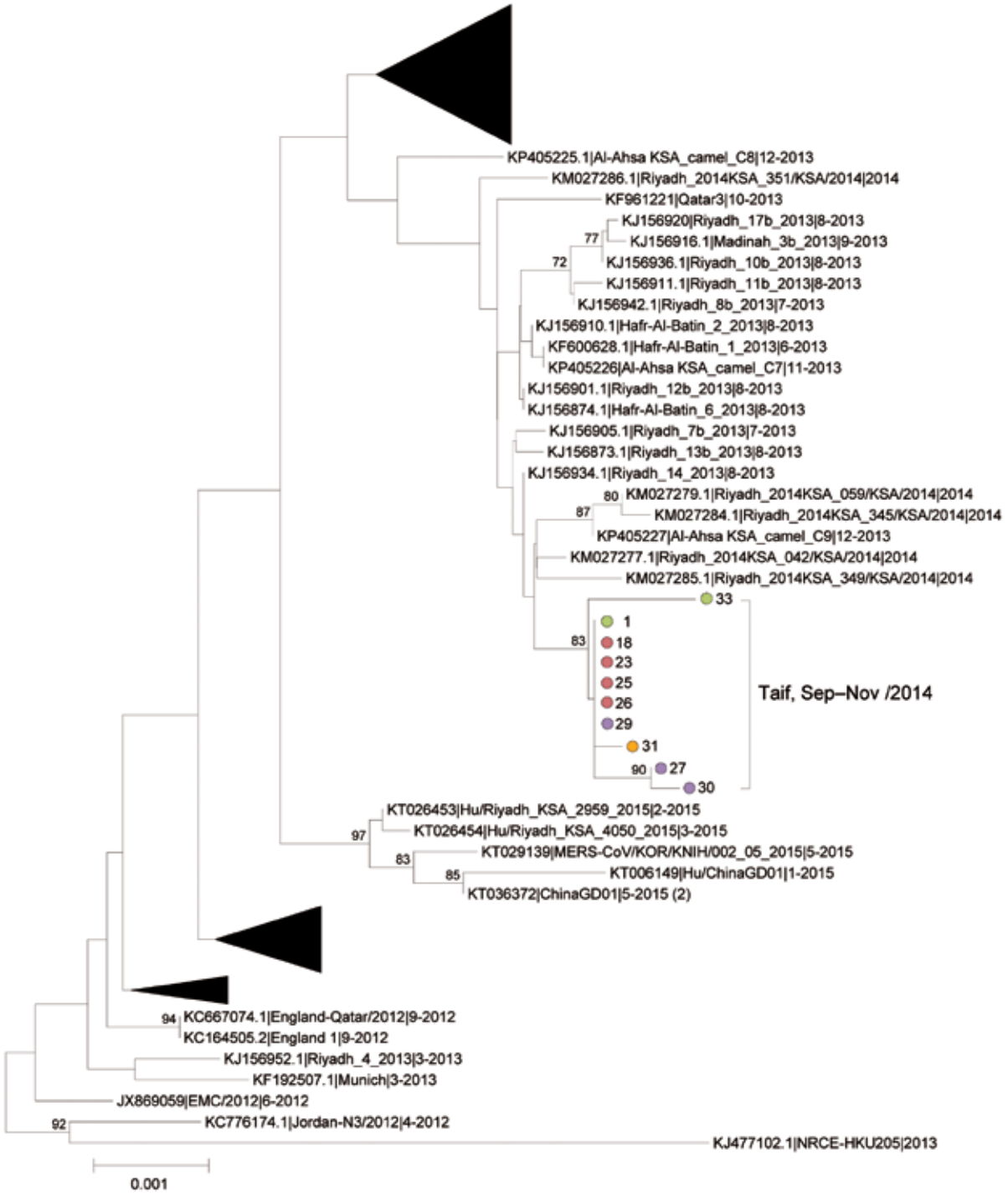

Figure 2. Midpoint-rooted phylogenetic tree inferred from multiple-sequence alignment of 10 new cases of Middle East respiratory syndrome coronavirus spike open reading frame sequences (4,062 nt) from Taif, Saudi Arabia (brackets). Patient numbers and healthcare facilities match those in Figure 1. Taif sequences are shown in context with the closest related sequences that comprise the Hafr-Al-Batin_1 clade, as originally defined by Cotton et al. (*17*), and with sequences related to the 2015 outbreak event in South Korea. For clarity, the remaining published sequences are collapsed into triangles. Published sequences are designated by GenBank accession number, strain name, and month and year of sample collection. The estimated neighbor-joining tree was constructed from nucleotide alignments by using MEGA version 6.06 (http://www.megasoftware.net). Bootstrap support values (1,000 replicates) ≥70% are plotted at the indicated internal branch nodes. Scale bar shows the genetic distance as the number of nucleotide substitutions per site. KSA, Kingdom of Saudi Arabia.

infections confirmed by rRT-PCR during a 3-week period. Eleven of the 15 were non-HCP patients who were regularly undergoing dialysis, and 8 (73%) died. The other 4 were HCP, 3 of whom were symptomatic. Our subsequent serologic investigation of HCP in the dialysis unit identified 3 additional and previously unrecognized HCP who were seropositive but denied symptoms at interview (online Technical Appendix). A total of 18 persons were involved in the dialysis unit transmission event. Although this investigation did not firmly establish modes of transmission, risk for respiratory droplet transmission in this setting might have been increased because of close spacing (<2 meters between beds) of patients who also were likely to be immunocompromised by end-stage renal disease and other underlying conditions such as diabetes. After implementation of recommended changes in infection-control practices, the number of cases reported in association with this dialysis unit quickly declined.

Our investigation is subject to several limitations. Our team had limited access to hospital A, although we were able to assess case reporting and investigation forms, discuss patients with providers, and receive patient specimens for further laboratory testing. This outbreak occurred among at least 4 facilities, and contact investigations were conducted by those facilities. Although the contact investigations were critical for detecting mildly ill patients in this outbreak, contact tracing and testing might not have been uniformly conducted in all facilities, potentially limiting the scope of our investigation. Although we were able to obtain partial MERS-CoV genome sequences from acute-phase serum samples from 10 of 12 patients, specimens were not available for all patients. Additional viral sequences from the unlinked cases would have been particularly useful in understanding whether these cases were possibly linked to the identified facility transmission events. The limited availability of specimens restricted our ability to obtain full-genome sequences that would likely provide greater epidemiologic power in resolving transmission events. Although we attempted to link the results of our epidemiologic investigation with the spike gene sequences from investigated cases, we cannot be certain whether the virus was introduced into the healthcare environment in Taif on one or multiple occasions. Circulation of MERS-CoV among camels in Taif has been documented (*19*), and the detection of phylogenetically common or closely related viruses in the human cases in this investigation might reflect multiple introductions of the same or similar viruses circulating in camels in Taif during this outbreak period. Notably, the 6 patients with identical spike gene sequences were in 3 clusters and had onset dates that spanned 64 days.

A comparison of the sensitivities of the MERS nucleocapsid ELISA and the MERS spike ELISA has not been published. Additional evaluation to better characterize the clinical sensitivity and specificity of the MERS-CoV serologic assays used in this study is necessary, and systematic cross-validation will be needed in the future.

Repeated introduction of MERS-CoV into healthcare facilities, resulting in transmission among patients, visitors, and HCP, has been a defining feature of MERS-CoV epidemiology since its emergence in 2012. Our investigation shows the persistence of MERS-CoV circulation in multiple healthcare settings over an extended period, despite lack of clearly defined epidemiologic links, and underscores the importance of identifying and monitoring exposed HCP, patients, and visitors. MERS-CoV transmission in any healthcare facility should trigger increased vigilance among all healthcare facilities that could potentially share patients and staff. Increased understanding of epidemiologic links among identified patients during transmission events is needed to inform surveillance strategies and infection prevention and control.

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Falling *Plasmodium knowlesi* Malaria Death Rate among Adults despite Rising Incidence, Sabah, Malaysia, 2010–2014

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Release date: December 17, 2015; Expiration date: December 17, 2016

Learning Objectives

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

- Describe notification-fatality rates of fatal *P. knowlesi* cases in Sabah during 2010–2014, based on a surveillance study using the Sabah Department of Health malaria notification database
- Identify clinical characteristics of fatal *P. knowlesi* cases in Sabah during 2012–2014
- Discuss management details of fatal *P. knowlesi* cases in Sabah during 2012–2014

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Deaths from *Plasmodium knowlesi* malaria have been linked to delayed parenteral treatment. In Malaysia, early intravenous artesunate is now recommended for all severe malaria cases. We describe *P. knowlesi* fatalities in Sabah, Malaysia, during 2012–2014 and report species-specific fatality rates based on 2010–2014 case notifications. Sixteen malaria-associated deaths (caused by PCR-confirmed *P. knowlesi* [7], *P. falciparum* [7], and *P. vivax* [1] and microscopy-diagnosed "*P. malariae*" [1]) were reported during 2012–2014. Six patients with severe *P. knowlesi* malaria received intravenous artesunate at hospital admission. For persons ≥15 years of age, overall fatality rates during 2010–2014 were 3.4, 4.2, and 1.0 deaths/1,000 *P. knowlesi*, *P. falciparum*, and *P. vivax* notifications, respectively; *P. knowlesi*–associated fatality rates fell from 9.2 to1.6 deaths/1,000 notifications. No *P. knowlesi*–associated deaths occurred among children, despite 373 notified cases. Although *P. knowlesi* malaria incidence is rising, the notification-fatality rate has decreased, likely due to improved use of intravenous artesunate.

Plasmodium knowlesi is the most common cause of malaria in East Malaysia, and the incidence of disease is increasing despite intensive control efforts that have substantially reduced the incidence of *P. falciparum* and *P. vivax* malaria in Malaysia (*1*–*3*). Although the greatest number of *P. knowlesi* cases has been reported in East Malaysia, the infection is also the predominant cause of malaria in Peninsular Malaysia (*4*) and is increasingly reported in other Southeast Asia countries and in travelers returning from these countries (*5*).

P. knowlesi infection can be associated with high parasitemia and is at least as likely as *P. falciparum* to cause severe malaria in adults (*6*). Age is strongly associated with parasitemia and, thus, a key risk factor for severe and fatal disease (*6*,*7*), neither of which has been reported in children with PCR-confirmed *P. knowlesi* malaria (*5*,*8*,*9*). In a tertiary referral hospital in Sabah, northeastern Malaysia, the rate of *P. knowlesi* malaria–associated deaths was low among persons ≥12 years of age who were promptly treated (including before hospital referral) with artesunate (*6*); however, *P. knowlesi* continues to cause fatal malaria among adults in Sabah (*10*,*11*). During 2010–2011, *P. knowlesi* was responsible for 6 of 14 fatal malaria cases in Sabah. Microscopy-based misdiagnosis of *P. knowlesi* malaria as the nearly identical, but more benign, *P. malariae* malaria was common, and fatal outcome was associated with delayed or lack of parenteral therapy: 2 of 6 patients with fatal *P. knowlesi* malaria received parenteral therapy (1 each with quinine and artesunate); the other 4 received chloroquine or sulfadoxine/pyrimethamine (*10*).

In the time since that study was conducted, recognition of *P. knowlesi* and its ability to cause severe disease has increased. The 2013 Management Guidelines of Malaria in Malaysia recommend that results for blood films with parasites resembling *P. malariae* be reported as *P. knowlesi*/*P. malariae* (*12*). The guidelines emphasize that PCR-confirmed *P. malariae* is rare in Sabah and that patients with a microscopy-based diagnosis of *P. malariae* infection should be assumed to have *P. knowlesi* malaria. Moreover, like recent World Health Organization (WHO) global guidelines (*13*–*15*), Malaysian guidelines now recommend intravenous artesunate for all patients with severe malaria caused by any *Plasmodium* spp. (*12*), and in western Sabah, the drug is being used earlier and more frequently for all malarial infections (*6*). In addition, oral artemisinin combination treatment is now recommended for uncomplicated *P. knowlesi* malaria (*12*).

We assessed clinical features and management of fatal *P. knowlesi* malaria cases in Sabah during 2012–2014. We also determined age-stratified death rates among patients with *Plasmodium* spp. malaria and assessed trends in fatality rates for *P. knowlesi* malaria during 2010–2014.

Methods

In Sabah, which has an area of 73,600 km^2 and population of 3.7 million (*16*), reporting of all malaria cases and associated deaths to the Sabah Department of Health (DoH) is mandatory; species are reported according to microscopy results. We obtained details of reported malaria-associated deaths during 2012–2014 from the Sabah DoH and reviewed district hospital case notes for clinical details. The study was approved by the ethics committees of the Malaysian Ministry of Health and Menzies School of Health Research.

We reviewed the Sabah DoH malaria notification database for the total number of microscopy-based *P. knowlesi*/*P. malariae, P. falciparum*, and *P. vivax* malaria case notifications during 2010–2014. These data were used to determine case-fatality rates (CFRs) among notified cases for each species (hereafter referred to as notified CFRs, defined as number of PCR-confirmed *P. knowlesi*, *P. falciparum*, and *P. vivax* malaria–associated deaths per 1,000 microscopy-based *P. malariae*/*P. knowlesi*, *P. falciparum*, and *P. vivax* malaria notifications). PCR-confirmed *P. malariae* infection is rare in Sabah, accounting for <1% of clinical samples diagnosed by microscopy as *P. malariae* or *P. knowlesi* (*1*); thus, most notifications of *P. malariae*/*P. knowlesi* can be assumed to be *P. knowlesi*. During 2010–2014 in Sabah, only 1 fatal malaria case, a microscopy-diagnosed *P. malariae* infection, lacked PCR confirmation; an adjusted CFR was calculated following inclusion of this case.

Results

Sixteen malaria-associated deaths were reported in Sabah during 2012–2014, of which 15 were confirmed by PCR to be caused by *P. knowlesi* (7 cases; cases 1–7, online

Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-1305-Techapp1.pdf); *P. falciparum* (7 cases); or *P. vivax* (1 case). Details of 1 *P. knowlesi* case (case 2) were previously reported (*11*). The remaining fatal case was microscopy diagnosed (without PCR confirmation) as *P. malariae* infection (online Technical Appendix).

Fatal PCR-Confirmed *P. knowlesi* Malaria

All 7 fatal *P. knowlesi* malaria cases occurred in adults (median age 61 [range 31–73] years); 4 were women. Six of these cases had been misdiagnosed by microscopy as *P. malariae* (4), *P. falciparum* (1), or *P. vivax* (1) infections. Severe malaria was recognized in 5 patients when they sought medical care; all received intravenous artesunate within 90 (median 30) minutes of diagnosis. Severity criteria at admission for these patients were jaundice (4 patients), acute kidney injury (3), metabolic acidosis (4), hyperparasitemia (2), respiratory distress (2), and coma (1) (online Technical Appendix Tables 1, 2). Shock and respiratory distress developed in all patients before death. All patients were intubated and ventilated; 2 received hemodialysis. Death occurred within 5–117 (median 41) hours of admission.

Two patients with fatal *P. knowlesi* malaria were not recognized to have severe malaria at admission; they received oral antimalarial treatment. One of these patients (case-patient 7, online Technical Appendix Table 1) had a blood film result reported as 22,666 *P. malariae* parasites/mL and was given oral artesunate/mefloquine for apparent uncomplicated malaria. Her creatinine level was 124 (reference 63–133) μmol/L; bilirubin, lactate, and bicarbonate results were not available. Within 12 hours, she became hypotensive and tachypneic; chest radiographs showed diffuse opacities in both lung fields. She was intubated and started on intravenous artesunate but died within 23 hours of admission. Subsequent reexamination of her initial blood slide showed 263,772 parasites/mL. The other patient (case 5, online Technical Appendix Table 1) was also thought to have uncomplicated malaria; her blood film result was reported as 9,866 *P. vivax* parasites/mL, her bilirubin level was 46 (reference <17) μmol/L, and her creatinine level was 143 μmol/L. She was treated with 1 dose of intravenous artesunate followed by chloroquine and primaquine. Blood film results the next day indicated *P. knowlesi* infection with 20,000 parasites/mL. Acute respiratory distress syndrome (ARDS) and metabolic acidosis developed, and the patient died on day 3, despite recommencement of intravenous artesunate. Postmortem reexamination of her initial blood slide showed 55,111 *P. knowlesi* parasites/mL.

One patient, a 56-year-old man, was comatose, a condition not previously reported in *P. knowlesi* malaria. He was unresponsive when brought into a health clinic by relatives, who reported a 1-day history of weakness and drowsiness and a 3-day history of fever, chills, arthralgia, and myalgia. At hospital referral, his blood pressure was 85/60 mm Hg, pulse rate 150 beats/min, and oxygen saturation 81% on room air. He had a Glasgow Coma Scale score of 6/15. Pupils were reactive but asymmetric (right 2 mm, left 4 mm). Meningism was not present, and neurologic examination showed normal tone, symmetrically reduced reflexes, and downgoing plantar reflexes. Blood investigations showed 6,471 *P. knowlesi* parasites/mL and metabolic acidosis. Computed tomography brain scan and lumbar puncture were not performed. The patient was intubated and begun on intravenous artesunate and ceftriaxone but died 41 hours after admission. Blood cultures for bacterial infections were negative.

Fatal PCR-Confirmed *P. vivax* Malaria

One person, a 53-year-old man, died from *P. vivax* malaria. At admission, he had a 7-day history of fever, rigors, myalgia, nonproductive cough, and abdominal pain. Physical examination results were unremarkable. Thrombocytopenia was present, and his bilirubin level was 21.5 μmol/L; creatinine and hemoglobin levels were normal. A blood film result was reported as 2,090 *P. vivax* parasites/mL; oral chloroquine and primaquine treatment were begun. The next day, the parasite count was 890 parasites/mL of blood, but the patient became hypotensive, and ARDS developed; a postintubation chest radiograph showed bilateral opacities. Intravenous artesunate and antibiotic drugs were initiated, and hemodialysis was performed for acute kidney injury (creatinine level 316 μmol/L), but the patient died 4 days after admission. Blood cultures for bacterial infections were negative.

Fatal PCR-Confirmed *P. falciparum* Malaria

Of the 7 *P. falciparum* malaria–associated deaths, 2 (29%) occurred in children (a boy and a girl 2–3 years of age, both Filipino) and 5 (71%) occurred in adults (3 men and 2 women 31–80 years of age; 2 Filipino, 2 Malaysian, and 1 Indonesian). At the initial examination, all patients met WHO criteria for severe malaria: jaundice (5 patients), cerebral malaria (4 patients), renal failure (3 patients), respiratory distress (3 patients), and anemia (2 patients). Within 2 hours of malaria diagnosis, 1 patient was given oral artesunate/mefloquine and all others were given intravenous artesunate. All patients were intubated and ventilated, 6 received inotropes, and dialysis was performed on 3. All patients died within 2–9 days of admission. Blood cultures for 1 child and 1 adult were positive for *Klebsiella pneumoniae* and coagulase-negative staphylococci, respectively; the latter was thought to represent contamination.

Review of 2010–2014 Malaria Notification Data

The overall notified CFR for *P. knowlesi* malaria was 3.08 deaths/1,000 cases, compared with 4.83 and 0.87

deaths/1,000 cases of *P. falciparum* and *P. vivax* malaria, respectively (Table). Among adults (persons ≥15 years of age), notified CFRs were 3.37, 4.17, and 1.02 deaths/1,000 cases for *P. knowlesi*, *P. falciparum*, and *P. vivax* malaria, respectively. Despite 373 notifications of *P. knowlesi* malaria and 611 notifications of *P. vivax* malaria among children (persons <15 years of age) during 2010–2014, no deaths from either species were reported. However, children with *P. falciparum* malaria had a notified CFR of 6.7 deaths/1,000 cases. Notified CFRs among adults with *P. knowlesi* malaria declined from 9.2 to 1.6 deaths/1,000 notifications in 2010 and 2014, respectively (χ^2 test for trend, p = 0.11).

During 2010–2014, female patients accounted for only 783 (19%) of the 4,217 *P. malariae/P. knowlesi* notifications, but they accounted for 6 (46%) of the 13 fatal *P. knowlesi* malaria cases. Thus, the notified CFR for *P. knowlesi* malaria was 7.66 deaths/1,000 cases for female patients, compared with 2.04 deaths/1,000 cases for male patients (Fisher exact test, p = 0.021). However, this difference was not significant in a multivariate logistic regression model adjusting for age (odds ratio 2.60, p = 0.095). For *P. falciparum* and *P. vivax* malaria, no difference was seen in the number of notified CFRs for male and female patients.

Discussion

Despite ongoing microscopy-based misdiagnoses of *P. knowlesi* infections, management of severe malaria in Sabah appears to have improved; all patients recognized to have severe *P. knowlesi* malaria on admission received intravenous artesunate as initial therapy. Although our findings clearly demonstrate the ability of *P. knowlesi* to cause fatal malaria despite optimal therapy, *P. knowlesi* notified CFRs in Sabah have fallen over the past 5 years in association with the increased early use of artesunate documented in this and other reports (*6*). Death from *P. knowlesi* malaria remains unreported in children, and all but 1 of the *P. knowlesi*–associated deaths in this series occurred in adults >50 years of age.

The absence of *P. knowlesi*–associated deaths among children, despite 373 *P. malariae*/*P. knowlesi* notified cases in this age group during 2010–2014, contrasts with the well-recognized risk for childhood deaths from *P. falciparum* malaria (*17*) and extends the lack of previous reports of either severe or fatal outcomes in children with *P. knowlesi* malaria (*5*,*8*,*9*). Furthermore, the large number of notified cases in children in this series suggests that the lack of *P. knowlesi*–associated deaths among children may not be due solely to the relative underrepresentation of children in previous series of *P. knowlesi* malaria (*6*,*7*,*18*,*19*). A lower risk for severe and fatal *P. knowlesi* malaria in children may be due to the previously documented strong association between age and parasitemia (*6*); the level of parasitemia in children is generally insufficient to cause severe and fatal disease (*20*). In addition, younger age may be associated with physiologic protection from severe and fatal *P. knowlesi* malaria, as suggested by previous findings of a lower risk of severe malaria after primary exposure to *P. falciparum* in nonimmune children compared with nonimmune adults (*21*).

Clinical and demographic characteristics for adult *P. knowlesi* malaria patients in this study were consistent with those in previous reports (*1*,*6*,*7*,*18*). Patients had a median age of 61 years. Because of the strong correlation between

Table. CFRs among persons with notified cases of *Plasmodium* spp. malaria, Sabah, Malaysia, 2010–2014*

	P. knowlesi†			*P. falciparum*			*P. vivax*		
Age group, year	No. notifications	No. deaths	Notified CFR‡	No. notifications	No. deaths	Notified CFR	No. notifications	No. deaths	Notified CFR
Persons ≥15 y of age									
2010	327	3	9.17	736	2	2.72	720	0	0.00
2011	608	3	4.93	467	2	4.28	479	1	2.09
2012	744	4§	5.38	543	2	3.68	390	0	
2013	927	1	1.08	239	1	4.18	211	1	4.74
2014	1,246	2	1.61	173	2	11.56	165	0	
Total	3,852	13	3.37	2,158	9	4.17	1,965	2	1.02
Persons <15 y of age									
2010	57	0	–	355	2	5.63	262	0	–
2011	95	0	–	138	1	7.25	149	0	–
2012	73	0	–	171	2	11.70	88	0	–
2013	69	0	–	58	0	–	52	0	–
2014	79	0	–	21	0	–	60	0	–
Total	373	0	–	743	5	6.73	611	0	–

*Notified CFR, case-fatality rate determined on the basis of the no. of PCR-confirmed malaria-associated deaths/1,000 notifications of microscopy-based malaria cases.
†Includes all cases notified as *P. knowlesi* or *P. malariae*. PCR-confirmed *P. malariae* infection is rare in Sabah, accounting for <1% of clinical samples diagnosed by microscopy as *P. malariae* or *P. knowlesi* (*1*); thus, most notifications of *P. malariae/P. knowlesi* can be assumed to be *P. knowlesi*.
‡p = 0.110 (χ^2 test for trend) for reduction in *P. knowlesi* fatality rate among adults during 2010–2014.
§Excludes 1 fatal case of microscopy-diagnosed *P. malariae*. The notified CFR in 2012 with this case included as a *P. knowlesi*–associated death was 6.72 deaths/1,000 notifications, and the overall notified CFR for *P. malariae/P. knowlesi* in adults was 3.63 deaths/1,000 notifications. The p value for the test for trend is unchanged at 0.11

age and parasitemia, older patients are known to be at increased risk for severe *P. knowlesi* malaria (*6*). Of the 7 patients who died, 4 were female; thus the notified CFR was significantly higher among female than male patients. Although this discrepancy appears to be primarily due to the older age of female patients with *P. knowlesi* malaria (*1*), a trend toward increased notified CFRs for female *P. knowlesi* patients remained even after adjusting for age. This finding is consistent with the increased risk for severe *P. knowlesi* malaria found for female patients in some (*7*,*18*), but not all (*6*), previous studies. Larger studies are needed to clarify the association between sex and risk for severe *P. knowlesi* malaria.

The complications experienced by the *P. knowlesi* malaria patients in this study were generally consistent with those in other reports; hyperparasitemia, respiratory distress, shock, jaundice, and acute kidney injury were common in this and previous reports (*6*,*7*,*18*,*22*–*24*). Metabolic acidosis occurred in 5 patients in this series. This complication of severe *P. knowlesi* malaria was uncommon in a previous tertiary referral hospital study that involved early, including prereferral, use of artesunate and in which no deaths occurred (*6*). However, metabolic acidosis has been reported in most fatal *P. knowlesi* malaria cases (*7*,*10*,*11*,*18*,*25*), and, as with *P. falciparum* malaria, is likely a late complication signifying poor outcome. Acute lung injury was present at admission in 2 patients and developed after treatment initiation in all remaining *P. knowlesi* patients; this finding is consistent with a posttreatment inflammatory response, as previously postulated (*6*).

Coma has not previously been reported in *P. knowlesi* malaria. Although decreased conscious state occurred in 1 patient in this study, blood cultures, lumber puncture, and computed tomography brain scan were not performed, and pupillary asymmetry, reported in this patient, is unusual in coma due to *P. falciparum* malaria. Therefore, while decreased consciousness directly associated with *P. knowlesi* remains possible, alternative causes are plausible.

Microscopy-based misdiagnosis of *P. knowlesi* infection occurred in 6 of 7 cases. Four of the 6 case-patients had misdiagnoses of *P. malariae* infection; all had high parasitemia (2 had >100,000 parasites/μL of blood), which is inconsistent with a diagnosis of *P. malariae* infection but highly suggestive of *P. knowlesi* infection. Malaysia's malaria guidelines recommend that blood films with parasites resembling *P. malariae* be reported as *P. knowlesi/P. malariae* (*12*); however, high parasitemia, particularly in the context of a very low statewide prevalence of *P. malariae* (*1*), makes *P. malariae* infection unlikely. The frequent microscopy-based misdiagnosis of *P. knowlesi* malaria in this and other reports (*26*) in Sabah highlights the need for alternative rapid diagnostic methods.

Despite the frequent misdiagnoses in this series, all patients with fatal *P. knowlesi* infection who were recognized as having severe malaria at admission were appropriately treated with early intravenous artesunate. In contrast, in our 2010–2011 review of malaria deaths (*10*), only 2 of 5 patients with severe *P. knowlesi* malaria received parenteral treatment; the other 3, who had misdiagnoses of *P. malariae* or *P. vivax* malaria, received oral chloroquine or sulfadoxine/pyramethamine. In the current study, 2 patients were thought to have uncomplicated malaria and received oral therapy (case-patient 5 was given chloroquine after 1 dose of intravenous artesunate; case-patient 7 was given artesunate/mefloquine). Postmortem reexamination of these patients' initial blood films showed a parasite count substantially higher than initially reported (55,111 parasites/μL vs. 9,866 parasites/μL for case-patient 5; 263,772 parasites/μL vs. 22,666 parasites/μL for case-patient 7). Parasitemia has been shown to be a major risk factor for severe *P. knowlesi* malaria: in a recent prospective study, severity criteria were present in >50% of patients with >20,000 parasites/μL of blood and >80% of patients with >100,000 parasites/μL of blood (*6*). WHO guidelines now recommend that intravenous artesunate be used for all patients with *P. knowlesi* malaria and >100,000 parasites/μL of blood or, if testing for laboratory criteria for severe malaria is not available, >20,000 parasites/μL blood (*14*,*15*). The failure of oral therapy in case-patient 7 (initial blood slide reported as 22,666 parasites/μL; bilirubin not available) highlights the value of this recommendation. Moreover, these 2 cases demonstrate that parasitemia must be accurately quantified in patients with *P. knowlesi* malaria.

The use of chloroquine in case-patient 5 may have contributed to the poor outcome. Compared with artesunate/mefloquine, chloroquine has been associated with reduced parasite clearance time in *P. knowlesi* malaria (*27*,*28*) and is no longer recommended as first-line treatment for *P. knowlesi* malaria in Malaysia (*12*). In Sabah, parasite clearance time for *P. vivax* malaria treated with chloroquine is reduced compared with that for cases treated with artesunate/mefloquine, and treatment failures are common (*29*). Moreover, *P. knowlesi* and *P. vivax* are frequently confused in microscopy examination (*26*); hence, a unified treatment approach should be considered in Sabah, using artemisinin for all malaria cases.

Although this case series highlights the ability of *P. knowlesi* malaria to cause fatal disease in adults even after prompt administration of intravenous artesunate, it must be noted that the number of deaths has not increased over recent years, despite a rise in *P. knowlesi* malaria notifications from 384 in 2010 to 1,325 in 2014. Thus, the adult notified CFR has declined from 9.2 deaths/1,000 notifications in 2010 to 1.6 deaths/1,000 notifications in 2014. This improvement likely resulted from increased

recognition of the ability of *P. knowlesi* to cause severe disease and to increased use of intravenous artesunate. Although intravenous artesunate has been recommended in Sabah since December 2008, intravenous quinine was still in use until at least 2010 (*10*). In a retrospective study of severe *P. knowlesi* malaria at a tertiary referral hospital in Sabah during 2007–2009, a total of 5 (31%) of 16 patients treated with intravenous quinine died. In contrast, at the same hospital during 2010–2011, none of the severe *P. knowlesi* malaria patients treated with early intravenous artesunate died (*6*). In addition, in Sabah, the increasing use of artemisinin combination treatment instead of chloroquine for uncomplicated *P. knowlesi* malaria (*6*) may also have contributed to the decline in notified CFRs, particularly for cases in which severe disease is unrecognized.

We also reported a case of fatal *P. vivax* malaria with ARDS, an increasingly well-recognized complication of *P. vivax* malaria that has resulted in fatalities (*30*–*34*). The pathophysiologic mechanism likely involves soluble mediators and endothelial damage, exacerbated by shock and leading to diffuse damage to alveolar membranes (*30*). As with the case in our study, most ARDS cases occur after treatment initiation (*32*,*35*), possibly resulting from an exacerbated inflammatory response to parasite killing. Most of the initial cases of *P. vivax*–associated ARDS were in returned travelers with single organ dysfunction and nonfatal outcome; however, more recent series from countries where *P. vivax* is endemic have reported ARDS cases with multiorgan dysfunction and considerable mortality (*35*,*36*).

This study had several limitations. First, the retrospective design of the case series resulted in unavoidably incomplete laboratory and clinical data. In particular, alternative diagnoses cannot be excluded in the case of possible *P. knowlesi*–associated coma. Second, our calculation of the microscopy-based notified CFR represents only an estimate of the true *P. knowlesi*–associated CFR. The accuracy of this estimate will depend on the accuracy of microscopy-based identification of all *Plasmodium* species, the notification rate of malaria cases, and the proportion of persons with malaria who seek care at a health clinic. We do not have data on the proportion of malaria cases in Sabah that are notified; it is probable, however, that some are not notified, so the notified CFR likely overestimates the true CFR. In addition, we cannot exclude the possibility that the reduction in the *P. knowlesi*–associated notified CFRs during 2010–2014 is due to an increase in the proportion of malaria cases that are notified. However, notification of malaria cases in Sabah has been mandatory since 1992, and there is no reason to suspect that the notification rate would have changed substantially since 2010. It is similarly unlikely that the proportion of *P. knowlesi* malaria cases diagnosed as *P. falciparum* malaria, and vice versa, changed sufficiently over the 5-year period to account for the observed decline in notified CFRs (*1*). Nonetheless, larger prospective studies involving molecular diagnostic methods are needed to obtain a more accurate assessment of the true *P. knowlesi* malaria CFR, including changes over time. Although we report notified CFRs for *P. knowlesi*, *P. falciparum*, and *P. vivax* malaria, these data may not reflect the relative virulence of each species. In this series, non-Malaysian citizens accounted for a higher proportion (5/7) of patients with fatal *P. falciparum* malaria than fatal *P. knowlesi* malaria, and it is possible that a delay in seeking care at a healthcare facility may be a confounding factor in comparing CFRs for malaria caused by these *Plasmodium* spp.

In conclusion, our findings show that despite increasing notifications of *P. knowlesi* malaria cases in Sabah, the number of fatal cases has not increased. The reduction in notified CFRs may be associated with the increased recognition of the ability of *P. knowlesi* to cause severe and fatal malaria and improved use of intravenous artesunate for severe malaria caused by any *Plasmodium* spp, as per recent policy changes (*6*,*12*). Nonetheless, this study demonstrates the ability of *P. knowlesi* to cause fatal malarial disease in adults, despite optimal therapy, and that *P. knowlesi* remains the most common cause of fatal malaria in adults in Sabah. In contrast, the study shows a notable absence of deaths among children with *P. knowlesi* malaria.

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Risk Factors for Primary Middle East Respiratory Syndrome Coronavirus Illness in Humans, Saudi Arabia, 2014

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Risk factors for primary Middle East respiratory syndrome coronavirus (MERS-CoV) illness in humans are incompletely understood. We identified all primary MERS-CoV cases reported in Saudi Arabia during March–November 2014 by excluding those with history of exposure to other cases of MERS-CoV or acute respiratory illness of unknown cause or exposure to healthcare settings within 14 days before illness onset. Using a case–control design, we assessed differences in underlying medical conditions and environmental exposures among primary case-patients and 2–4 controls matched by age, sex, and neighborhood. Using multivariable analysis, we found that direct exposure to dromedary camels during the 2 weeks before illness onset, as well as diabetes mellitus, heart disease, and smoking, were each independently associated with MERS-CoV illness. Further investigation is needed to better understand animal-to-human transmission of MERS-CoV.

Middle East respiratory syndrome coronavirus (MERS-CoV) is a newly recognized respiratory pathogen first identified in a patient from Saudi Arabia in June 2012 (*1*). MERS-CoV causes acute respiratory disease that has a high case-fatality rate (*2*). All cases have been linked to countries in or near the Arabian Peninsula; >85% of cases have been reported from Saudi Arabia (*2*). Outbreaks of MERS-CoV have been associated primarily with transmission in healthcare settings (*3*–*5*). Transmission among household contacts of case-patients has been documented (*6*), but sustained human-to-human transmission has not (*7*). Low-level infections with MERS-CoV have been reported, but seroprevalence of MERS-CoV antibodies in the general population in Saudi Arabia is low (*8*). Strategies to prevent and control infection are recommended to limit secondary transmission in healthcare settings and among household contacts (*9*,*10*). MERS-CoV cases continue to be reported in Saudi Arabia in healthcare settings and in the community (*2*).

Animals have been suspected as a source of primary infection since early in the emergence of MERS-CoV, particularly given the similarities to severe acute respiratory syndrome coronavirus, a zoonosis known to cause human respiratory disease, often severe, with sustained human-to-human transmission and amplification in healthcare settings (*11*). Persons with early cases of MERS-CoV infection were observed to have had exposure to dromedary camels (henceforth dromedaries), and subsequent serologic studies from the Arabian Peninsula confirmed high seroprevalence of MERS-CoV neutralizing antibodies in dromedaries (*12*–*14*). Other studies have detected partial genome sequences of MERS-CoV from dromedary specimens (*15*–*17*), and more recently infectious MERS-CoV has been isolated from dromedaries (*16*,*18*–*21*). Additionally, a recent report provided virologic and serologic evidence of transmission of MERS-CoV from a sick dromedary to a human in Saudi Arabia (*19*).

Despite these reports, risk factors for primary illness with MERS-CoV (i.e., cases in persons without apparent exposure to other infected persons) are not well understood.

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No risk factors for primary transmission of MERS-CoV to humans have been confirmed by epidemiologic studies, including a link with exposure to dromedaries or any other animal species. We conducted a case–control study to assess exposures in primary cases and to identify risk factors associated with primary MERS-CoV illness in humans.

Methods

Study Design

In Saudi Arabia, all laboratory-confirmed MERS-CoV cases are reported to the Ministry of Health (MoH) and routinely investigated to assess preillness exposures. All cases reported during March 16–November 13, 2014, were screened for inclusion. For cases reported before May 13, 2014, a confirmed case was defined as illness in any person hospitalized with bilateral pneumonia and laboratory confirmation of MERS-CoV infection on the basis of a positive real-time reverse transcription PCR targeting 2 genes: the upstream of E gene and the open reading frame 1a gene (*22*). The case definition was revised on May 13, after which a confirmed case was defined as laboratory confirmation and any 1 of the following 4 clinical definitions: 1) fever and community-acquired pneumonia or acute respiratory distress syndrome based on clinical or radiologic evidence; 2) healthcare-associated pneumonia based on clinical and radiologic evidence in a hospitalized person; 3a) acute febrile (≥38°C) illness, b) body aches, headache, diarrhea, or nausea/vomiting, with or without respiratory symptoms, and c) unexplained leucopenia (leukocytes $<3.5 \times 10^9$ cells/L) and thrombocytopenia (platelets $<150 \times 10^9$/L); 4) protected or unprotected exposure of a person (including a healthcare worker) to a confirmed or probable MERS-CoV infection and upper or lower respiratory illness within 2 weeks after exposure (*23–25*). For this study, case-patients were selected from among symptomatic patients whose illness met the case definition in place at the time of report and who met the study inclusion criteria described below.

Case and Control Selection

Primary MERS-CoV cases were defined as cases in persons without known exposure to other MERS-CoV cases or recent (within 14 days) exposure to healthcare settings (*3*,*5*). MERS-CoV case-patients meeting this definition were presumed to have acquired infection through nonhuman contact. A trained MoH interviewer contacted the case-patient or proxy by phone or in person to conduct an initial screening. Case-patients were excluded if, within 14 days before onset of their MERS-CoV illness, they had been admitted to or visited any healthcare facility; had worked in a healthcare facility in any capacity; had a recognized epidemiologic link with another person either with confirmed MERS-CoV infection or with an acute respiratory illness (as perceived by the participant) of unknown cause; were <18 years of age; or did not provide consent for interview either personally or by proxy (i.e., a family member or close friend familiar with the preillness activities and usual habits of the case-patient) for case-patients who had died or were too ill to give consent personally.

For each case-patient, we randomly selected up to 4 neighborhood controls matched by age and sex. For case-patients 18 to ≤25 years old, controls were matched within 5 years of age, and for those >25 years old, controls were matched within 10 years of age. First, starting at the case-patient's household, a random direction was selected by flipping a coin. Second, the distance in number of houses from the case-patient's residence was randomly determined from 1 to 10 by choosing from a random number list. For multifamily structures, the starting floor and apartment were randomly chosen. Once a household was identified, 1 control was selected on the basis of the matching criteria; the exclusion criteria used for case-patients were also applied for all controls. If >1 person in the household met matching criteria, 1 was randomly chosen. If no matching control was found in the selected household, the next house in the same direction was visited, and so on, until an eligible control was enrolled.

Interview Process

A case–control protocol developed by the World Health Organization was adapted to create a standardized questionnaire for assessing risk factors associated with MERS-CoV illness. This questionnaire was used by MoH staff to conduct in-person interviews with case-patients (or their proxies) and controls (*26*). The 14-day period before illness onset was defined as the exposure period both for case-patients and their corresponding controls.

Data Collection

The questionnaire addressed demographic information; medical history; travel history; and information about human, food, and animal exposures. Human exposure questions addressed preillness exposures to healthcare settings or persons with acute respiratory illness. Food exposure questions assessed consumption of fruit, vegetables, unpasteurized milk, meats, urine, or chewing of siwak (a twig from the *Salvadora persica* tree, traditionally used for teeth cleaning). Animal exposure questions addressed multiple species (dromedaries, goats, sheep, horses, cattle) and whether any direct or indirect exposure to animals occurred. Direct animal exposure in the 14-days before illness onset was defined as physical contact with animals or animal products (carcasses, body fluids, secretions, urine, excrement, or raw meat) in any setting (farm, livestock market, slaughterhouse, racetrack, stable, or other animal-related venue) or engaging in certain animal-related

activities (feeding animals, cleaning housing, slaughtering, assisting with birth, milking, kissing or hugging, or other related tasks). Indirect animal exposure in the 14 days before illness onset was defined as having visited settings where animals were kept but without having direct contact; or exposure to household members who themselves had direct animal exposure. When assessing animal exposure during the previous 6 months, participants self-defined direct physical contact. Interviews with case-patients and controls were conducted in Arabic or English.

Because this investigation was part of a public health response, MoH and the US Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) determined it to be nonresearch and therefore not subject to institutional review board review. We obtained written informed consent from all participants or their proxies.

Statistical Analysis

We used Epi Info 7 (CDC, Atlanta GA, USA) for data entry and SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) for data analysis. Characteristics that were not part of the matching process for case-patients and controls were compared using χ^2 tests, Fisher exact tests, or *t* tests. We used exact conditional logistic regression to estimate odds ratios (ORs), 95% mid-p CIs, and exact p values for potential risk factors for MERS-CoV illness. Factors found to be significant ($p<0.05$) in the univariate analysis were further evaluated in multivariable analyses. We created a final multivariable model through stepwise elimination of nonsignificant variables until all remaining variables in the multivariate model were significant at $p<0.05$. Interactions between risk factors were also evaluated in the multivariable analyses.

Results

During March 16–November 13, 2014, a total of 535 patients with laboratory-confirmed MERS-CoV infection were reported to the MoH. After screening based on the exclusion criteria, 34 patients were identified as possible primary case-patients. Two persons refused to participate, and 2 did not meet the age criteria for inclusion. The remaining 30 case-patients, representing 8 of 13 regions in Saudi Arabia, were enrolled in the study (Figure). Symptom onset dates for enrolled case-patients ranged from February 25 through November 2, 2014.

We identified and included 116 controls: 4 age-, sex- and neighborhood-matched controls for each of 28 case-patients and 2 controls for each of the 2 remaining case-patients. Of the 116 controls, ages for 6 exceeded the age-matching criteria by 1–5 years. One case-patient did not have a formal residence; after his interview, 4 controls were selected from the vicinity of the area where he spent his nights.

Case-patients and controls were interviewed during June 8–November 29, 2014. Interviews with case-patients and controls were conducted on the same day, a median of

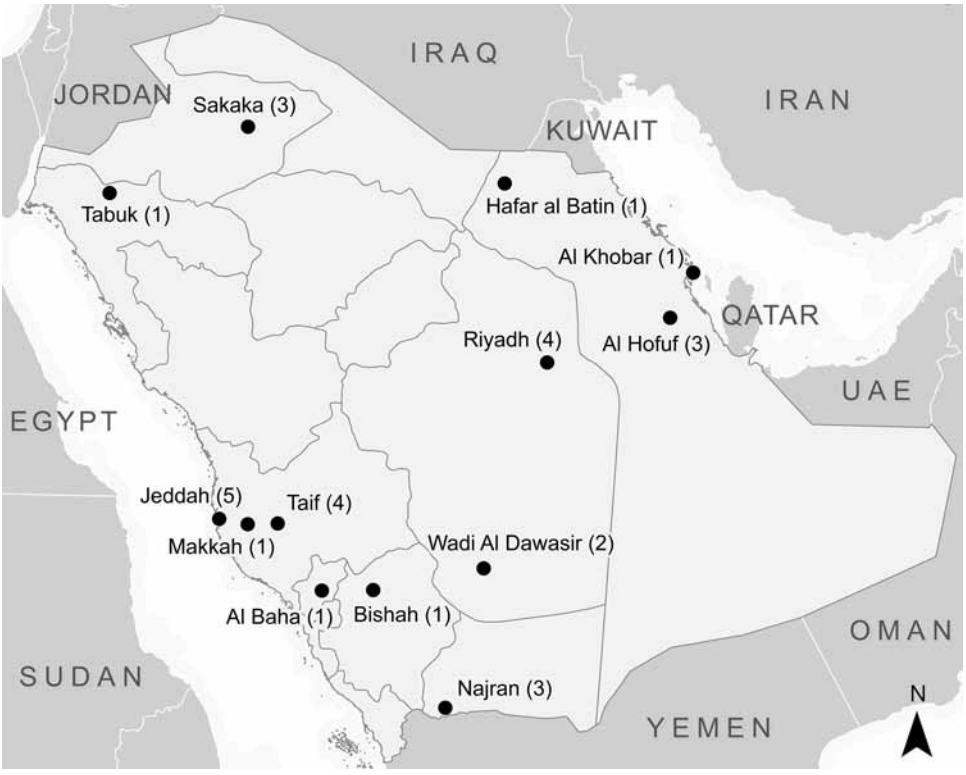

Figure. City or governorate of residence of persons with primary Middle East respiratory syndrome coronavirus included in the study, Saudi Arabia, March 16–November 13, 2014.

27 days (range 0–192 days, interquartile range 12–70 days) after case-patients' illness onsets.

Median age of case-patients was 49 years; 97% were male (Table 1). Rate of ownership of a barn or farm with animals was similar between case-patients and controls, but income was higher for case-patients than for controls (53% vs. 34%, respectively, had a monthly income of >6,000 SAR [US $1,600]; 2013 gross national income per capita is $2,188/month in Saudi Arabia [*27*]). Other demographic features between the groups were similar. At the time of interview, 7 (23%) case-patients were deceased, and 10 (33%) were too ill to be interviewed. For these 17 (57%) case-patients and for 1 (1%) control, a family member served as proxy. Case-patients interviewed by proxy were more likely than those interviewed directly to have underlying medical conditions but were similar in age, other demographic characteristics, and animal-related exposures.

Several exposures were associated with MERS-CoV by univariate analysis (Table 2). During the 14 days before illness onset, case-patients were more likely than controls to have had direct dromedary exposure (33% vs. 15%, OR 3.73, 95% CI 1.24–11.80); to keep dromedaries in or around the home (30% vs. 15%, OR 3.34, 95% CI 1.04–10.98); or to have visited a farm where dromedaries were present (90% vs. 53%, OR 11.57, 95% CI 2.67–∞); Among those who visited a farm where livestock were kept during the exposure period, case-patients were more likely than controls to have milked dromedaries (50% vs. 23%, OR 10.36, 95% CI 2.47–∞). Case-patients also were more likely than controls to live in the same household as someone who had visited a farm with dromedaries during the previous 14 days (30% vs. 12%, OR 3.95, 95% CI 1.23–13.72) and to have had direct contact with a dromedary while there (40% vs. 15%, OR 5.03, 95% CI 1.66–16.88). Case-patients also were more likely than controls to have had direct physical contact with dromedaries in the previous 6 months (37% vs. 13%, OR 7.67, 95% CI 2.10–36.08). Case-patients were no more likely than controls to report exposure to bats, goats, horses, sheep, or the products of these animals; however, direct cattle exposure was significantly associated with illness (13% vs. 3%, OR 6.00, 95% CI 1.02–48.44). No differences were noted in consumption of fruits; vegetables; or animal products, including uncooked meat, unpasteurized animal milk, or dromedary urine (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/01/15-1340-Techapp1.pdf). We observed no significant differences in dromedary exposures between case-patients interviewed directly and those interviewed by proxy.

Case-patients were more likely than controls to have ≥1 underlying medical condition (70% vs. 42%, OR 5.11, 95% CI 1.70–18.67). Diabetes mellitus (55% vs. 28%, OR 3.72, 95% CI 1.45–10.25); heart disease (37% vs. 12%, OR 5.11, 95% CI 1.81–15.46); and chronic lung disease (13% vs. 1%, OR 17.68, 95% CI 4.22–∞) were each reported significantly more frequently among case-patients than among controls. No significant differences were identified in other reported health conditions (asthma, kidney failure, chronic liver disease, cancer, blood disorders, or conditions requiring corticosteroid use). Case-patients also were more likely than controls to currently smoke tobacco (37% vs. 19%, OR 3.14, 95% CI 1.10–9.24). Using siwak during the exposure period was associated with a lower risk for MERS-CoV illness (25% vs. 49%, OR 0.24, 95% CI, 0.06–0.77).

Table 1. Demographic characteristics of Middle East respiratory syndrome coronavirus case-patients compared with age- and sex-matched neighborhood controls. Saudi Arabia, March 16–November 13, 2014

Variable*	Total, no. (%), n = 146	Case-patients, no. (%), n = 30	Controls, no. (%), n = 116	p value
Sex				1.000†
F	5 (3)	1 (3)	4 (3)	
M	141 (97)	29 (97)	112 (97)	
Interview respondent‡				<0.001†
Self	128 (88)	13 (43)	115 (99)	
Proxy (relative)	18 (12)	17 (57)	1 (1)	
Nationality				0.620§
Saudi	98 (67)	19 (63)	79 (68)	
Non-Saudi	48 (33)	11 (37)	37 (32)	
Education				0.850§
Primary school or less	65 (45)	14 (47)	51 (44)	
More than primary school	81 (55)	16 (53)	65 (56)	
Household income (monthly)‡				0.047§
≤6,000 SAR	91 (62)	14 (47)	77 (66)	
>6,000 SAR	55 (38)	16 (53)	39 (34)	
Marital status				0.475§
Never married	8 (5)	3 (10)	5 (4)	
Married	133 (91)	26 (87)	107 (92)	
Widowed	5 (3)	1 (3)	4 (3)	

*Median ages (ranges) in years are as follows: case-patients, 49 (20–72); controls, 50 (19–74); all, 50 (19–74). p<0.846, pooled t test.
†Fisher exact test.
‡Statistically significant (p<0.05).
§χ^2 test.

Table 2. Animal-related exposures, underlying health conditions, current tobacco use, and siwak use for Middle East respiratory syndrome coronavirus case-patients compared with matched controls. Saudi Arabia, March 16–November 13, 2014

Variable	No. (%) with exposure* Case-patients, n = 30	Controls, n = 116	Odds ratio (95% CI)	p value
Animal-related exposures				
Household members frequently visit farms with dromedaries†‡	12/30 (40)	14/115 (12)	7.06 (2.23–26.46)	<0.001
Household members visited a farm with dromedaries during exposure period‡	9/30 (30)	14/115 (12)	3.95 (1.23–13.72)	0.018
Household members had direct contact with dromedaries during exposure period‡§	12/30 (40)	17/114 (15)	5.03 (1.66–16.88)	0.004
Spouse	4/30 (13)	4/116 (3)	4.26 (0.86–23.41)	0.065
Other relatives‡	7/30 (23)	7/116 (6)	4.59 (1.36–16.27)	0.012
Domestic help‡	5/30 (17)	3/116 (3)	15.04 (1.96–369.59)	0.006
Dromedaries kept in/around home during exposure period‡§	9/30 (30)	17/115 (15)	3.34 (1.04–10.98)	0.047
Goats kept in/around home during exposure period‡§	1/30 (3)	22/115 (19)	0.08 (0.003–0.58)	0.011
Horses kept in/around home during exposure period§	1/29 (3)	0/115 (0)	4.00 (0.44–∞)	0.200
Bats in/around house during exposure period§	3/28 (11)	11/112 (10)	1.60 (0.24–9.23)	0.646
Sheep kept in/around home during exposure period§	10/30 (33)	22/115 (19)	3.34 (0.97–12.19)	0.057
Sheep present at a slaughterhouse visited during exposure period‡§	1/30 (3)	18/116 (16)	0.15 (<0.001–0.56)	0.040
Visited farm where livestock were kept during exposure period§	10/29 (34)	32/116 (28)	1.67 (0.52–5.42)	0.393
Dromedary present on farm‡	9/10 (90)	17/32 (53)	11.57 (2.67–∞)	0.013
Milked dromedaries while on farm‡	5/10 (50)	7/31 (23)	10.36 (2.47–∞)	0.013
Visited other livestock venue (i.e., not farm, market, slaughterhouse, racetrack, or stable) during exposure period‡§	7/29 (24)	12/111 (11)	3.33 (1.001–11.05)	0.040
Direct physical contact with dromedary during last 6 mo‡	11/30 (37)	15/116 (13)	7.67 (2.10–36.08)	0.001
Any direct contact with a dromedary during exposure period‡§¶	10/30 (33)	17/116 (15)	3.73 (1.24–11.80)	0.020
Any direct contact with a goat during exposure period§	4/30 (13)	22/116 (19)	0.64 (0.17–2.02)	0.584
Any direct contact with a§ sheep during exposure period§¶	10/30 (33)	38/116 (33)	1.03 (0.37–2.77)	1.000
Any direct contact with a horse during exposure period§¶	1/30 (3)	0/116 (0)	4.00 (0.44–∞)	0.200
Any direct contact with cattle during exposure period§¶	4/30 (13)	4/116 (3)	6.00 (1.02–48.44)	0.043
Underlying health conditions and behaviors				
Diabetes‡	16/29 (55)	32/116 (28)	3.72 (1.45–10.25)	0.005
Emphysema, chronic bronchitis, or other chronic lung disease‡	4/30 (13)	1/113 (1)	17.68 (4.22-∞)	0.003
Heart disease‡	11/30 (37)	14/114 (12)	5.11 (1.81–15.46)	0.002
Current smoker‡	11/30 (37)	22/116 (19)	3.14 (1.10–9.24)	0.030
Any underlying condition‡#	21/30 (70)	49/116 (42)	5.11 (1.70–18.67)	0.004
Any underlying condition, including current smoking‡	27/30 (90)	64/116 (55)	7.55 (2.32–33.45)	<0.001
Using *siwak* during exposure period‡§	7/28 (25)	56/114 (49)	0.24 (0.06–0.77)	0.023

*Denominators vary on the basis of completeness of responses or reflect subsets.
†Dromedaries, dromedary camels.
‡Statistically significant (p<0.05).
§The exposure period of cases is defined as the 14 days before the date of the first symptom onset. For controls, the exposure period is the same as for the case to which they are matched.
¶Direct animal contact includes any of the following specific exposures: physical contact with animals or animal products (i.e., carcasses, body fluids, secretions, urine, excrement, or raw meat) in any setting (i.e., farm, livestock market, slaughterhouse, racetrack or stable, or other animal-related venues) or engaging in certain animal-related activities (i.e., feeding animals, cleaning their housing, slaughtering them, assisting with their birth, milking them, kissing or hugging them, or other related tasks).
#Diabetes, asthma, emphysema, chronic bronchitis, other chronic lung disease, kidney failure, chronic liver disease, heart disease, history of cancer treatment, blood disorder.

Multivariable analysis yielded a final model in which direct dromedary exposure in the 2 weeks before illness onset was associated with MERS-CoV illness (adjusted OR 7.45, 95% CI 1.57–35.28), along with having diabetes (adjusted OR 6.99, 95% CI 1.89–25.86) or heart disease (adjusted OR 6.87, 95% CI 1.81–25.99) or currently smoking tobacco (adjusted OR 6.84, 95% CI 1.68–27.94) (online Technical Appendix Table 2). When substituting direct physical contact with dromedaries in the previous 6 months for direct dromedary exposure in the past 2 weeks, we found this exposure to be significantly associated with MERS-CoV illness (adjusted OR 14.59, 95% CI 2.38–89.55) along with previously identified risk factors: having diabetes (adjusted OR 6.95, 95% CI 1.85–26.12) or heart disease (adjusted OR 6.09, 95% CI 1.61–22.94) or currently smoking tobacco (adjusted OR 7.36, 95% CI 1.75–30.94). We identified no significant interactions for direct dromedary exposure, having diabetes, having heart disease, or currently smoking tobacco and other exposures, underlying conditions, or behaviors.

Discussion

By carefully identifying persons with primary MERS-CoV infections and systematically comparing their characteristics to age- and sex-matched neighborhood controls, our study supports a link between exposure to dromedaries and

human MERS-CoV illness, as well as host risk factors (i.e., diabetes, heart disease, and smoking). Exposure to bats, goats, horses, sheep, or the products of these animals were not associated with MERS CoV illness in our study. The role of an animal reservoir in the transmission of MERS-CoV to humans has been actively considered since the first reported cases in 2012. Our investigation was designed to broadly assess the possible routes and modes of transmission of MERS-CoV and to determine the risk associated with exposure to different animal species and general environmental factors.

In our study, direct contact with dromedaries in the 2 weeks before illness onset was associated with MERS-CoV illness. The proportions reporting direct contact with dromedaries was limited among both case-patients and controls (33% vs. 15%). Among specific direct exposures that we investigated, only milking dromedaries was significantly associated with illness. However, we noted a significant association when considering together all reported activities that involve direct dromedary exposure. When we controlled for underlying conditions, direct exposure to dromedaries (whether in the previous 2 weeks or in the previous 6 months) remained an independent risk factor for MERS-CoV illness. Additionally, living in the same household with persons who reported working on or visiting a farm where dromedaries were kept was a risk factor for illness; although the numbers were small, the highest risks were associated with other relatives and domestic helpers. Indirect contact with dromedaries might explain primary MERS-CoV illness in case-patients without direct dromedary contact and should be further explored. Other potential explanations of MERS-CoV illness in primary case-patients who did not have direct contact with dromedaries include unrecognized community exposure to patients with mild or subclinical MERS-CoV infection or exposure to other sources of primary MERS-CoV infection not ascertained in our study. A recent nationwide serosurvey from Saudi Arabia estimated that >44,000 persons might be seropositive for MERS-CoV and might be the source of infection to patients with confirmed primary MERS-CoV illness but with no dromedary exposure (*8*). Although we found that direct and indirect dromedary exposure were significantly associated with MERS-CoV illness, our study had limited power to detect specific behaviors or practices associated with illness. Future studies should be designed to further explore this association.

Case-patients in our study were significantly more likely than controls to report diabetes; this finding provides epidemiologic evidence of diabetes as a risk factor for MERS-CoV illness. Smoking and heart disease were also significantly associated with MERS-CoV illness. Of note was the overwhelming male preponderance in our study; only 1 of the 30 case-patients with primary infection was female. The fact that men in Saudi Arabia are much more likely than women to have contact with dromedaries might explain this observation. Previous studies have reported some male preponderance, but those findings were not as striking as our results, probably because MERS-CoV infections in most patients in other studies were healthcare associated and transmitted from human to human (*2*,*4*)

Our study is subject to several limitations. First, the delay between illness and interview might have affected recall among study participants. Second, our study was a nationwide investigation that covered a large area, and interviews were conducted by different teams at different times. However, all interviewers received training designed to limit interview variability. Third, MERS-CoV is a highly lethal disease, and 17 of the 30 case-patients in our study were interviewed by proxy (i.e., information was collected from a family member), which might have affected the reliability of the exposure information collected. Fourth, choosing neighborhood controls could have resulted in an underestimation of certain risk factors because of possible similarities between case-patients and controls. However, the study still identified an association with dromedary exposure. Fifth, the occurrence of primary MERS-CoV cases is a relatively rare event, limiting the number of cases available for inclusion in our study and the power to detect differences from controls. We applied stringent criteria for enrollment and attempted to exclude persons who might have acquired infection through human-to-human transmission, but the possibility of misclassification remains. However, inadvertent inclusion of secondary cases is likely to mean that the true risk associated with dromedary exposure was higher than we estimated. Sixth, we did not investigate dromedary husbandry practices or ascertain whether dromedaries were infected with MERS-CoV. Additionally, the surveillance system in place in Saudi Arabia might be more likely to detect persons severely affected by MERS-CoV, who also might be more likely to have underlying conditions. This fact might have overstated the role of underlying conditions as a risk factor for disease. Finally, as in any study where a large number of parameters are tested, the expected type 1 error rate is 5%; therefore, one could anticipate that 1 in 20 significant results would incorrectly reject the null hypothesis.

In conclusion, our findings represent an important initial step in understanding the risk factors for MERS-CoV infection, including zoonotic transmission. Control of MERS-CoV ultimately depends on the interruption of transmission to prevent primary MERS-CoV cases. Future longitudinal studies to assess specific human–dromedary interactions are needed to inform preventive measures.

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Human Papillomavirus Prevalence and Herd Immunity after Introduction of Vaccination Program, Scotland, 2009–2013

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In 2008, a national human papillomavirus (HPV) immunization program using a bivalent vaccine against HPV types 16 and 18 was implemented in Scotland along with a national surveillance program designed to determine the longitudinal effects of vaccination on HPV infection at the population level. Each year during 2009–2013, the surveillance program conducted HPV testing on a proportion of liquid-based cytology samples from women undergoing their first cervical screening test for precancerous cervical disease. By linking vaccination, cervical screening, and HPV testing data, over the study period we found a decline in HPV types 16 and 18, significant decreases in HPV types 31, 33, and 45 (suggesting cross-protection), and a nonsignificant increase in HPV 51. In addition, among nonvaccinated women, HPV types 16 and 18 infections were significantly lower in 2013 than in 2009. Our results preliminarily indicate herd immunity and sustained effectiveness of the bivalent vaccine on virologic outcomes at the population level.

Human papillomavirus (HPV) vaccination programs are established in several countries worldwide (*1*–*3*). The national vaccination program in Scotland began in 2008; a bivalent vaccine that conferred protection against HPV types 16 and 18 was offered at school to girls 12–13 years of age (routine cohort). In addition, girls 13–17 years of age (3-year catch-up cohort) were offered the vaccine from September 2008 through August 2011. Starting in September 2012, the licensed quadrivalent vaccine replaced the bivalent vaccine in the program. Consequently, data in this article reflect the effects of the bivalent vaccine only. Since the 2008–09 school year, receipt of all 3 doses was >90% for girls in the routine cohort, and during 2008–2011, it was lower (65%) for girls in the catch-up cohort (*4*).

Previously, we reported that sustained high uptake of HPV vaccination was associated with reduced prevalence of HPV types 16 and 18 and evidence of cross-protection against nonvaccine types HPV 31, 33, and 45 among women who had undergone their first cervical screening test for precancerous disease from 2009 through 2012 (*5*). These data reconcile with studies undertaken in other settings. In England, an ecologic study showed that 19.1% and 6.5% of vaginal swab samples were positive for HPV 16 and 18 in the pre- and postvaccination periods, respectively (*6*). Markowitz et al. also demonstrated that despite low vaccine coverage, HPV 16 and 18 prevalence among girls who had received the quadrivalent vaccine in the routine and catch-up programs was reduced by 56% (*7*). Evidence is also emerging with regard to the effectiveness of HPV vaccination for reducing the incidence and prevalence of low- and high-grade precancerous cervical lesions (*8*–*11*). In Australia, recent studies designed to assess the extent of herd immunity to vaccine-type HPV have shown evidence for potential development of herd immunity in the nonvaccinated population (*12*,*13*). However, few studies of the extent of herd immunity have been published, particularly studies in which vaccination status can be directly linked to viral outcomes.

The ability to directly link large datasets (including cervical screening, vaccination, and disease registers) in Scotland through a unique personal identifier, the Community Health Index (CHI), enables us to comprehensively assess the effects of vaccination, including the extent of potential herd immunity. By including samples from women undergoing their first cervical smear testing in 2013, we further assessed the effects of HPV vaccination among young women in Scotland by comparing prevalence of HPV 16 and 18; HPV 31, 33, and 45; and other high-risk HPV types among women who were fully vaccinated as part of the catch-up cohort with prevalence among

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nonvaccinated women in the same birth cohorts. Additionally, we investigated whether the prevalence of any nonvaccine HPV types was greater among vaccinated women. Using these updated data, we determined whether high uptake of the vaccine protects nonvaccinated women by assessing the trend, over birth cohort, for the proportion of nonvaccinated women with positive results for each HPV outcome.

Materials and Methods

Surveillance Program and Sample Population

The Scottish Cervical Screening Programme is an organized, national, call–recall program that invites women 20–60 years of age to visit their general practitioner for a cervical smear test (*14*). The program is facilitated through the electronic Scottish Cytology Call–Recall System, which records which women are eligible for screening and contains information about cytology, histology, vaccination status, recall, and management.

During 2009–2013, the National Health Service cytopathology laboratories that serve the Screening Programme collected ≈1,000 liquid-based cytology samples per year from women 20–21 years of age who were undergoing their first cervical smear testing. All samples collected during 2009–2013 were subjected to HPV genotyping, and the results from the 2009–2010 samples constituted a prevaccination baseline. The sampling methods used in this study are described elsewhere (*5*).

Data and Linkage

Liquid-based cytology samples collected by the cytology laboratories were labeled with an anonymous study identification number and underwent HPV genotyping at the Scottish HPV Reference Laboratory. The study identification numbers and the CHI number were sent to the Information Services Division of the Scottish National Health Service, where CHI numbers were used to link data from the Scottish Cytology Call–Recall System, the Scottish Immunisation Call–Recall System, and the Child Health Schools Program-System. The postal code of the patient's residence was used to rank the geographic data zone for each sample according to the Scottish Index of Multiple Deprivation (1 = most deprived and 5 = least deprived; http://www.gov.scot/Topics/Statistics/SIMD/BackgroundMethodology).

HPV Testing

A detailed account of the testing procedures has been described elsewhere (*5*). In brief, HPV genotyping was performed by using the Multimetrix HPV Genotyping Kit (Diamex, Heidelberg, Germany), which can detect 24 HPV types, including all established high-risk carcinogenic types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59); probable carcinogenic types (HPV 68); and some possibly carcinogenic types (HPV 26, 53, 66, 70, 73, and 82), according to the latest International Agency for Research on Cancer groupings (*15*). This assay can also detect 5 low-risk HPV types (HPV 6, 11, 42, 43, and 44). International Agency for Research on Cancer guidelines also include HPV 67 as possibly carcinogenic, but this type is currently undetectable by use of the Multimetrix HPV kit (*16*).

Statistical Analyses

Power calculations for the liquid-based cytology samples are described elsewhere (*5*). The prevalence of each detectable HPV type, along with 95% CIs, was calculated. A z-test of 2 proportions was used to assess differences in HPV type–specific prevalence among women who received all 3 doses of the vaccine and those who received none. The Bonferroni correction (significance level $\alpha = 0.05/22$) was used because of the multiple statistical testing conducted for the 22 nonvaccine HPV types detected by the assay. Significance was assessed at $\alpha = 0.05$ for HPV types 16 and 18. Association between the number of doses of vaccine received and HPV outcome was measured by using logistic regression adjusted for deprivation score, birth cohort year, and age at vaccination. A linear trend test was used to assess evidence for a linear change in positivity over the range of the previously mentioned variables. HPV outcomes were positivity for HPV types 16 or 18; HPV 31, 33 or 45; other nonvaccine high-risk types (HPV 35, 39, 51, 52, 56, 58, 59, and 68) in the carcinogenic and probably carcinogenic categories; or any HPV type detected by the Multimetrix HPV assay. Potential herd immunity was evaluated by using logistic regression and testing for a linear trend over time in the prevalence of HPV 16 and 18, the cross-protective types, other nonvaccine high-risk types, and any HPV among women who were not vaccinated during 2009–2013.

Results

Sample Characteristics

We analyzed 5,765 liquid-based cytology samples from women 20–21 years of age who underwent their first cervical smear testing during 2009–2013. The number of samples received each year was distributed evenly between the quintiles of the Scottish Index of Multiple Deprivation (≈20% samples/quintile) (Table 1). Overall, valid HPV test results were available for 5,715 samples, of which 57.1% (95% CI 55.8%–58.3%) were positive for any HPV type and 46.9% (95% CI 45.6%–48.2%) were positive for any high-risk HPV type (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, or 68). As expected, because of eligibility criteria, vaccination status differed greatly by collection year; 38% of women received 3 doses in 2011, 67%

Table 1. Yearly distribution of 5,765 liquid-based cytology samples collected from women 20–21 years of age undergoing their first cervical smear collection, Scotland, 2009–2013*

Year	Total	No. (%) samples									No. (%) samples with valid HPV results
		No. vaccine doses received				SIMD score					
		0	1	2	3	1	2	3	4	5	
2009	1,673	1,652 (98.74)	5 (0.30)	1 (0.06)	15 (0.90)	386 (23.07)	389 (23.25)	335 (20.02)	271 (16.20)	292 (17.45)	1,652 (98.74)
2010	1,074	1,012 (94.23)	7 (0.65)	7 (0.65)	48 (4.47)	260 (24.21)	208 (19.37)	219 (20.39)	193 (17.97)	194 (18.06)	1,053 (98.04)
2011	1,005	557 (55.42)	18 (1.79)	48 (4.78)	382 (38.01)	235 (23.38)	190 (18.91)	185 (18.41)	201 (20.00)	194 (19.30)	1,001 (99.60)
2012	997	245 (24.57)	26 (2.61)	52 (5.22)	674 (67.60)	216 (21.66)	201 (20.16)	172 (17.25)	191 (19.16)	217 (21.77)	993 (99.60)
2013	1,016	198 (19.49)	33 (3.25)	46 (4.53)	739 (72.74)	251 (24.70)	211 (20.77)	191 (18.80)	141 (13.88)	222 (21.85)	1,018 (100.00)

*HPV, human papillomavirus; SIMD, Scottish Index of Multiple Deprivation (1 = most deprived; 5 = least deprived).

in 2012, and 72% in 2013. The samples received in 2009 and 2010 were from women who were not eligible for the catch-up campaign; therefore, 98% and 94% of the samples from these years, respectively, came from nonvaccinated women (Figure).

Effect on Vaccine-Type Infections

We observed a statistically significant decrease in HPV 16 and 18 among vaccinated compared with nonvaccinated women (p<0.0001) (Figure). Positivity for HPV 16 and 18 in the samples was 11% (95% CI 9.7%–12.5%) among fully vaccinated women and 29.4% (95% CI 27.9%–30.9%) among nonvaccinated women (Table 2). Overall, annual prevalence of HPV 16 and 18 decreased over time; 10.1% (95% CI 8.4%–12.2%) of the samples collected in 2013 were positive for HPV 16 and 18, whereas 28.8% (95% CI 26.7%–31%) of the samples collected in 2009 were positive (*5*).

Unadjusted analysis showed a significant linear trend for birth cohort and number of doses a woman received; prevalence of HPV 16 and 18 was lower among women from later birth cohorts and women who had received more doses (both p<0.0001) (Table 2). The linear trend remained significant for both variables in the adjusted analysis (both

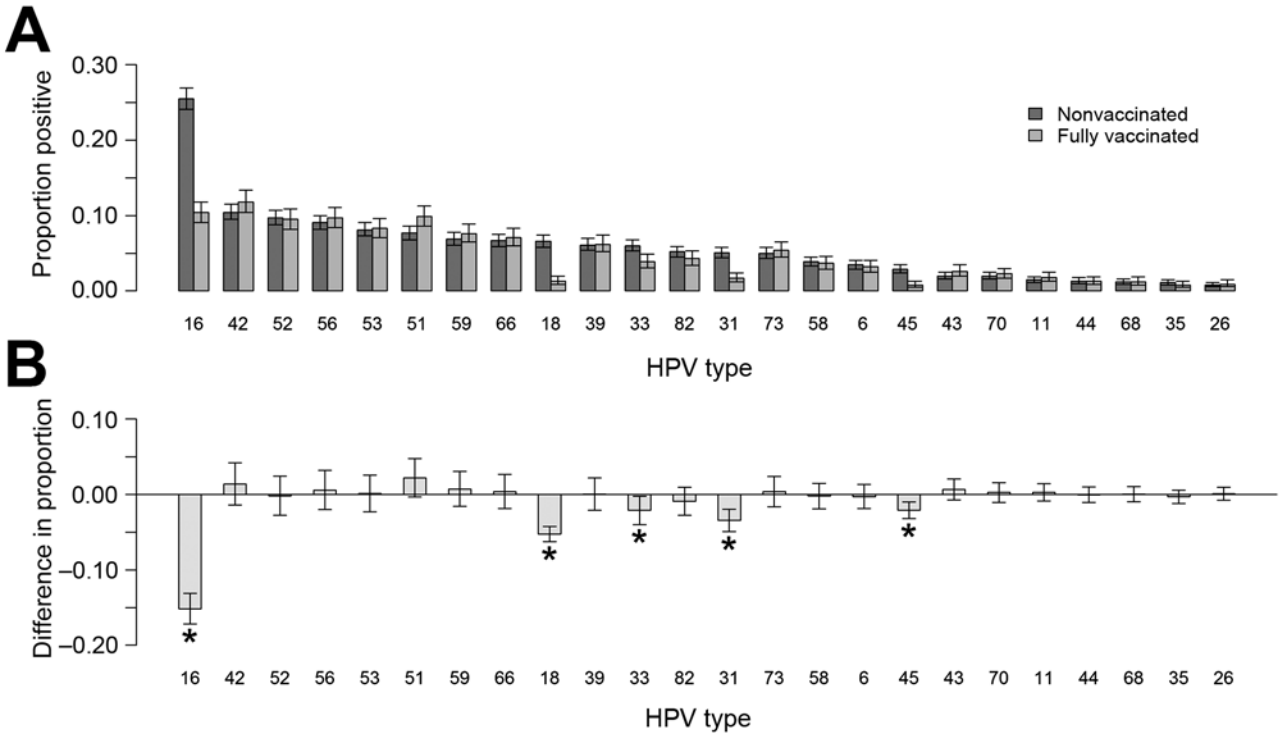

Figure. Analyses for 5,715 liquid-based cytology cervical samples from vaccinated and nonvaccinated women, for which valid human papillomavirus (HPV) testing results were available, Scotland, 2009–2013. A) Proportion and 95% CIs for samples with positive results for each HPV type. B) Difference in the proportion positive and associated 95% CIs for the difference between vaccinated and nonvaccinated women, by HPV type. Other than HPV types 16 and 18, the 95% CIs of the difference were corrected for multiple testing using by using the Bonferroni correction. *Significant change.

Table 2. Prevalence and unadjusted odds of positivity for HPV types 16 or 18 and cross-protective types stratified by year of sample collection, number of doses received, birth year, and age at vaccination, Scotland, 2009–2013 (N = 5,765)*

		HPV 16 or 18			Cross-protective HPV types†		
Variable	No.	No. pos	% Pos (95% CI)	Unadjusted OR (95% CI)	No. pos	% Pos (95% CI)	Unadjusted OR (95% CI)
Collection year							
2009	1,652	476	28.8 (26.7–31.0)	1 (reference)	215	13.0 (11.5–14.7)	1 (reference)
2010	1,053	333	31.6 (28.9–34.5)	1.14 (0.97–1.35)	143	13.6 (11.6–15.8)	1.05 (0.84–1.32)
2011	1,001	233	23.3 (20.7–26.0)	0.75 (0.63–0.90)	104	10.4 (8.7–12.4)	0.78 (0.60–0.99)
2012	993	169	17.0 (14.8–19.5)	0.51 (0.42–0.62)	83	8.4 (6.8–10.2)	0.61 (0.47–0.79)
2013	1,016	103	10.1 (8.40–12.2)	0.28 (0.22–0.35)	64	6.3 (5.0–8.0)	0.45 (0.33–0.60)
No. doses vaccine received							
0	3,619	1062	29.4 (27.9–30.9)	1 (reference)	468	12.9 (11.9–14.1)	1 (reference)
1	89	20	22.5 (15.0–32.2)	0.70 (0.41–1.13)	15	16.9 (10.5–26.0)	1.37 (0.75–2.33)
2	154	28	18.2 (12.9–25.0)	0.54 (0.35–0.80)	11	7.1 (4.0–12.3)	0.52 (0.26–0.92)
3	1,853	204	11.0 (9.70–12.5)	0.30 (0.25–0.35)	115	6.2 (5.2–7.4)	0.45 (0.36–0.55)
Birth year							
1988	844	251	29.7 (26.8–32.9)	1 (reference)	119	14.1 (11.9–16.6)	1 (reference)
1989	1,196	343	28.7 (26.2–31.3)	0.96 (0.79–1.17)	140	11.7 (10–13.7)	0.82 (0.63–1.06)
1990	1,204	349	29.0 (26.5–31.6)	0.97 (0.80–1.18)	155	12.9 (11.1–14.9)	0.91 (0.70–1.17)
1991	867	175	20.2 (17.6–23.0)	0.60 (0.48–0.74)	80	9.2 (7.5–11.3)	0.62 (0.46–0.83)
1992	1,261	169	13.4 (11.6–15.4)	0.36 (0.29–0.45)	90	7.1 (5.8–8.7)	0.47 (0.35–0.62)
1993	393	27	6.90 (4.8–9.8)	0.17 (0.11–0.26)	25	6.4 (4.3–9.2)	0.41 (0.26–0.63)
Age at vaccination, y‡							
15–16	970	75	7.7 (6.2–9.6)	1 (reference)	52	5.4 (4.1–7.0)	1 (reference)
17	631	79	12.5 (10.2–15.3)	1.70 (1.22–2.38)	47	7.4 (5.6–9.8)	1.42 (0.94–2.13)
18	391	65	16.6 (13.3–20.6)	2.38 (1.67–3.40)	30	7.7 (5.4–10.7)	1.47 (0.91–2.32)
>18	109	33	30.3 (22.4–39.5)	5.31 (3.28–8.48)	12	11 (6.4–18.3)	2.23 (1.1–4.18)

*HPV, human papillomavirus; OR, odds ratio; pos, positive.
†HPV types 31, 33, or 45.
‡For those vaccinated.

p<0.0001); however, the adjusted odds of positivity were tempered for birth cohort (Table 3). The adjusted odds ratio (OR) of being infected with HPV 16 or 18 decreased with every dose. For 1 dose, OR was 0.45 (95% CI 0.24–0.84); 2 doses, OR 0.39 (95% CI 0.23–0.67); and 3 doses, OR 0.27 (95% CI 0.19–0.37). Women from least deprived areas were significantly less likely to have positive results for HPV 16 and 18 than were those from more deprived areas (p = 0.0322). The linear trend for age at vaccination was significant; odds of infection with HPV 16 and 18 were greater for women vaccinated at older ages than for those vaccinated at 15–16 years of age (p<0.0001) (Table 3).

Evidence for Cross-Protection

Prevalence of HPV types 31, 33, and 45 decreased among vaccinated compared with nonvaccinated women (p<0.0001, p = 0.0012, and p<0.0001, respectively) (Figure). The positivity for cross-protective HPV types was 12.9% (95% CI 11.9%–14.1%) among nonvaccinated women and 6.2% (95% CI 5.2%–7.4%) among fully vaccinated women (Table 2). During 2009–2013, overall cross-protective type prevalence also declined, from 13% (95% CI 11.5%–14.7%) in 2009 to 6.3% (95% CI 5%–8%) in 2013 (*5*).

According to unadjusted analyses, the odds of being infected with cross-protective types decreased significantly according to birth cohort year (p = 0.0001), but adjusted analyses showed no such significant effect (p = 0.2413) because of confounding of the effect with vaccination status (Tables 2, 3). A strong significant linear trend was observed according to the number of doses received; the adjusted odds of positivity decreased with the number of doses received (1-dose OR 1.15 [95% CI 0.54–2.33] vs. 3-dose OR 0.45 [95% CI 0.29–0.68]; p<0.0001). Odds of positivity for cross-protective types were significantly reduced among women from the least deprived backgrounds (p = 0.0028); however, no significant difference was observed according to age at vaccination (p = 0.3736).

Positivity for High-Risk HPV Types other than 16, 18, 31, 33, and 45

The overall prevalence of nonvaccine, non–cross-protective high-risk HPV types (HPV 35, 39, 51, 52, 56, 58, 59 and 68) significantly increased, from 29.1% (95% CI 26.9%–31.3%) in 2009 to 33.9% (95% CI 31.0%-36.8%) in 2013 (p = 0.0128) (*5*). Prevalence of nonvaccine, non–cross-protective high-risk HPV types did not differ significantly (p = 0.959) between nonvaccinated women (32.5% [95% CI 31%–34%]) and fully vaccinated women (32.9% ([95% CI 30.8%–35%]) (Table 4). Prevalence of HPV 51 was marginally and nonsignificantly increased among vaccinated women compared with nonvaccinated women (p = 0.0059).

Odds of nonvaccine or cross-protective high-risk HPV type infection were significantly higher for women in later birth cohorts than for those in earlier birth cohorts (p

Table 3. Adjusted odds of positivity for HPV 16 or 18 and cross-protective HPV types by birth year, number of doses of vaccine received, SIMD score, and age at vaccination, Scotland, 2009–2013*

Variable	HPV type 16 or 18		Cross-protective HPV types†	
	Adjusted OR (95% CI)‡	Linear trend p value	Adjusted OR (95% CI)‡	Linear trend p value
Birth year		0.0005		0.2413
1988	1 (reference)		1 (reference)	
1989	0.96 (0.79–1.17)		0.82 (0.63–1.06)	
1990	1.02 (0.84–1.24)		0.97 (0.75–1.27)	
1991	0.97 (0.75–1.25)		0.86 (0.60–1.21)	
1992	0.84 (0.63–1.11)		0.77 (0.53–1.12)	
1993	0.43 (0.26–0.67)		0.72 (0.42–1.20)	
No. doses vaccine received		<0.0001		<0.0001
0	1 (reference)		1 (reference)	
1	0.45 (0.24–0.84)		1.15 (0.54–2.33)	
2	0.39 (0.23–0.67)		0.46 (0.21–0.94)	
3	0.27 (0.19–0.37)		0.45 (0.29–0.68)	
SIMD quintile		0.0322		0.0028
1 (most deprived)	1 (reference)		1 (reference)	
2	0.84 (0.70–1.02)		1.05 (0.82–1.33)	
3	0.85 (0.70–1.03)		0.93 (0.72–1.19)	
4	0.91 (0.75–1.11)		0.72 (0.54–0.94)	
5 (least deprived)	0.75 (0.62–0.92)		0.76 (0.58–0.99)	
Age at vaccination, y§		<0.0001		0.3736
15–16	1 (reference)		1 (reference)	
17	1.33 (0.92–1.91)		1.26 (0.80–1.98)	
18	1.65 (1.07–2.53)		1.19 (0.67–2.08)	
>18	3.41 (1.98–5.82)		1.50 (0.68–3.12)	

*HPV, human papillomavirus; OR, odds ratio; SIMD, Scottish Index of Multiple Deprivation.
†HPV types 31, 33, or 45.
‡Adjusted for birth year, SIMD score, and age at vaccination.
§For those vaccinated.

= 0.0147) (Table 5). According to adjusted analysis, the odds of positivity for a nonvaccine, non–cross-protective, high-risk HPV type was 1.5 times higher for those born in 1992 and 1993 than for those born in 1988 (reference birth cohort). Although the unadjusted analysis shows some tempering of this effect, a linear trend was still present (p = 0.04) (Table 4). When adjusted for birth cohort, odds of infection were slightly reduced for women who received 3 doses of vaccine compared with women who received no vaccine, but this difference was not significant (p = 0.2953). No significant linear trend was found for nonvaccine, non–cross-protective, high-risk HPV type positivity according to deprivation status (p = 0.1378) or age at vaccination (p = 0.4541).

Overall Positivity for any HPV Type

Prevalence of all 24 HPV types detected by the assay remained unchanged from 2009 to 2012 (58.1% [95% CI 55.7%–60.4%] in 2009 and 58.4% [95% CI 55.3%–61.4%] in 2012) but decreased to 53.8% (95% CI 50.8%–56.9%) in 2013 (Table 4) (*5*). Overall HPV positivity was 53.1% (95% CI 50.8%–55.3%) among fully vaccinated women and higher (59.7% [95% CI 58.1%–61.3%]) among nonvaccinated women.

According to unadjusted analyses, overall HPV positivity showed a significant linear trend by birth cohort year; HPV infection was more likely among women in later birth cohorts than among than those born in 1988 (p = 0.03171). According to adjusted analyses, however, this trend was not significant (p = 0.115) (Tables 4, 5). HPV infection was significantly less likely among women who had received 3 doses of vaccine than among those who had received no doses (p<0.004) and was also less likely among women from the least deprived backgrounds than among those from the most deprived backgrounds (linear trend test p = 0.0002). Furthermore, overall HPV positivity did not differ significantly between women vaccinated at different ages (p = 0.331).

Prevalence of HPV among Nonvaccinated Women (Herd Immunity)

Prevalence of HPV 16 and 18 among nonvaccinated women remained relatively stable at ≈30% during 2009–2012 but decreased to 21.2% in 2013 (Table 6). During 2010–2013, prevalence of HPV types 31, 33, or 45 declined gradually, from 13.7% in 2010 to 9.6% in 2013. In 2013, the odds of infection with HPV types 16 and 18 was reduced among nonvaccinated women (OR 0.67 [95% CI 0.47–0.96]) compared with the baseline odds in 2009, and testing for trend over all years showed a marginal decrease over time (p = 0.054) (Table 6). Odds of infection with HPV types 31, 33, or 45 were reduced in 2012 and 2013 compared with 2009, but these odds were not significant, and no significant linear trend was observed (p = 0.104). The odds of infection with nonvaccine, non–cross-protective, high-risk HPV types were significantly

Table 4. Prevalence and unadjusted odds of high-risk HPV excluding vaccine and cross-protective types and any HPV by year of sample collection, number of doses received, birth year, and age at vaccination, Scotland, 2009–2013 (N = 5,765)*

		High-risk HPV, excluding vaccine and cross-protective types†			Any HPV		
Variable	No.	No. pos	% Pos (95% CI)	Unadjusted OR (95% CI)	No. pos	% Pos (95% CI)	Unadjusted OR (95% CI)
Collection year							
2009	1,652	480	29.1 (26.9–31.3)	1 (reference)	959	58.1 (55.7–60.4)	1 (reference)
2010	1,053	364	34.6 (31.7–37.5)	1.29 (1.09–1.52)	618	58.7 (55.7–61.6)	1.01 (0.86–1.18)
2011	1,001	330	33.0 (30.1–35.9)	1.20 (1.01–1.42)	587	58.6 (55.6–61.7)	1.05 (0.89–1.23)
2012	993	352	35.5 (32.5–38.4)	1.34 (1.13–1.59)	580	58.4 (55.3–61.4)	1.04 (0.88–1.21)
2013	1,016	344	33.9 (31.0–36.8)	1.25 (1.06–1.48)	547	53.8 (50.8–56.8)	0.87 (0.74–1.02)
No. doses							
0	3,619	1,176	32.5 (31.0–34.0)	1 (reference)	2162	59.7 (58.1–61.3)	1 (reference)
1	89	32	36.0 (26.8–46.3)	1.17 (0.75–1.80)	55	61.8 (51.4–71.2)	1.12 (0.73–1.75)
2	154	53	34.4 (27.4–42.2)	1.09 (0.77–1.52)	91	59.0 (51.2–66.5)	1.00 (0.73–1.40)
3	1,853	609	32.9 (30.8–35.0)	1.02 (0.90–1.15)	983	53.1 (50.7–55.3)	0.78 (0.70–0.87)
Birth year							
1988	844	235	27.8 (24.9–31.0)	1 (reference)	477	56.5 (53.2–59.8)	1 (reference)
1989	1,196	371	31.0 (28.5–33.7)	1.18 (0.98–1.44)	700	58.5 (55.7–61.3)	1.09 (0.91–1.30)
1990	1,204	413	34.3 (31.7–37.0)	1.37 (1.13–1.66)	697	57.9 (55.1–60.6)	1.06 (0.89–1.26)
1991	867	287	33.1 (30.1–36.3)	1.28 (1.04–1.58)	515	59.4 (56.1–62.6)	1.13 (0.93–1.36)
1992	1,261	435	34.5 (31.9–37.2)	1.36 (1.12–1.64)	706	56.0 (53.2–58.7)	0.98 (0.82–1.17)
1993	393	129	32.8 (28.4–37.6)	1.25 (0.97–1.62)	196	49.9 (45.0–54.8)	0.77 (0.60–0.97)
Age at vaccination, y‡							
15–16	970	305	31.4 (28.6–34.4)	1 (reference)	491	50.6 (47.5–53.8)	1 (reference)
17	631	241	38.2 (34.5–42.0)	1.34 (1.09–1.66)	358	56.7 (52.8–60.5)	1.28 (1.05–1.57)
18	391	116	29.7 (25.4–34.4)	0.92 (0.71–1.19)	220	56.3 (51.3–61.1)	1.26 (0.99–1.59)
>18	109	32	29.4 (21.6–38.5)	0.93 (0.59–1.42)	60	55.0 (45.7–64.1)	1.19 (0.80–1.78)

*HPV, human papillomavirus; OR, odds ratio; pos, positive.
†HPV 35, 39, 51, 52, 56, 58, 59, or 68.
‡For those vaccinated.

higher among nonvaccinated women in 2010, 2011, and 2012 (OR 1.26 [95% CI 1.07–1.5], OR 1.5 [95% 1.22–1.83], and OR 1.44 [95% CI 1.09–1.91], respectively) than in 2009 (Table 7). Odds of infection with any HPV were increased in 2011 and 2012 (OR 1.3 [95% CI 1.07–1.59] and OR 1.56 [95% CI 1.18–2.09], respectively) over odds in 2009, but this linear trend was not significant (p = 0.0576) (Table 7).

Discussion

Decline of HPV prevalence in Scotland has been reported (*5*). We show a further decline in the prevalence of HPV types 16 and 18 among women in Scotland, associated with high rates of vaccination with the bivalent HPV vaccine. There is evidence that each dose administered conferred protection against HPV type 16 or 18 and that the odds of infection were significantly reduced after 2 and 3 doses. Our findings are comparable with those of a nested analysis of a randomized controlled trial, which reported efficacy of 1, 2, and 3 doses of bivalent HPV vaccine against HPV 16 and 18 infection (*17*). However, because of the small number of women in our study who had received 2 doses and because our study was powered to detect an effect of 3 doses, the findings with regard to effectiveness of <3 doses should be considered preliminary and may be confounded by other factors. Our evidence of the vaccine conferring cross-protection against HPV types 31, 33, and 45 is also consistent with findings of a previous study (*18*). Of note, published results of a recent study in England (*6*) do not report similar reductions in cross-protective types; however, the authors of that article concluded that further analysis of samples from women in birth cohorts with high vaccine coverage was needed for full evaluation of the effects of vaccination on prevalence of nonvaccine HPV types.

We observed a reduction in the prevalence of HPV types 16 and 18 among nonvaccinated women undergoing their first cervical smear testing in 2013. This finding is encouraging evidence of herd immunity and corroborates the findings of 2 studies from Australia: a sexual health clinic–based national surveillance study that observed a decline in genital warts among heterosexual men and a cross-sectional study that showed a decrease in HPV prevalence among nonvaccinated women (*12*,*13*). These data and our data are welcome because protecting the nonvaccinated population from infection with HPV 16 or 18 depends on development of herd immunity. However, the limited number of nonvaccinated women, particularly in later years since the introduction of the program, makes interpretation of these results somewhat challenging, as reflected in the CIs. In addition, although in 2009, nonvaccinated women were not vaccinated because of ineligibility, in 2013, nonvaccinated women may not have been vaccinated for other reasons (religious, cultural, or

Table 5. Adjusted odds of positivity for high-risk HPV excluding vaccine and cross-protective types and any HPV type by birth year, number of doses received, SIMD score, and age at vaccination, Scotland, 2009–2013*

Variable	High-risk HPV types, excluding vaccine and cross-protective types†		Any HPV	
	Adjusted OR (95% CI)‡	Linear trend p value	Adjusted OR (95% CI)‡	Linear trend p value
Birth year		0.0147		0.1158
1988	1 (reference)		1 (reference)	
1989	1.18 (0.97–1.44)		1.08 (0.91–1.29)	
1990	1.41 (1.16–1.72)		1.10 (0.92–1.32)	
1991	1.32 (1.04–1.69)		1.36 (1.08–1.71)	
1992	1.49 (1.16–1.91)		1.39 (1.10–1.77)	
1993	1.46 (1.06–2.00)		1.13 (0.84–1.53)	
No. doses vaccine received		0.2953		0.004
0	1 (reference)		1 (reference)	
1	0.89 (0.53–1.48)		0.78 (0.48–1.30)	
2	0.84 (0.56–1.27)		0.72 (0.48–1.07)	
3	0.80 (0.63–1.02)		0.60 (0.48–0.76)	
SIMD quintile		0.1378		0.0002
1 (most deprived)	1 (reference)		1 (reference)	
2	0.99 (0.84–1.17)		0.93 (0.80–1.10)	
3	0.99 (0.84–1.18)		0.83 (0.70–0.97)	
4	0.98 (0.82–1.17)		0.87 (0.74–1.03)	
5 (least deprived)	0.87 (0.73–1.03)		0.73 (0.62–0.86)	
Age at vaccination, y§		0.4541		0.331
15–16	1 (reference)		1 (reference)	
17	1.40 (1.10–1.77)		1.2 (0.95–1.51)	
18	0.98 (0.71–1.35)		1.24 (0.92–1.68)	
>18	0.98 (0.60–1.57)		1.25 (0.80–1.96)	

*HPV, human papillomavirus; OR, odds ratio; SIMD, Scottish Index of Multiple Deprivation.
†HPV 35, 39, 51, 52, 56, 58, 59, or 68.
‡Adjusted for birth cohort year, SIMD score, and age at vaccination.
§For those vaccinated.

societal), which may influence their likelihood of being infected by HPV types 16 or 18 relative to women in the 2009 cohort. However, the increased odds of infection with nonvaccine, non–cross-protective, high-risk HPV types and any HPV type among nonvaccinated women in years after 2009 suggests that these reasons are probably not a major confounding factor. Data from future cohorts will show whether the reduction of infection among nonvaccinated women is sustained.

It has been postulated that the reduction of infection with HPV types 16 and 18 and cross-protective types could leave a vacant niche, leading to increased infections with less oncogenic, nonvaccine HPV types among vaccinated women (*19*,*20*). We found that odds of infection with nonvaccine, non–cross-protective, high-risk HPV types were higher among women in later birth cohorts than among those in earlier birth cohorts. Furthermore, we observed that the most common high-risk type infecting fully vaccinated women in later cohorts was HPV 51, replacing HPV 16 as the most prevalent type but at lower rates. However, because of the increased overall prevalence between 2009 and 2013, comparison of nonvaccine high-risk HPV type prevalence between nonvaccinated and fully vaccinated women will be confounded. It is feasible that rather than truly replacing vaccine types, the other high-risk types are simply being unmasked because of less competition for the resources within molecular amplification assays (*21*). Consequently, we found no strong evidence for type replacement occurring in Scotland; these results are consistent with data from Australia (*22*). Continued follow-up is needed for evaluation of the potential for type replacement after high uptake of the bivalent HPV vaccine. Our ongoing analysis of HPV prevalence among women with histologically confirmed cervical lesions and linkage to colposcopy data to assess cervical disease in the female population of Scotland will aid in addressing the issue of clinically relevant type replacement (*7*).

Table 6. Prevalence and odds of infection with HPV types 16 or 18 and for HPV cross-protective types among nonvaccinated women, by study year, Scotland, 2009–2013*

Study year	No. women	HPV 16 or 18			Cross-protective HPV types†		
		No. pos	% Pos (95% CI)	OR (95% CI)	No. pos	% Pos (95% CI)	OR (95% CI)
2009	1,652	468	28.3 (26.2–30.6)	1 (reference)	211	12.8 (11.2–14.5)	1 (reference)
2010	1,012	310	30.6 (27.9–33.5)	1.13 (0.95–1.34)	139	13.7 (11.8–16.0)	1.10 (0.87–1.38)
2011	557	164	29.4 (25.8–33.4)	1.05 (0.85–1.29)	71	12.7 (10.2–15.8)	0.99 (0.74–1.32)
2012	245	78	31.8 (26.3–37.9)	1.18 (0.88–1.57)	28	11.4 (8.0–16.0)	0.88 (0.58–1.33)
2013	198	42	21.2 (16.1–27.4)	0.67 (0.47–0.96)	19	9.6 (6.2–14.5)	0.71 (0.44–1.17)

*HPV, human papillomavirus; OR, odds ratio; pos, positive.
†HPV 31, 33, or 45.

Table 7. Prevalence and odds of infection with high-risk HPV excluding vaccine and cross-protective types and for any HPV among nonvaccinated women, by study year, Scotland, 2009–2013*

Study year	No. women	High-risk HPV excluding vaccine and cross-protective types†			Any HPV		
		No. pos	% Pos (95% CI)	OR (95% CI)	No. pos	% Pos (95% CI)	OR (95% CI)
2009	1.652	473	28.6 (26.5–30.9)	1	946	57.3 (54.9–59.6)	1
2010	1,012	338	33.4 (30.6–36.4)	1.26 (1.07–1.5)	578	57.1 (54.0–60.1)	0.99 (0.85–1.16)
2011	557	210	37.7 (33.8–41.8)	1.50 (1.22–1.83)	354	59.5 (59.5–67.4)	1.30 (1.07–1.59)
2012	245	90	36.7 (30.9–42.9)	1.44 (1.09–1.91)	166	61.7 (61.7–73.3)	1.56 (1.18–2.09)
2013	198	65	32.8 (26.7–39.6)	1.20 (0.87–1.64)	118	52.6 (53.6–66.2)	1.10 (0.82–1.49)

*HPV, human papillomavirus; OR, odds ratio; pos, positive.
†HPV 35, 39, 51, 52, 56, 58, 59, or 68.

The main strength of this study is that vaccination and screening data can be linked at the individual level. Coupled with the high levels of vaccine uptake and age of screening in Scotland, this linkage enables us to directly evaluate the effects of HPV vaccination on HPV prevalence.

Nevertheless, our study has some limitations. Data from a screened population are not representative of the whole population and possibly underestimate the true prevalence of HPV infection. However, the prevalence of HPV among 20-year-old women who did not undergo a cervical screening test but were invited to send a self-collected urine sample or vaginal swab sample did not differ significantly from prevalence among those who had undergone cervical screening testing in a previous study (*23*). We were also unable to collect sexual history data and so could not determine an overall change in sexual practices among the population, which might confound our results. However, our observed increase in prevalence of nonvaccine, non–cross-protective, high-risk HPV types among vaccinated and nonvaccinated women and the relative stability of the prevalence of HPV overall suggests either an increase or stabilization of sexual behavior between 2009 and 2013. Also, the results of the third National Surveys of Sexual Attitudes and Lifestyles study, conducted in 2013, showed that the number of women's lifetime sex partners, a known risk factor for HPV infection, has increased since 2000, and it is therefore more likely that the effect of the vaccine has been underestimated in our study (*24*). In addition, the incidence of genital herpes and gonorrhea in Scotland increased from 2005 to 2014, suggesting that sexual activity has increased over time (*25*). However, women in later birth cohorts within the catch-up campaign are more likely to have received the vaccine within the school-based program than were those in earlier birth cohorts, who are more likely to have received the vaccine out of school (in general practice). Previous studies have shown that those who leave school are more likely to be from high-deprivation backgrounds and are consequently more likely to be infected with HPV (*4*,*26*). Therefore, the effect of the HPV vaccine may be confounded by differences between those who leave school and those who stay in school.

Our data preliminarily suggest the presence of herd immunity in the nonvaccinated female population of Scotland. However, we could not assess whether herd immunity is conferring protection to the male population, who are not routinely sampled as part of the surveillance program. We plan to use genital wart consultation data from men to act as a proxy for detecting herd immunity in the male population (*13*). In the meantime, the first girls who received the vaccine as part of the routine vaccination program will be eligible for cervical screening toward the end of 2015. Those data will enable us to demonstrate the effect of equitable >90% vaccine uptake on HPV prevalence and cervical disease among young, presumed HPV naive, women in Scotland.

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Decline in Decreased Cephalosporin Susceptibility and Increase in Azithromycin Resistance in *Neisseria gonorrhoeae*, Canada

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Antimicrobial resistance profiles were determined for *Neisseria gonorrhoeae* strains isolated in Canada during 2010–2014. The proportion of isolates with decreased susceptibility to cephalosporins declined significantly between 2011 and 2014, whereas azithromycin resistance increased significantly during that period. Continued surveillance of antimicrobial drug susceptibilities is imperative to inform treatment guidelines.

Gonorrhea, caused by *Neisseria gonorrhoeae*, is the second most commonly reported sexually transmitted infection in Canada; ≈13,000 cases occur yearly, and rates have increased from 20.1 cases/100,000 population in 2000 to 39.2 cases/100,000 in 2013 (*1*). The infection is also a global public health threat, with ≈106 million cases/year occurring worldwide (*2*). Gonococci have acquired resistance to many antimicrobial agents used for treatment (*3*), however, which makes it imperative to conduct surveillance programs so appropriate treatment recommendations can be determined. In 2011, the increases in MICs of cephalosporins prompted the authors of the Canadian Sexually Transmitted Infections Guidelines to update the recommended gonorrhea treatment from a single antimicrobial drug to combination therapy with ceftriaxone (250 mg intramuscularly) and azithromycin (1 g orally in a single dose) as the first-line treatment for uncomplicated anogenital and pharyngeal *N. gonorrhoeae* infections in adults (*4*). We analyzed antimicrobial drug susceptibility levels of *N. gonorrhoeae* to cephalosporins and azithromycin in Canada since the recommended treatments were updated in 2011.

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The Study

The National Microbiology Laboratory (NML) in Winnipeg, Manitoba, Canada, has conducted ongoing monitoring of antimicrobial drug susceptibilities in *N. gonorrhoeae* isolates since 1985. Isolates are submitted to NML by provincial laboratories when they identify a resistant isolate or by laboratories that do not conduct antimicrobial susceptibility testing. To determine the proportion of antimicrobial drug resistance, we used the total number of isolates identified in each province as the denominator.

Antimicrobial drug susceptibilities of *N. gonorrhoeae* to ceftriaxone, azithromycin, and cefixime (Sigma-Aldrich, Oakville, Ontario, Canada) were determined by using agar dilution as previously described (*5*,*6*). MIC breakpoints used were the following: decreased susceptibility to cefixime, MIC ≥0.25 mg/L; decreased susceptibility to ceftriaxone, MIC ≥0.125 mg/L (*2*); resistance to azithromycin, MIC ≥2.0 mg/L (*7*). For controls, we used *N. gonorrhoeae* reference cultures ATCC49226, WHOF, WHOG, WHOK, and WHOP. Statistical analysis was determined by using EpiCalc 2000 version 1.02 (http://www.brixtonhealth.com/epicalc.html). A 2 × 2 χ^2 test was used to compare proportions of resistance per year to identify significant differences between years (p values calculated with 99% CI).

From 2010 through 2014, ≈59,400 cases of *N. gonorrhoeae* infection were reported in Canada; 16,370 (≈28%) were diagnosed by culture. Provincial public health laboratories submitted 6,728 isolates to NML for testing (2010 = 1,235; 2011 = 1,173; 2012 = 1,035; 2013 = 1,184; 2014 = 2,101). Sex and age data of patients were available for 6,468 (96.1%) isolates. Of these, 5,221 (80.7%) were from male patients (mean age 32.6 years; range <1–83 years) and 1,247 (19.3%) were from female patients (mean age 27.9 years; range <1–83 years). Source specimens included urethral (n = 2,320), rectal (n = 981), pharyngeal (n = 592),

cervical (n = 365), vaginal (n = 154), and other sources (n = 85); sources for 2,231 isolates were not given. The sexual orientation of patients and information on cases of treatment failure were not available.

In 2010, a total of 98 (3.3%) of 2,970 isolates had decreased susceptibility to cefixime. This proportion increased to 4.2% (140/3,360) in 2011 and then decreased significantly to 1.1% (42/3,809; p<0.001) in 2014 (Figure). Similarly, decreased ceftriaxone susceptibility was 7.3% (218/2,970) in 2010 and declined to 2.7% (102/3,809; p<0.001) by 2014 (Figure).

Before 2010, the proportion of azithromycin resistance was ≤0.4% (data not shown). Azithromycin resistance increased to 1.2% (37/2,970) in 2010 and then decreased to 0.4% (13/3,360) in 2011. From 2011 to 2014, azithromycin resistance increased significantly to 3.3% (127/3,809; p<0.001). Thirty-eight isolates were identified as part of an outbreak in 1 province. When the outbreak-related isolates were excluded, 2.3% of all the isolates were azithromycin resistant in 2014, still a significant increase (p<0.001) from 2011. In 2014, azithromycin resistance was identified in 5 provinces across Canada. During 2009–2012 in Canada, 5 isolates with a high level of azithromycin resistance (MIC of azithromycin ≥256 mg/L) were identified.

In 2012, seven isolates with combined decreased susceptibility to cephalosporins and resistance to azithromycin were identified (0.2%, 7/3,036). In 2013, eight (0.3%, 8/3,195) and in 2014 one (0.03%, 1/3,809) of these isolates were identified. These isolates with both decreased susceptibility to cephalosporins and resistance to azithromycin may threaten the success of the currently recommended therapy.

From 2010 to 2014 in Canada, the proportion of *N. gonorrhoeae* isolates with decreased susceptibility to cephalosporins decreased significantly. The timing of the decrease corresponded to changes in treatment guidelines from monotherapy with third-generation cephalosporins to combination therapy with ceftriaxone/azithromycin. Although causality cannot be attributed to this decline, the higher dose of ceftriaxone plus azithromycin could be more effectively treating gonococcal infections and diminishing the spread of isolates with reduced cephalosporin susceptibility. Similar data have been reported from the United Kingdom and the United States. In 2011, the United Kingdom recommended dual antimicrobial therapy with ceftriaxone (500 mg intramuscularly) and azithromycin (1 g orally in a single dose) for treatment of uncomplicated gonococcal infection (*8*). Isolates with decreased susceptibility to cefixime (MIC ≥0.125 mg/L) declined significantly from 10.8% in 2011 to 5.7% in 2012 and then to 5.2% in 2013 after implementation of the new guidelines (*9*). In the United States in 2012, ceftriaxone (250 mg intramuscularly) combined with azithromycin (1 g orally) or doxycycline was the recommended therapy (*10*). Decreased susceptibility to cefixime (MIC ≥0.25 mg/L) declined from 1.4% in 2011 to 0.4% in 2013 and decreased susceptibility to ceftriaxone (MIC ≥0.125 mg/L) declined from 0.4% in 2011 to 0.05% in 2013 (*11*,*12*). Although the observed decline in decreased susceptibility to cephalosporins is encouraging, during 2010–2014 in Canada, the proportion of azithromycin-resistant isolates increased significantly, to 3.3%. This increase is alarming because it approaches the 5% level at which the World Health Organization recommends reviewing and modifying national guidelines for treatment of sexually transmitted infections (*2*). Azithromycin resistance levels in Canadian isolates were higher than that reported in the United States (0.5%, 0.3%, and 0.4% in 2010, 2011, 2012, respectively) (*12*) and the United Kingdom (0.8% in 2012 and 1.6% in 2013 [MIC≥1 mg/L]) (*9*) but similar to resistance levels in Australia (2.1% in 2013) (*13*).

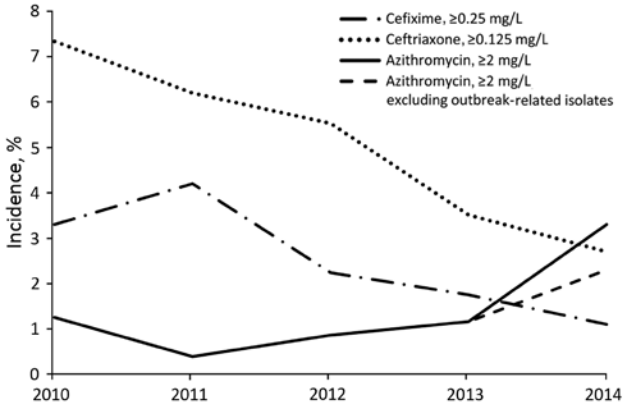

Figure. MICs for *Neisseria gonorrhoeae* isolates with decreased susceptibility to cefixime and ceftriaxone and resistance to azithromycin, Canada, 2010–2014. Percentages are based on the total number of isolates tested nationally per year: 2010 = 2,970; 2011 = 3,360; 2012 = 3,036; 2013 = 3,195; 2014 = 3,809.

Limitations of this study include the representativeness of isolates, because ≈70% of gonococcal infections in Canada are diagnosed by nucleic acid amplification tests (*14*), and the current passive surveillance system collects predominately resistant isolates from provinces with different susceptibility testing methods. The proportion of resistance could be higher than that indicated by our numbers.

Conclusions

Continued surveillance of gonococcal antimicrobial susceptibilities is vital to inform treatment guidelines and mitigate the spread of isolates with decreased susceptibility to cephalosporins and resistance to azithromycin. The data we present further support efforts to limit the spread of *N. gonorrhoeae* antimicrobial drug resistance and prevent the emergence of untreatable multidrug-resistant gonorrhea.

Dr. Martin is the head of the Streptococcus and STI Unit at the National Microbiology Laboratory, Public Health Agency of Canada. Her research interests focus on antimicrobial resistance mechanisms in *Neisseria gonorrhoeae* and the development of novel technologies to characterize these isolates.

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Rapid Emergence and Clonal Dissemination of CTX-M-15–Producing *Salmonella enterica* Serotype Virchow, South Korea

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The prevalence of cefotaxime-resistant *Salmonella enterica* serotype Virchow has dramatically increased in South Korea since the first isolation in 2011. Of 68 isolates collected over 10 years, 28 cefotaxime-resistant isolates harbored the $bla_{\text{CTX-M-15}}$ extended-spectrum β-lactamase gene and were closely related genetically, demonstrating the clonal dissemination of CTX-M-15–producing *Salmonella* Virchow in South Korea.

Nontyphoidal *salmonella*, a foodborne pathogen, causes human gastroenteritis worldwide. Among >2,500 different *Salmonella enterica* serotypes, *Salmonella Enteritidis* and *Salmonella Typhimurium* are the most common serotypes responsible for human salmonellosis (*1*). In Europe, *Salmonella* Virchow has recently increased in prevalence, and a high proportion of isolated strains are resistant to multiple antimicrobial drugs (*2*–*4*).

Third-generation cephalosporins are widely used to treat major bacterial infections in humans and animals (*5*). However, the emergence and rapid spread of drug-resistant bacteria has become a serious public health concern. Extended-spectrum β-lactamases (ESBLs) are known to confer antimicrobial drug resistance by hydrolyzing most β-lactam antimicrobial drugs, including third-generation cephalosporins (*5*). Since the first report from Spain in 2000 of strains producing CTX-M-9 (*6*), which confers resistance to cefotaxime, various CTX-M–type ESBLs have been identified in *Salmonella* Virchow. In Spain, Belgium, and France, CTX-M-9 and CTX-M-2 producers spread clonally in humans and poultry (*7*,*8*). In addition, the $bla_{\text{CTX-M-15}}$ gene was identified in porcine isolates in South Korea (*9*). These reports demonstrate that CTX-M–producing *Salmonella* Virchow clones can be easily transmitted to humans through food products of animal origin. In South Korea, the incidence of *Salmonella* Virchow infections in humans has increased over the years, necessitating a nationwide survey of antimicrobial drug resistance in *Salmonella* Virchow isolates.

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The Study

During 2005–2014 in South Korea, local public health laboratories participating in the national surveillance network isolated 68 *Salmonella* Virchow strains from feces samples from patients with acute diarrhea. Until 2010, <5 *Salmonella* Virchow strains were isolated per year, but this number gradually increased to 17 in 2014 (Figure). *Salmonella* Virchow consistently ranked among the top 10 serotypes in prevalence during each study year in South Korea, accounting for ≈1.5%–2% of salmonellosis cases.

We used the broth microdilution method (*10*) to perform antimicrobial susceptibility testing of *Salmonella* Virchow; results showed that 54 (79.4%) of the 68 isolates were resistant to ≥1 of the 15 antimicrobial agents tested (Table; online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/1/16-1220-Techapp1.pdf). The highest resistance rates were noted for nalidixic acid (77.9%), followed by ampicillin (44.1%), cefotaxime (44.1%), tetracycline (42.6%), and gentamicin (23.5%). Only 2 (2.9%) isolates were resistant to ciprofloxacin, but 50 (73.5%) had intermediate resistance. All of the isolates were susceptible to chloramphenicol, amikacin, or imipenem. Multidrug resistance, defined as resistance to at least 3 different classes of antimicrobial agents, was found in 30 (44.1%) isolates (Table).

All multidrug-resistant isolates showed resistance to third-generation cephalosporins. In South Korea, cefotaxime-resistant strains were first isolated in 2011. Since then, 4, 9, and 14 isolates were collected in 2012, 2013, and 2014, respectively (Figure). The rates of cefotaxime resistance in *Salmonella* Virchow have increased markedly, from 21.4% in 2011 to 82.3% in 2014. This annual trend of increasing cefotaxime resistance in South Korea is of interest because the rates were substantially higher than those reported in Spain during 2002–2006 (15%) (*11*) and Belgium during 2009–2013 (<10%) (*12*). Moreover, even in Israel and Switzerland, where the incidence of *Salmonella* Virchow was relatively higher than that in South Korea, antimicrobial drug resistance to third-generation cephalosporins was rare (*3*,*4*).

Among the 30 cefotaxime-resistant *Salmonella* Virchow isolates, 28 were confirmed to be ESBL-producers.

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

[2]Members of the PulseNet Korea Working Group are listed at the end of the article.

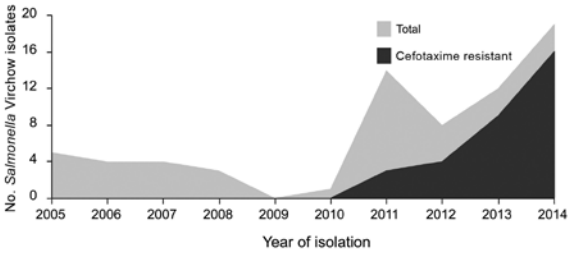

Figure. Temporal distribution of *Salmonella enterica* serotype Virchow isolates in South Korea, 2005–2014.

PCR and sequencing of β-lactamase genes (*13*) confirmed that these 28 isolates harbored a $bla_{\text{CTX-M-15}}$ gene, whereas the other 2 contained a $bla_{\text{CMY-2}}$ gene. Cefotaxime resistance was transferred by conjugation from 9 *Salmonella* Virchow isolates to *Escherichia coli* J53 recipients, and the $bla_{\text{CTX-M-15}}$ gene was identified in transconjugants. Southern blotting and PCR-based replicon typing (*14*) showed that all plasmids in transconjugants were ≈215 kb in size and possessed an IncHI2 plasmid, which was further assigned to sequence type (ST) 2 by plasmid double locus sequence typing (*15*). The analysis of the genetic environment surrounding the $bla_{\text{CTX-M-15}}$ gene (*13*) in transconjugants showed that insertion sequences IS*Ecp1* and o*rf477* were detected 48 bp upstream and downstream of the $bla_{\text{CTX-M-15}}$ gene, respectively. This IS*Ecp1*-$bla_{\text{CTX-M-15}}$–open reading frame 477 transposable unit was also identified in other incompatibility groups of the plasmids in *Enterobacteriaceae*. Furthermore, it was identical to that of the ST2-IncHI2 plasmid of *Salmonella* Enteritidis isolated from humans and poultry meat in South Korea (J. Kim, unpub. data), suggesting that the $bla_{\text{CTX-M-15}}$ gene in *Salmonella* Virchow might have originated from IS*Ecp1*-mediated transposition followed by interspecies spread through the IncHI2-type plasmid studied here.

All of the CTX-M-15–producing strains had reduced ciprofloxacin susceptibility (MICs of 0.25–0.5 μg/mL). All 10 randomly selected isolates harbored a single substitution within the quinolone resistance–determining region of GyrA at codon 83 (Ser→Phe), which is the major mutation described in *Salmonella* species (*8*). Because fluoroquinolones and third-generation cephalosporins are the drugs of choice for treating severe *salmonella* infections in humans, the reduced ciprofloxacin susceptibility in cefotaxime-resistant *Salmonella* Virchow is considered a critical risk factor for infections with these strains.

The genetic relationship of the 68 *Salmonella* Virchow isolates was determined by using multilocus sequence typing (http://mlst.warwick.ac.uk/mlst/dbs/Senterica) and pulsed-field gel electrophoresis (PFGE) according to a standardized protocol. Seven multilocus sequence typing loci displayed 4 different profiles, and most isolates belonged to sequence type (ST) 16 (n = 59), followed by ST197 (n = 6), ST359 (n = 2), and ST426 (n = 1). All of the 28 CTX-M-15–producing strains were typed as ST16, but the cefotaxime-susceptible isolates were also assigned to this type. The PFGE analysis yielded sufficient discriminatory power in typing *Salmonella* Virchow isolates; 22 *Xba*I and 21 *Bln*I PFGE patterns were generated. Although the isolates shared ≥70% similarity, CTX-M-15–producing strains clustered on the basis of a similarity value of 90% (online Technical Appendix Figure), indicating the clonality of cefotaxime-resistant strains.

For humans, the main route of *Salmonella* infection is the consumption of contaminated food of animal origin, and *Salmonella* Virchow is one of the most prevalent serotypes identified in poultry and poultry products. The use of cephalosporins in animal production has led to emergence of antimicrobial drug–resistant *Salmonella* Virchow clones among food animals (*8*), posing a threat to public health because of the possible transmission of these bacteria through the food chain. In fact, the *Xba*I PFGE pattern identified among human isolates in this study was identical to that in 2 cefotaxime-resistant *Salmonella* Virchow strains isolated in 2012 from poultry meat in our collection (online Technical Appendix Figure). Furthermore, this pattern appeared similar to the patterns of *Salmonella* Virchow harboring the $bla_{\text{CTX-M-15}}$ gene on an ST2-IncHI2 plasmid that was isolated from organically raised pigs in South Korea (*9*). Resistant clinical strains were collected from 13 of 15 local public health laboratories in South Korea during 2011–2014; thus, although the mode of the spread of *Salmonella* Virchow in humans is not established, it has been postulated that CTX-M-15–producing *Salmonella* Virchow might have disseminated clonally through the nationwide distribution of contaminated food products rather than through independent emergence.

Table. Antimicrobial resistance profiles of *Salmonella enterica* serotype Virchow isolates in South Korea, 2005–2014*

Isolate no.	Antimicrobial drug	No. resistant isolates
1	–	14
2	NAL	18
3	TCY	2
4	NAL, SXT	2
5	TCY, NAL	2
6	AMP, CEF, CTX, NAL	5
7	AMP, CEF, CTX, NAL, TCY	7
8	AMP, CEF, CTX, GEN, NAL	1
9	AMP, CEF, CTX, GEN, NAL, TCY	15
10	AMC, AMP, CEF, CTX, FOX, SAM, NAL	1
11	AMC, AMP, CEF, CTX, FOX, SAM, NAL, TCY	1
Total		68

*AMC, amoxicillin/clavulanic acid; AMP, ampicillin; CEF, cephalothin; CTX, cefotaxime; FOX, cefoxitin; GEN, gentamicin; NAL, nalidixic acid; SAM, ampicillin/sulbactam; SXT, trimethoprim/sulfamethoxazole; TCY, tetracycline; –, pansusceptible.

Conclusions

We analyzed the antimicrobial drug susceptibility and genetic relatedness of *Salmonella* Virchow isolates from patients with diarrhea in South Korea. Of 68 isolates obtained during 2005–2014, a total of 30 were resistant to third-generation cephalosporins. The prevalence rate of the resistant strains has dramatically increased since the isolation of cefotaxime-resistant strains in 2011. These findings suggest that the cefotaxime-resistant isolates are genetically closely related and harbor a plasmid carrying the $bla_{\text{CTX-M-15}}$ gene of the same compatibility group (ST2-IncHI2), representing clonal dissemination of CTX-M-15–producing *Salmonella* Virchow in South Korea and posing an urgent threat to public health. Therefore, more comprehensive surveillance is required to prevent further spread of resistant clonal strains.

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Avian Influenza A(H7N9) Virus Infection in 2 Travelers Returning from China to Canada, January 2015[1]

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In January 2015, British Columbia, Canada, reported avian influenza A(H7N9) virus infection in 2 travelers returning from China who sought outpatient care for typical influenza-like illness. There was no further spread, but serosurvey findings showed broad population susceptibility to H7N9 virus. Travel history and timely notification are critical to emerging pathogen detection and response.

Since February 2013, >600 human cases of avian influenza A(H7N9) infection have been reported from eastern China, where the virus is considered enzootic in poultry (*1*). Travel-associated cases have previously been reported in Asia (*2*); however, in January 2015, Canada reported 2 travel-associated cases, which are described here.

The Study

A married couple, both 56 years of age, from British Columbia (BC), Canada, traveled in Hong Kong (December 29, 2014–January 3, 2015, and January 6–7); Taipei, Taiwan (January 3–6); and Fujian Province, China (January 7–11), returning home on January 12. They recalled seeing live poultry and copious droppings while visiting Fujian on January 8 but recollected no other poultry contact.

Around January 3–7, the previously healthy woman experienced mild cough, sore throat, and hoarseness. She recovered, but influenza-like illness (ILI), including fever, cough, myalgia, and fatigue, developed on January 14. On January 15, she sought outpatient care. A healthcare worker (HCW) collected a nasal swab specimen, which was sent to the BC Public Health Microbiology and Reference Laboratory (BC-PHMRL), where most influenza testing is centralized in BC. On January 16, the sample tested positive for influenza A virus by reverse transcription PCR (RT-PCR), and the HCW was informed; the next business day (January 19), the patient was prescribed oseltamivir.

For surveillance purposes, BC-PHMRL conducts subtyping of all detected influenza A viruses. Despite a high virus titer (cycle threshold [C_t] 23.43), the specimen could not be subtyped for human influenza H1 or H3 virus by matrix gene–based RT-PCR. Further subtyping using RT-PCR–based targets for the hemagglutinin gene indicated an H7 virus. Sequence analysis of a matrix gene fragment showed 99% identity with H7N9 and H9N2 viruses, the latter of which is known to have donated internal genes to H7N9 virus (*1,3*). On January 23, BC-PHMRL notified public health authorities of a presumptive diagnosis of H7N9 virus infection in the woman (index case), and on January 26, Canada's National Microbiology Laboratory (NML) confirmed the diagnosis by RT-PCR.

On January 13, a day before the woman became ill, her husband, who had a history of asthma, had onset of ILI symptoms (fever, productive cough, chest pain, dyspnea, headache, myalgia, and fatigue) and visited the same HCW. The HCW prescribed doxycycline but did not collect a specimen. On January 19, after his wife received a diagnosis of influenza, the man was prescribed oseltamivir. A throat swab specimen collected from him on January 23 was RT-PCR–positive for influenza A (C_t 29.79). On January 29, NML confirmed H7N9 virus infection.

Neither patient experienced conjunctivitis, which has been reported with H7N7 and H7N3 infections (*3*,*4*), and both recovered in self-isolation at home. Follow-up respiratory specimens were still positive by RT-PCR on January 26 (day 12 after ILI onset for the woman [C_t 36.82]; day 13 for the man [C_t 33.80]) and on January 28 (day 15 after ILI onset) for the man (C_t 30.21).

The HCW remained asymptomatic; however, because of the patients' travel history, the HCW began oseltamivir prophylaxis after learning the index patient had laboratory-confirmed influenza. Throat swab specimens collected from the HCW on January 26 were influenza RT-PCR–negative. Approximately 20 other close contacts of the patients were placed under 10-day surveillance (from last exposure), including 1 who received oseltamivir prophylaxis.

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[1]Preliminary results from the population serosurvey were presented at the CACMID-AMMI Canada 2014 Annual Conference, April 3–5, 2014, Victoria, British Columbia, Canada.

All contacts remained asymptomatic. Passengers on the flight taken by the patients while they were asymptomatic were not included in active surveillance because >10 days had elapsed (*5*); however, media communications included flight details and public health advice.

Virus cultures in Madin-Darby canine kidney cells were attempted with all patient samples; only the woman's January 15 sample was culture-positive for influenza virus (A/British Columbia/1/2015[H7N9]). Phylogenetic analysis of the hemagglutinin and neuraminidase genes showed that the strain clustered with 2014 and 2015 H7N9 human isolates from Jiangsu, Zhejiang, and Fujian Provinces, China, and 2014 chicken isolates from Jiangxi, China, belonging to clade W2-C (*1*). Similar to genomes of previous human H7N9 isolates, the genome of the patient's isolate showed clinically relevant markers: substitutions G186V (H3 numbering), Q226L, and T160L in the hemagglutinin for increased human receptor affinity; substitution E627K in polymerase basic 2 for mammalian replication; substitutions S31N and V27I in matrix 2 for amantadine resistance; deletion in the neuraminidase stalk (positions 69–73); and neuraminidase inhibitor susceptibility (*3*).

Antibody titers to H7N9 and recent human influenza A(H3N2) and A(H1N1)pdm09 strains in paired serum samples from the case-patients and HCW were measured by hemagglutination inhibition assay at NML (Table 1) (*6*). At ≈7 weeks after ILI onset, the case-patients showed seroconversion (≥4-fold antibody rise) to H7N9 virus; the HCW had no detectable H7N9 antibody (Table 1).

As part of population risk assessment, we also previously measured hemagglutination inhibition antibody titers to H7N9 virus in anonymized residual serum samples collected and banked from patients attending community-based laboratory test sites in 2010 (n = 1,116; ≈100 samples/10-year age group) (*7*) and 2013 (n = 496; ≈50 samples/10-year age group) (*8*) across the same BC region to which the couple returned. The assessment was conducted as described (*7,8*) and approved by the University of British Columbia Research Ethics Board. Results showed broad population serosusceptibility to H7N9 virus: 5% (10/201) of serum samples collected from patients ≥60 years of age in 2013 had low-level titers (≥10 but <40), but no other samples had detectable H7N9 virus antibody (Table 2). Three samples with titers ≥20 showed titers <10 by microneutralization assay (Table 2).

Conclusions

The onset of ILI in 2 BC patients 5–6 days after observing poultry in China is consistent with the median 5-day incubation period reported elsewhere for H7N9 virus (*9*) and with common-source acquisition of the virus in Fujian. However, we cannot rule out other unrecognized exposures or person-to-person transmission between the couple. Continued RT-PCR detection in respiratory specimens 2 weeks after ILI onset has been documented (*9*) but does not necessarily signify ongoing shedding of infectious virus. Although previous case series have reported inconsistent antibody responses to H7N9 virus, often with low avidity and potentially correlated with clinical outcome (*10–12*), both BC case-patients with typical outpatient ILI demonstrated seroconversion.

Reported human cases of H7N9 infection have mostly been in older men, and two thirds have been categorized as severe (*2,3,9*). Mild illness has occasionally

Table 1. Antibody titers to avian influenza A(H7N9) virus and recent human influenza A(H3N2) and A(H1N1)pdm09 virus strains for 2 persons with virologically confirmed H7N9 virus infection and for an HCW contact, British Columbia, Canada, January 2015*

Person, date of specimen collection	Duplicate inverse HI titers and GMT for influenza antibody								
	A/British Columbia/1/2015(H7N9)†			A/Switzerland/9715293/2013(H3N2)‡§			A/California/07/2009(H1N1)‡¶		
	Titer 1	Titer 2	GMT	Titer 1	Titer 2	GMT	Titer 1	Titer 2	GMT
Index patient#									
January 26	20	20	20	10	10	10	10	10	10
March 5	80	160	113	10	10	10	10	10	10
Second patient#									
January 26	20	40	28	10	10	10	10	10	10
March 5	160	160	160	20	20	20	20	20	20
HCW contact**									
January 27	<10	<10	<10	40	40	40	20	20	20
May 1	<10	<10	<10	40	40	40	20	20	20

*The 2 H7N9 virus–infected persons were a married couple; the woman was the index patient, and the man was the second patient. GMT, geometric mean titer; HCW, healthcare worker; HI, hemagglutination inhibition.
†Assay was conducted by using homologous H7N9 virus isolated from the index patient (Global Initiative on Sharing Avian Influenza Data accession no. EPI_ISL_171342); the virus was antigenically equivalent to influenza A/Anhui/1/2013(H7N9), which was used in the population serosurvey reported in Table 2. The HI assay was conducted by using horse erythrocytes, as previously described (*6*).
‡Assay was conducted by using viruses of each human influenza A H1 and H3 subtype to which strains identified globally during the 2014–15 influenza season were considered antigenically related (see http://www.who.int/influenza/vaccines/virus/recommendations/2015_16_north/en/). Titers were measured according to standard assay protocols of the National Microbiology Laboratory, Canada's influenza reference laboratory.
§Assay was conducted by using guinea pig erythrocytes and in the presence of oseltamivir carboxylate to address potential neuraminidase-mediated binding of influenza A(H3N2) viruses to erythrocytes.
¶Assay was conducted by using turkey erythrocytes.
#Received neither the 2013–14 nor 2014–15 influenza vaccine nor prior pneumococcal vaccine.
**Received the 2013–14 and the 2014–15 influenza vaccines.

Table 2. Antibody titers to influenza A/Anhui/1/2013(H7N9) in an anonymized population serosurvey, Lower Mainland, British Columbia, Canada, May 2013*

Age group, y	No. patients†	Median age, y	% Female	Mean GMT (95% CI)‡	No. (%, 95% CI)§	
					With titer ≥10	With titer ≥20
<10	49	4	47	5	0	0
10–19	48	16	67	5	0	0
20–29	49	27	69	5	0	0
30–39	50	34	90	5	0	0
40–49	50	46	60	5	0	0
50–59	49	55	49	5	0	0
60–69	50	65.5	56	5.3 (4.9–5.7)	3 (6.0, 0.0–12.6)	1 (2.0, 0–5.9)¶
70–79	50	75	42	5	0	0
80–89	50	83	40	5.4 (5–5.9)	4 (8.0, 0.5–15.5)	1 (2.0, 0–5.9)¶
≥90	51	92	73	5.3 (4.9–5.6)	3 (5.9, 0.0–12.4)	1 (2.0, 0–5.8)¶
All	496	50	59	5.1 (5–5.2)	10 (0.9, 0.2–1.7)#	3 (0.3, –0.1 to 0.7)#

*Titers were measured by hemagglutination inhibition assay by using horse erythrocytes as previously described (*6*); assays were conducted at the National Microbiology Laboratory, Canada's influenza reference laboratory in July 2013. GMT, geometric mean titer.
†Serum samples were collected in May 2013; 5 samples had insufficient serum and were excluded from the analyses (*8*).
‡Titers were assessed in duplicate. Titers <10 were assigned a value of 5. GMT of duplicate titers derived as individual titers and group GMTs derived by age and overall.
§No patients had a titer ≥40.
¶Further assessed by microneutralization (MN) assay, according to procedures described by the Centers for Disease Control and Prevention (Atlanta, GA, USA); available by request. MN titers for all 3 samples were <10.
#Age-standardized (direct method) to the 2013 Fraser Valley and Greater Vancouver, British Columbia, Canada, population projections (BC Stats, 2013: http://www.bcstats.gov.bc.ca/StatisticsBySubject/Demography/PopulationProjections.aspx).

been reported in children (*2,13,14*), but, as exemplified by the BC cases, adults can also experience milder infection. Imported cases of novel influenza are less likely to be recognized if they are mild. In that regard, identification of H7N9 virus in an outpatient setting was adventitious. Travel history triggered specimen collection by the HCW, and identification of nonsubtypeable influenza by the provincial laboratory prompted further investigation and public health notification.

Despite broad susceptibility and instances of household or familial transmission, H7N9 virus has not demonstrated easy person-to-person spread. Poultry exposure remains the major risk factor for human H7N9 infection (*2,3,9*). Primary prevention messages should emphasize to travelers that they avoid exposure to poultry and uncooked poultry products while visiting affected areas. As illustrated by a prior imported case of avian influenza A(H5N1) virus to Alberta, Canada, however, such exposures may not always be recognized or avoidable (*15*). Screening should therefore begin with travel history in the 2 weeks before onset of acute respiratory illness. Patients should be encouraged to volunteer recent travel histories, and HCWs should elicit information regarding travel to affected areas. Public health and laboratory partners should be notified of suspect cases, as appropriate, during the diagnostic work-up, so that emerging pathogen screening, risk assessment, and advice can be guided in a timely manner.

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Surveillance of Bacterial Meningitis, Ethiopia, 2012–2013

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Among 139 patients with suspected bacterial meningitis in Ethiopia, 2012–2013, meningococci (19.4%) and pneumococci (12.9%) were the major disease-causing organisms. Meningococcal serogroups detected were A (n = 11), W (n = 7), C (n = 1), and X (n = 1). Affordable, multivalent meningitis vaccines for the African meningitis belt are urgently needed.

Ethiopia has the second-largest population (≈94 million in 2013) among the meningitis belt countries of sub-Saharan Africa (*1*). However, during 2001–2010, a median of only 1,056 suspected meningitis cases per year (range 5–8,571/year) was reported to the World Health Organization (*2*). The largest meningitis epidemics occurred in 1981 (*3*) and 1989 (*4*), resulting in ≈45,000 and ≈50,000 cases, respectively. Serogroup A meningococci were the major cause of these epidemics, although serogroup C strains were also identified in 1981, 1983–84, and during outbreaks in 2000 and 2003 (*5*). Conjugate vaccines against *Haemophilus influenzae* serotype b, *Streptococcus pneumoniae* (pneumococcal conjugate vaccine [PCV] 10), and *Neisseria meningitidis* serogroup A (MenAfriVac) were introduced in 2007, 2011, and 2013–2015, respectively. Because data permitting assessment of these vaccines are limited, we implemented a surveillance study.

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The Study

Patients with symptoms of meningitis admitted to 3 referral teaching university hospitals in Ethiopia (Hawassa Referral Hospital [Southern Nations, Nationalities and Peoples Region], Tikur Anbessa Referral Hospital [Addis Ababa], and Gondar University Hospital [Amhara region]) (Figure 1) during February 2012–June 2013 received a lumbar puncture as part of routine diagnostic procedures. If cerebrospinal fluid (CSF) was turbid, the patient was included in the study. The study was approved by ethical review committees in Norway (Regional Ethics Committee reference 2011/825b) and Ethiopia (National Research Ethics Review Committee reference 3-10/6/5-04).

Demographic and clinical data were recorded in predefined case record forms. CSF samples were inoculated into a transisolate medium vial and sent to Armauer Hansen Research Institute (AHRI) in Addis Ababa for bacteriologic verification by standard procedures (*6*).

Bacterial DNA was extracted by using the QiAmp DNA extraction kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Conventional multiplex PCR for species identification was performed with primers specific for amplifying the genes *porA* (*N. meningitidis*), *lytA* (*S. pneumoniae*), and *bexA* (*H. influenzae*) for all samples, and capsule genogrouping was carried out for *porA* PCR-positive samples (*7*,*8*). Transisolate samples were also analyzed by multiplex real-time PCR with primers targeting genes *ctrA* (*N. meningitidis*), *ompP* (*H. influenzae*), and *lytA* (*S. pneumoniae*). Real-time PCR reactions (3 parallel) were run on an ABI 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA); a cycle threshold (C_t) value of ≤40 indicated a positive result. If C_t was >35, the sample was retested. Samples positive for the *ctr*A gene were subjected to capsule genogrouping by singleplex real-time PCR for verification of *N. meningitidis* serogroups A, B, C, Y, W, and X (*9*). CSF samples positive for *N. meningitidis* were further tested in a nested *porA* PCR, followed by sequencing the *porA* gene to identify the 2 variable region peptide loops of the PorA protein (*5*). Multilocus sequence typing and ferric enterobacin transport genotyping were performed (*6*).

During the surveillance period, 139 patients met criteria for suspected bacterial meningitis and were included in our analysis (Table 1). Of these, 92 patients (66.2%) were admitted in Gondar, 27 in Hawassa (19.4%), and 20 in

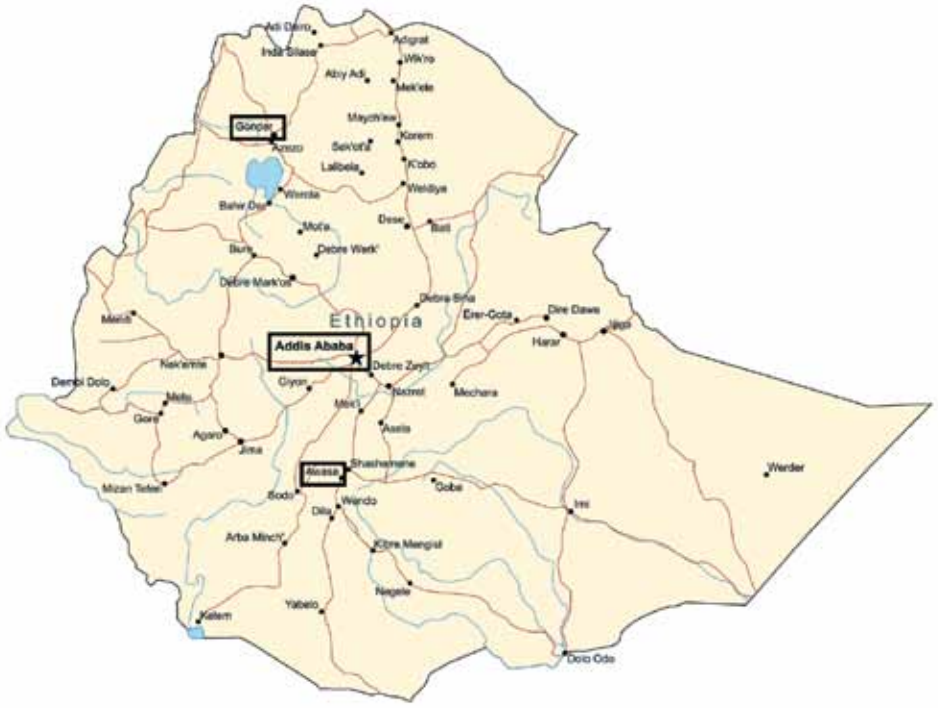

Figure 1. Locations (boxes) of the 3 meningitis surveillance study hospitals in Gondar, Addis Ababa, and Hawassa (also spelled Awasa or Awassa), Ethiopia. Air distances from Addis Ababa to Gondar and Hawassa are ≈420 km and 220 km, respectively. Modified with permission from http://www.MapResources.com.

Addis Ababa (14.4%). Culturing performed at AHRI identified a pathogen in 15 (10.8%) of the 139 patients: *N. meningitidis* (n = 4), *S. pneumoniae* (n = 9), and *H. influenzae* (n = 1). Conventional multiplex PCR performed at AHRI identified DNA from the same 3 pathogens in 18 (12.9%) CSF samples: *N. meningitidis* (n = 7), *S. pneumoniae* (n = 10), and *H. influenzae* (n = 1). By multiplex real-time PCR of the same CSF samples, etiologic agent could be verified in 46 (33.1%) samples: *N. meningitidis* (n = 27; 19.4%), *S. pneumoniae* (n = 18; 12.9%), and *H. influenzae* (n = 1; 0.7%). For the remaining 93 patients, an etiologic agent for the meningitis episode was not determined.

The proportion of CSF samples with etiologic agent identified by real-time PCR varied between sites, peaking in Hawassa with 19 (70.4%) of 27 samples, followed by Addis Ababa with 7 (35.0%) of 20 samples and Gondar with 20 (21.7%) of 92 samples (Table 2). Of 27 CSF samples positive for *N. meningitidis*, genogroup could be determined for 20 (Table 2). For the remaining 7 samples, genogroup could not be determined by real-time PCR because DNA concentration was low.

Serogroup distribution differed substantially by geographic region: W dominated in Gondar, A in Hawassa (Figure 2). One case of meningococcal disease caused by serogroup C and 1 caused by serogroup X meningococci were identified in Gondar and Hawassa, respectively. *PorA* genosubtyping results were available for 19 of the 27 CSF samples containing *N. meningitidis* DNA; both PorA variable regions were indicated for 15 samples. Samples from Hawassa were P1.20,9 (n = 7), P1.5–11,10–1 (n = 1), P1.5,_ (n = 1) and P1._,9 (n = 1), whereas those from Gondar were P1.5,2 (n = 7), P1._4 (n = 1) and P1._,2 (n = 1). Genotyping of the 4 meningococcal strains isolated showed that 1 was serogroup W, P1.5,2:F1–1:ST11, whereas 3 were serogroup A, P1.20,9:F3–1:ST7. The 3 pneumococcal isolates that were recovered for multilocus sequence typing were

Table 1. Age and sex distribution of 139 patients with suspected bacterial meningitis and breakdown of identified pathogen types, Ethiopia, 2012–2013

Patient characteristic	Total no. (%) cases	No. (%) *Neisseria meningitidis* infections, n = 28	No. (%) *Streptococcus pneumoniae* infections, n = 18
Age, y			
≤4	48 (34.5)	9 (33.3)	6 (33.3)
5–12	26 (18.7)	11 (40.7)	4 (22.2)
13–19	13 (9.4)	4 (14.8)	1 (5.6)
20–29	20 (14.4)	2 (7.4)	4 (22.2)
30–39	12 (8.6)	1 (3.7)	0
≥40	20 (14.4)	0	3 (16.7)
Sex			
M	83 (59.7)	17 (63.0)	10 (55.6)
F	56 (40.3)	10 (37.0)	8 (44.4)

Table 2. Organisms detected by real-time PCR in CSF samples from 139 meningitis patients, by location, Ethiopia, 2012–2013*

Location	*Neisseria meningitidis*† serogroup					*Streptococcus pneumoniae*‡	*Haemophilus influenzae*§
	A	C	W	X	NG		
Gondar	0	1	6	0	3	10	0
Hawassa	9	0	1	1	2	5	1
Addis Ababa	2	0	0	0	2	3	0
Total	11	1	7	1	7	18	1

*CSF, cerebrospinal fluid; NG; nongroupable.
†n = 27.
‡n = 18.
§n = 1.

sequence types (ST) 8875 (n = 2) and ST289 (n = 1), all from Gondar.

The case-fatality rate (CFR) for meningococcal patients was 11.1% (3/27), whereas that for pneumococcal patients was 16.7% (3/18). Among the total 20 fatal bacterial meningitis cases, 8 were reported from Hawassa; 2 were caused by pneumococci, 3 by meningococci (1 serogroup A, 1 nongroupable, and 1 W), and 1 by *H. influenzae*. Of the 2 fatal cases from Addis Ababa, 1 was caused by *S. pneumoniae.* No samples from fatal cases from Gondar were positive by real-time PCR. The proportion of meningococcal case-patients with serogroup A infection in the MenAfriVac target group (1–29 years of age) was 31.0% (9/29).

These case-based demographic data and laboratory-verified analyses of CSF samples from 139 bacterial meningitis patients in 3 hospitals in Ethiopia indicate baseline data before MenAfriVac vaccination. The dominance of serogroups W and A among the cases of known etiology in Gondar and Hawassa, respectively, suggests geographic variation in meningoccocal serogroup distribution in Ethiopia (Figure 2). The presence of serogroup W and X in Ethiopia is in line with trends in the rest of the meningitis belt (*10–12*) and may diminish the effects of the monovalent serogroup A conjugate vaccine on overall meningococcal disease incidence. Molecular typing showed that serogroup A meningococci isolated in Ethiopia in 2012–2013 were the same ST (ST7) as those causing the 2002–2003 outbreaks; both expressed PorA P1.20,9 (*4*,*5*). The serogroup W isolates were ST11 with PorA P1.5,2, the same found among outbreak strains in other meningitis belt countries (*11–13*).

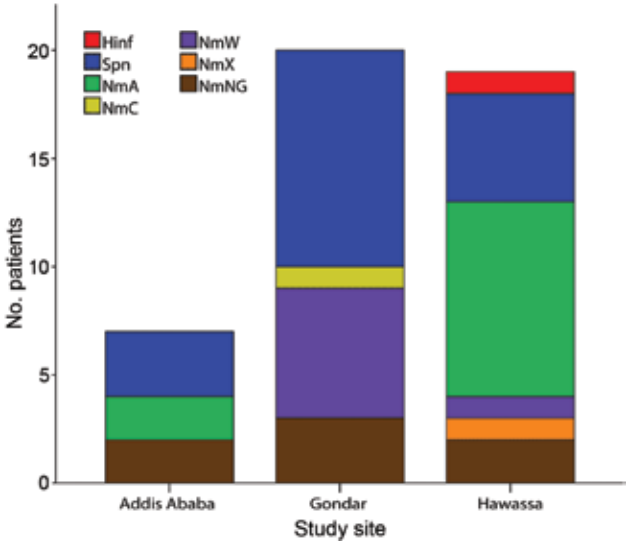

Figure 2. Distribution of causative organisms among 139 patients with clinical symptoms of bacterial meningitis in Ethiopia, 2012–2013, as verified by DNA from either meningococci (*Neisseria meningitidis,* serogroups A, NmA; C; NmC; X, NmX; ; W, NmW; and NG, not serogrouped as A, C, Y, W, or X), *Streptococcus pneumoniae* (Spn), or *Haemophilus influenzae* (Hinf) in cerebrospinal fluid.

Variation between sites and the overall low rates of etiologic agent identification may be explained by differences in interpreting meningococcal symptoms and CSF turbidity, as well as delay in transporting samples to the laboratory. CFR among meningococcal disease patients (11.1%) was comparable with that observed in other meningitis belt countries, whereas CFR among pneumococcal disease patients (16.7%) was lower than typically observed (≈50%) (*14*).

Conclusions

This study highlights the need for reinforcement of case-based, laboratory confirmed surveillance of bacterial meningitis in Ethiopia to enable mapping of distribution of causative organisms across the country and determine the potential effects of existing vaccines. The high proportion of serogroup W meningococci observed in northern Ethiopia is cause for concern, as is the presence of serogroup X. Recent outbreaks of meningitis caused by serogroup W in Burkina Faso and C in Nigeria (*15*) have been met with reactive vaccination campaigns with polysaccharide vaccines in areas where MenAfriVac has been implemented. This situation is suboptimal and calls for fast-tracking the development of affordable, multivalent conjugate vaccines against serogroups A, C, Y, W, and X meningococci (*10*).

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Identification of Source of *Brucella suis* Infection in Human by Using Whole-Genome Sequencing, United States and Tonga

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Brucella suis infection was diagnosed in a man from Tonga, Polynesia, who had butchered swine in Oregon, USA. Although the US commercial swine herd is designated brucellosis-free, exposure history suggested infection from commercial pigs. We used whole-genome sequencing to determine that the man was infected in Tonga, averting a field investigation.

In August 2013, a man in his 20s from Tonga, Polynesia, who had moved to the United States in June 2010, was examined in a hospital in Portland, Oregon, after experiencing 4 weeks of fever, night sweats, headache, productive cough, shortness of breath, and weight loss. He also reported pleuritic chest pain and abdominal pain radiating to his back. A computed tomography scan showed lung and liver abnormalities. Blood cultures grew *Brucella suis* biovar 1. After treatment with oral sulfamethoxazole (800 mg 3×/d), trimethoprim (160 mg 3×/d), and doxycycline (100 mg 1×/d); and intravenous gentamicin (580 mg 3×/d), the infection resolved. Because *Brucella* infection is a reportable condition in Oregon, the case was referred to the Oregon Health Authority Acute and Communicable Disease office, and authority personnel informed the Oregon Department of Agriculture's veterinary officials that the patient had routinely purchased pigs from a local farm for home slaughter, suggesting the patient may have contracted *B. suis* from commercial swine.

The US commercial swine herd is considered to be free of *B. suis*; however, *B. suis* is endemic among feral swine and occasionally has infected domestic swine (*1*,*2*). Slaughter surveillance, primarily by using the buffered acidified plate antigen test, is conducted routinely to identify such events to prevent the re-establishment of *B. suis* in the commercial swine herd and to protect workers.

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The Study

Serum from sows culled on the farm in question had been collected during routine slaughter surveillance. Two weakly positive results during the previous 3 years were investigated by following the guidelines in the USDA Swine Brucellosis Control/Eradication State-Federal-Industry Uniform Methods and Rules (*3*); no brucellosis was confirmed. Although feral swine reside in Oregon, none had recently been reported near the farm.

The case-patient's lack of exposure to feral swine and a known exposure to commercial swine required further investigation. An epidemiologic investigation to evaluate the commercial herd's infection status would require testing of swine on the premises and related farms, and tasks such as tracing sales from the herd and testing swine possibly exposed to swine brucellosis by temporary movement of boars to or from farms for breeding purposes. Such investigations can be costly, especially if there has been extensive movement of swine in and out of the herd.

Whole-genome sequencing (WGS) and single-nucleotide polymorphism (SNP) analyses can provide increased resolution to identify the source of infections without conducting more expensive field investigations (*4*). The National Veterinary Services Laboratories (NVSL) had implemented WGS and SNP analysis as the primary means of genotyping *B. abortus* and *Mycobacterium bovis* isolates and applied this information to identify sources of other outbreaks. Although a project to sequence *B. suis* isolates from animals of US origin was in process at the time of this investigation, few had been sequenced. To rapidly investigate the case in Oregon, laboratory staff from the Centers for Disease Control and Prevention extracted and provided DNA from the isolate recovered from the Oregon patient, and the NVSL sequenced 59 *B. suis* biovar 1 isolates recovered from US-origin animals. Oregon was declared free of swine brucellosis in 1987 (http://www.oregon.gov/ODA/programs/AnimalHealthFeedsLivestockID/AnimalDiseases/Pages/AnimalDiseases.aspx), and the NVSL did not have archived isolates from Oregon or surrounding states. Consequently, isolates selected for sequencing were mostly from the southern United States and had been re-

covered from domestic swine and cattle during 1993–2013 (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/21/1/15-0843-Techapp1.pdf). A few isolates were selected from dogs, horses, and humans, all of whom, based on data from epidemiologic investigations, likely had contact with feral swine.

To obtain the whole-genome sequences, we sequenced *B. suis* DNA on a MiSeq instrument (Illumina, San Diego, CA, USA) using 2×250 paired-end chemistry and the Nextera XT library preparation kit (Illumina), targeting 100× coverage. FASTQ files from the instrument were put through the NVSL in-house analysis pipeline (https://github.com/USDA-VS). Reads were aligned to *B. suis* isolate 1330 (GenBank Reference Sequence accession nos. NC_017250 and NC_017251) by using BWA (*5*) and Samtools (*6*). We processed BAM files (*6*) by using the Genome Analysis

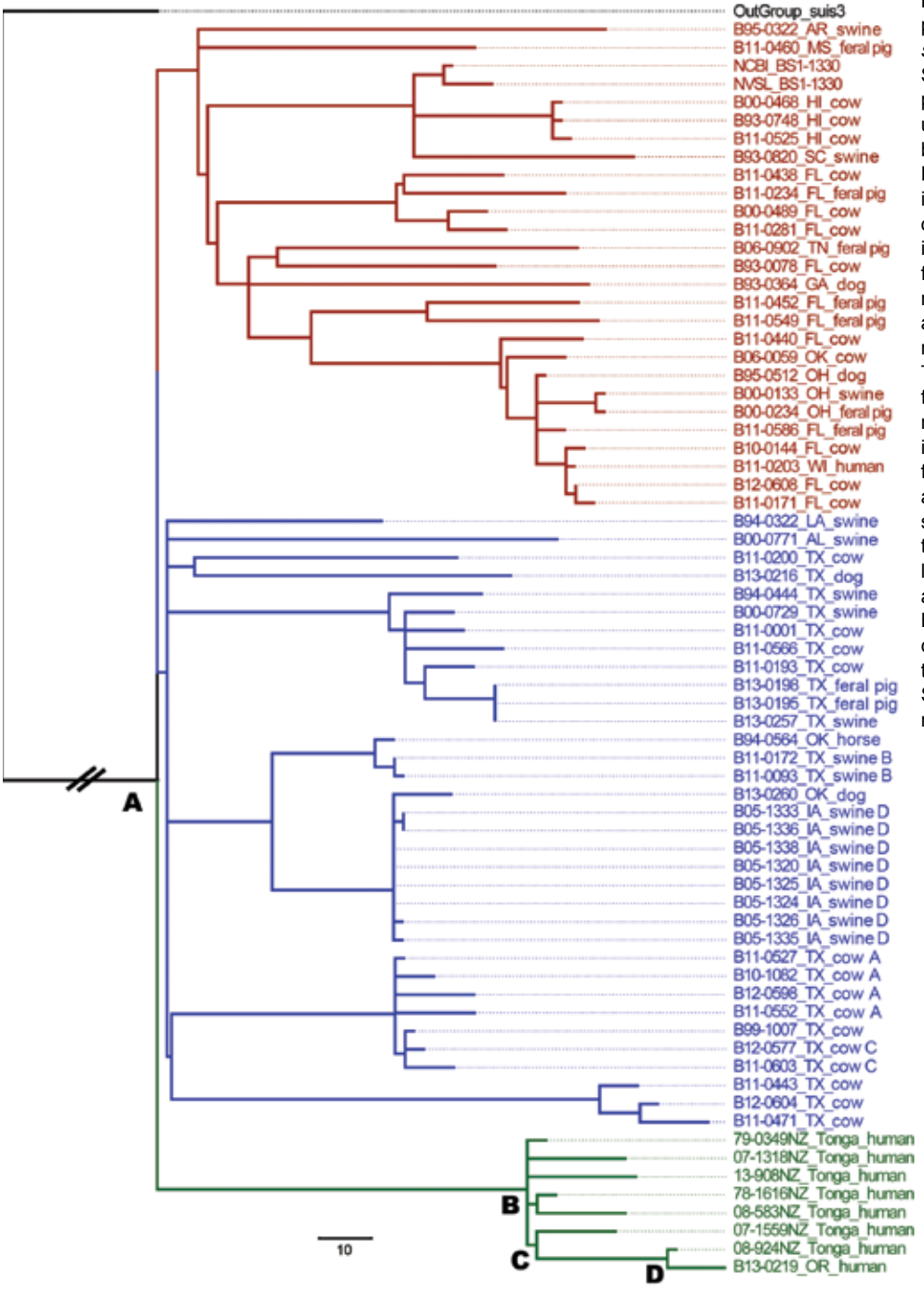

Figure. Maximum-likelihood phylogenetic tree of *Brucella suis* isolates from the United States and Tonga. The phylogenetic tree was rooted using a truncated *B. suis* biovar 3 isolate (black text). Red and blue text indicate 59 isolates recovered from US origin sources. Green text indicates the isolate recovered from the immigrant from Tonga residing in Oregon, B13-0219, and 7 additional isolates recovered from patients from Tonga in New Zealand. The first 2 digits of the sample number indicate the year isolated. Isolates recovered from different animals within a herd are labeled with the same letter designation after the species information. The letter A designates the common ancestor between all isolates; B, C, and D identify the common ancestors between the Tonga and Oregon isolates. Scale bar indicates 10 single-nucleotide polymorphisms.

Toolkit best-practice workflow (*7*). SNPs were called by using the UnifiedGenotyper from the toolkit, outputting SNP to variant call files (*7*–*9*). Results were filtered by using a minimum Phred (http://www.1000genomes.org/node/101) quality score (scaled probability of SNP presence) of 300 and allele count of 2. From the variant call files, SNPs gathered were output in 3 formats: an aligned FASTA file (http://www.ncbi.nlm.nih.gov/BLAST/blastcgihelp.shtml); a tab-delimited file with the position location and SNPs grouped and sorted; and a phylogenetic tree created by using RAxML (*10*). We visually validated SNPs using the Integrative Genomics Viewer (*11*). Sequencing files were deposited in the National Center for Biotechnology Information Sequence Read Archive (SRA) under the Bioproject PRJNA251693 (http://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA251693; online Technical Appendix 1).

Initially, as evidenced by the 60 isolates described, the United States could not be ruled out as a source because of a lack of resolution. Two approaches were considered to improve the resolution of the *B. suis* database: sequence enough isolates originating in the United States to assess the likelihood that any US-origin isolate would closely match, or sequence isolates originating from Tonga and determine whether they were clustered with the isolate from the case-patient in Oregon. The difficulty in obtaining isolates from representative feral swine throughout the United States precluded the first option as a viable solution. To obtain isolates from Tonga, we contacted the Ministry of Primary Industries in New Zealand for assistance; its staff members provided DNA from 7 *B. suis* isolates recovered from patients who were from Tonga. New Zealand is not known to have *B. suis* in its feral or commercial swine populations; therefore, humans with diagnoses of *B. suis* had likely been infected in another country.

We constructed a maximum-likelihood phylogenetic tree comprising the Oregon human isolate, the 59 field isolates from the United States, and the 7 isolates from New Zealand recovered from patients from Tonga (Figure). The branch labeled as OutGroup_suis3 roots the phylogenetic tree, and the A node is the most recent common ancestor (MRCA) for all isolates. Three lineages evolved from the MRCA. Initially, the Oregon human isolate was the only representative in its lineage. Without the perspective of the Tonga isolates, a US source for this isolate could not be ruled out because it shared the same MRCA as 2 other lineages occurring within the United States. All additional Tonga–origin isolates clustered tightly with the Oregon human isolate share a common ancestor at the B node. The Oregon human isolate is anchored by 2 additional common ancestors: C and D. In addition to the phylogenetic tree, a table displaying divergent SNPs of closely related isolates, including nucleotide calls and the positions within the genome, was created for transparency and clarity (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/21/1/15-0843-Techapp2.pdf). Often, 1 or 2 SNP calls inform the epidemiology of a case. For example, the 08-924 isolate recovered from a patient in Tonga in 2008 has 1 additional SNP (a thymine at position 1809039 on chromosome 2) from sharing a common ancestor with the Oregon isolate.

Conclusions

WGS and SNP analysis effectively concluded that this case-patient was infected in Tonga and not by swine in the United States. Thus, widespread testing of domestic swine was not conducted; agricultural trade continued without restrictions, and postexposure treatment of contacts participating in home slaughter or meat preparation was not needed. This case also demonstrates the value of and need for an international database of validated WGS isolates that can be used by both human and animal health officials in their respective and collaborative epidemiologic investigations. Finally, this case highlights the benefits of a One Health (http://onehealthinitiative.com/) approach between public and animal health, including state, federal, and international authorities.

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Porcine Epidemic Diarrhea Virus and Discovery of a Recombinant Swine Enteric Coronavirus, Italy

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Porcine epidemic diarrhea virus (PEDV) has been detected sporadically in Italy since the 1990s. We report the phylogenetic relationship of swine enteric coronaviruses collected in Italy during 2007–2014 and identify a drastic shift in PEDV strain variability and a new swine enteric coronavirus generated by recombination of transmissible gastroenteritis virus and PEDV.

Porcine epidemic diarrhea virus (PEDV) and *Transmissible gastroenteritis virus* (TGEV) (family Coronaviridae, genus *Alphacoronavirus*) are enveloped viruses that contain a single-stranded, positive-sense RNA genome of ≈28 kb. Infection with these viruses causes watery diarrhea, dehydration, and a high mortality rate among suckling pigs. Coronaviruses (CoVs) are prone to genetic evolution through accumulation of point mutations and homologous recombination among members of the same genus (*1*). Porcine respiratory coronavirus (PRCV), a mutant of TGEV, appeared in pigs in the 1980s (*2*). The spread of PRCV, which conserved most of the antigenic sites and causes cross-protection against TGEV (*3*), led to the gradual disappearance of TGEV. Newly emerging CoVs pose a potential threat to human and animal health because multiple human CoV infections have been derived from animal hosts. Emerging swine coronaviruses are of great concern to swine health because of the potential increase in viral pathogenesis.

In 1978, PEDV was first identified in Europe; subsequent reports occurred in many countries in Asia, including China, Japan, Korea, and Thailand. In 2010–2012, genetically different PEDV strains emerged, causing severe outbreaks in China (*4*). PEDV spread to the United States, Canada, and Mexico in 2013–2014, and genetically related strains were detected in South Korea and Taiwan (*5–7).* The PEDV outbreak caused large global economic losses to the swine industry. In Europe, a severe PEDV epidemic occurred in Italy during 2005–2006 (*8*), and in 2014–2015, PEDV was detected in Germany, France, and Belgium. These strains have a high nucleotide identity to PEDV strains that contain distinct insertions and deletions (INDELs) in the S gene (S-INDELs) from the United States (*9–11*). We report the detection and genetic characterization of swine enteric CoVs circulating in Italy during 2007–2014. We also report a recombinant TGEV and PEDV strain (identified as the species *Swine enteric coronavirus* [SeCoV]) circulating from June 2009 through 2012. Finally, we describe the phylogenetic relationship of the 2014 PEDV S-INDELs to the recent PEDV strains circulating in Europe.

The Study

During 2007–2014, we collected 27 fecal and 24 intestinal samples from pigs with suspected PEDV or TGEV infections; the pigs came from swine farms in northern Italy (Po Valley), which contains the regions of Piemonte, Lombardia, Emilia Romagna, and Veneto (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/22/1/15-0544-Techapp1.pdf). The Po Valley contains 70% of Italy's swine. Clinical signs included watery diarrhea in sows and a death rate in piglets of 5%–10%, lower than is typical with PEDV or TGEV infections. Samples were submitted for testing by electron microscopy, PEDV ELISA, viral isolation, pan-CoV reverse transcription PCR (RT-PCR), and RT-PCR for PRCV and TGEV; selected positive pan-CoV samples were sequenced (*12–14*) (online Technical Appendix).

Results of electron microscopy showed that 25 (49%) of the 51 samples contained CoV-like particles, but all samples were negative for viral isolation. Although only 38 samples (74%) were positive by pan-CoV RT-PCR, 47 (92%) were positive by the PEDV ELISA (Table 1) (*12,13*). Of the 38 pan-CoV–positive samples, 18 were selected for partial RNA-dependent RNA polymerase (RdRp), spike (S1) (*14*), and membrane (M) sequencing (Table 1). All samples were negative for PRCV and TGEV by RT-PCR, ruling out co-infection with PEDV and TGEV or PRCV (*15*).

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

[2]Deceased.

Table 1. Distribution of test results of samples from pig farms in study of swine enteric coronaviruses in northern Italy, 2007–2014*

Sample no.	Farm no.	Year	Region	EM	PEDV ELISA	Pan-CoV RT-PCR	TGEV/ PRCV S1	Sequences		
								RdRp	S1	M
222654	1	2007	Emilia Romagna	–	+	+	NA	NA	NA	NA
1448	2	2007	Emilia Romagna	–	+	–	NA	NA	NA	NA
19908	3	2007	Emilia Romagna	–	+	+	–	Cluster I	Cluster I	Cluster I
70323	4	2007	Lombardia	+	+	+	NA	NA	†	NA
114372	5	2007	Lombardia	+	+	+	NA	NA	NA	NA
200079	6	2007	Lombardia	–	+	+	NA	NA	†	NA
320855/5	7	2007	Lombardia	+	+	+	–	Cluster I	Cluster I	Cluster I
320855/6	7	2007	Lombardia	+	+	+	NA	†	†	NA
3936/1	8	2008	Lombardia	–	+	+	–	Cluster I	Cluster I	Cluster I
3936/2	8	2008	Lombardia	–	+	+	NA	†	NA	NA
29177	9	2008	Veneto	+	+	+	–	Cluster I	Cluster I	Cluster I
43853	10	2008	Lombardia	+	+	–	NA	NA	NA	NA
7239‡	11	2009	Lombardia	–	+	+	–	Cluster I	Cluster I	Cluster I
20001	12	2009	Lombardia	–	+	+	–	Cluster I	Cluster I	Cluster I
20416	13	2009	Lombardia	–	+	+	NA	†	†	NA
22603	14	2009	Lombardia	–	+	+	–	Cluster I	Cluster I	Cluster I
26199/2	15	2009	Lombardia	–	+	–	NA	NA	NA	NA
87565	16	2009	Emilia Romagna	–	+	+	NA	NA	†	NA
111357/7	17	2009	Lombardia	NA	+	–	NA	NA	NA	NA
137442	18	2009	Lombardia	+	+	+	–	Cluster II	Cluster II	Cluster II
205396	19	2009	Lombardia	+	+	–	NA	NA	NA	NA
208995	20	2009	Lombardia	+	–	+	NA	†	NA	NA
213306‡	21	2009	Lombardia	+	+	+	–	Cluster II	Cluster II	Cluster II
244945	22	2009	Emilia Romagna	+	+	–	NA	NA	NA	NA
245242	22	2009	Emilia Romagna	+	+	+	NA	†	NA	NA
274771	23	2009	Veneto	+	–	–	NA	NA	NA	NA
307121	24	2009	Emilia Romagna	+	+	+	–	Cluster II	Cluster II	Cluster II
315994	25	2009	Lombardia	+	–	–	NA	NA	NA	NA
320695	26	2009	Lombardia	+	+	+	NA	NA	†	†
320825	26	2009	Lombardia	+	+	+	–	Cluster II	Cluster II	Cluster II
324345	27	2009	Lombardia	+	+	+	NA	†	†	†
324374	27	2009	Lombardia	+	+	+	NA	†	†	†
324397	27	2009	Lombardia	+	+	+	–	Cluster II	Cluster II	Cluster II
324507/1	28	2010	Lombardia	+	+	+	–	Cluster II	Cluster II	Cluster II
324507/2	28	2010	Lombardia	+	+	+	NA	NA	†	†
324507/3	28	2010	Lombardia	+	+	+	NA	NA	NA	†
324507/4	28	2010	Lombardia	+	+	+	NA	NA	†	†
5448/2	29	2011	Emilia Romagna	NA	+	–	NA	NA	NA	NA
28607	30	2012	Lombardia	–	+	+	NA	NA	†	†
29742	30	2012	Lombardia	+	+	+	–	Cluster II	Cluster II	Cluster II
30917	31	2012	Lombardia	+	+	–	NA	NA	NA	NA
35621/1	32	2012	Lombardia	+	+	–	NA	NA	NA	NA
35621/2	32	2012	Lombardia	–	+	+	NA	NA	NA	NA
41906	33	2012	Lombardia	–	+	+	NA	NA	NA	NA
44833	34	2012	Lombardia	NA	+	+	–	Cluster II	Cluster II	Cluster II
67322	8	2012	Lombardia	–	+	+	NA	NA	NA	†
273992	35	2012	Lombardia	–	+	+	–	Cluster II	Cluster II	Cluster II
32961	36	2013	Piemonte	–	+	–	NA	NA	NA	NA
32963	36	2013	Piemonte	+	+	–	NA	NA	NA	NA
178509	37	2014	Emilia Romagna	NA	NA	+	–	Cluster III	Cluster III	Cluster III
200885	38	2014	Emilia Romagna	+	+	+	–	Cluster III	Cluster III	Cluster III

*Cluster I represents strains circulating from 2007 through mid-2009; cluster II represents strains circulating from mid-2009 through 2012; and cluster III represents strains circulating since 2014. EM, electron microscopy; M, membrane; pan-CoV RT-PCR, pan-coronavirus reverse transcription PCR; PEDV, porcine epidemic diarrhea virus; PRCV, porcine respiratory coronavirus; RdRp, RNA-dependent RNA polymerase; S1, spike; TGEV, transmissible gastroenteritis virus; +, positive; –, negative; NA, not tested or sequenced.
†Sequence available but not included in this study.
‡Samples selected for whole genome sequencing.

On the basis of the partial sequences from RdRp and the S1 and M genes, the strains from Italy clustered into 3 temporally divided groups, suggesting 3 independent virus entries. Cluster I represents strains circulating from 2007 through mid-2009; cluster II represents strains circulating from mid-2009 through 2012; and cluster III represents strains circulating since 2014 (online Technical Appendix Figure 2, panels A–C). Cluster I was identified in Emilia Romagna (n = 1), Lombardia (n = 5), and Veneto (n = 1). Cluster II was identified in Emilia Romagna (n = 1) and Lombardia (n = 8). Cluster III was identified in Emilia Romagna at 2 swine farms. To help explain the temporal clustering, a

single S1 gene segment was sequenced from clusters I and II (PEDV/Italy/7239/2009 and SeCoV/Italy/213306/2009, respectively). Because of the recent outbreak of PEDV in Europe, the 2 positive samples from cluster III (PEDV/Italy/178509/2014 and PEDV/Italy/200885/2014) were sequenced (Figure 1, panel A).

One strain from each cluster was selected for whole genome sequencing (online Technical Appendix). Unfortunately, the whole genome was obtained from only clusters I and II (PEDV/Italy/7239/2009 and SeCoV/Italy/213306/2009, respectively; Figure 1, panel B). Recombination analysis was conducted on the 2 whole genomes and was not detected in PEDV/Italy/7239/2009. Recombination was detected in SeCoV/Italy/213306/2009 at position 20636 and 24867 of PEDV CV777 and at position 20366 and 24996 of TGEV H16 (Figure 2), suggesting the occurrence of a recombination event between a PEDV and a TGEV. The complete S gene of SeCoV/Italy/213306/2009 shared 92% and 90% nt identity with the prototype European strain PEDV CV777 and the original highly virulent North American strain Colorado 2013, respectively, and the remaining genome shared a 97% nt identity with the virulent strains TGEV H16 and TGEV Miller M6. Whole-genome analysis of PEDV/Italy/7239/2009 showed that it grouped with the global PEDV strains (*6*) and shared ≈97% nt identity with PEDV strains CV777, DR13 virulent, and North American S-INDEL strain OH851 (Table 2).

Conclusions

During 2007–2014, most (92%) samples collected from the Po Valley in Italy were positive for PEDV by ELISA; only 72% were positive by pan-CoV PCR. However, because we were investigating the presence of PEDV or TGEV in samples with clinical signs of diarrhea, the high occurrence of PEDV may not reflect the actual prevalence of PEDV in Italy. The increased percentage

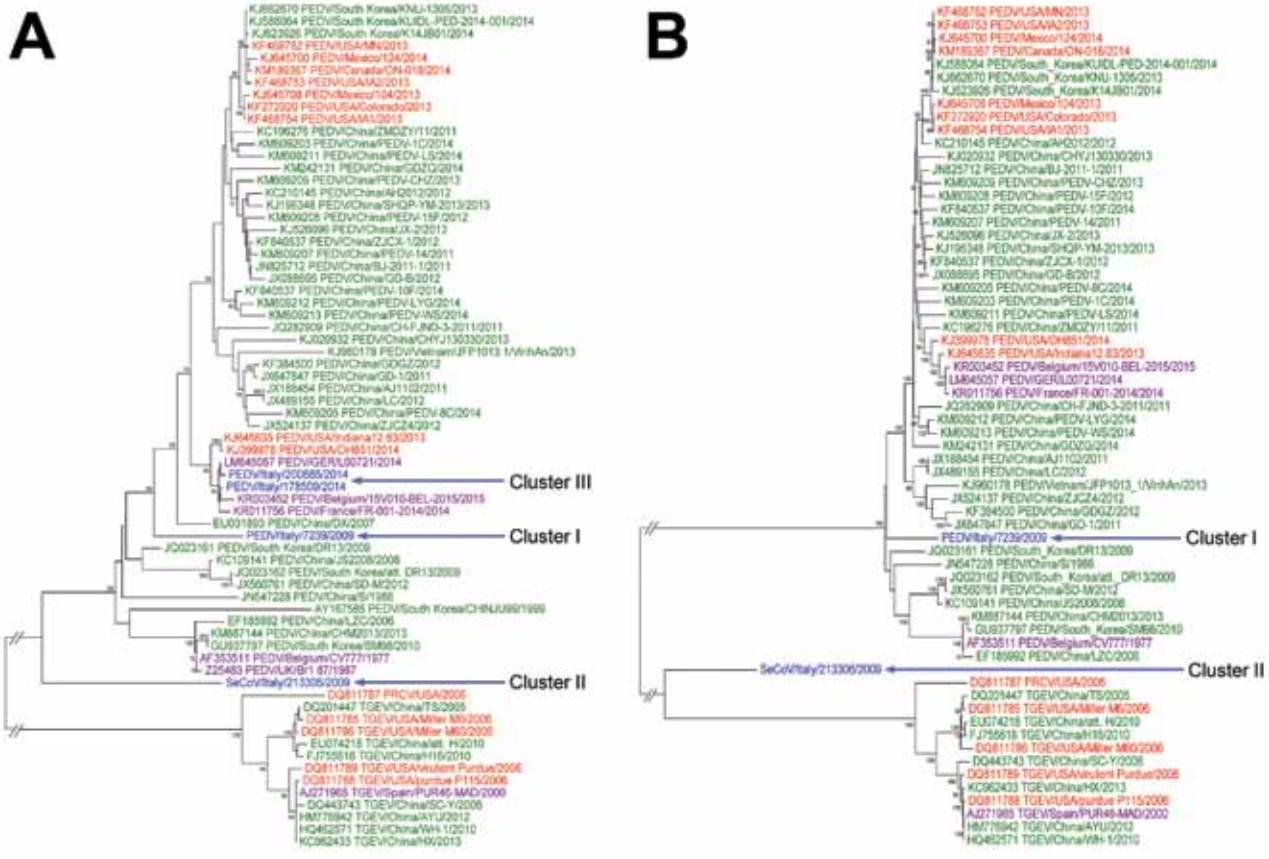

Figure 1. Phylogenetic analyses of swine enteric coronaviruses in Italy. A) Analysis performed on the basis of the nucleotide sequence of the complete spike (S1) gene of 4 representative strains from the 3 clusters and B) whole genome of 2 positive strains from clusters I and II. Cluster I represents strains circulating from 2007 through mid-2009; cluster II represents strains circulating from mid-2009 through 2012; and cluster III represents strains circulating since 2014. Bootstrap values >70% (1,000 replicates) are indicated. Reference sequences are identified by GenBank accession no. and strain name. The strains from this study are represented in blue; strains from China are green; strains from North America are red; and strains from Europe are purple. PEDV, porcine epidemic diarrhea virus; PRCV, porcine respiratory coronavirus; TGEV, transmissible gastroenteritis virus; SeCoV, swine enteric coronavirus.

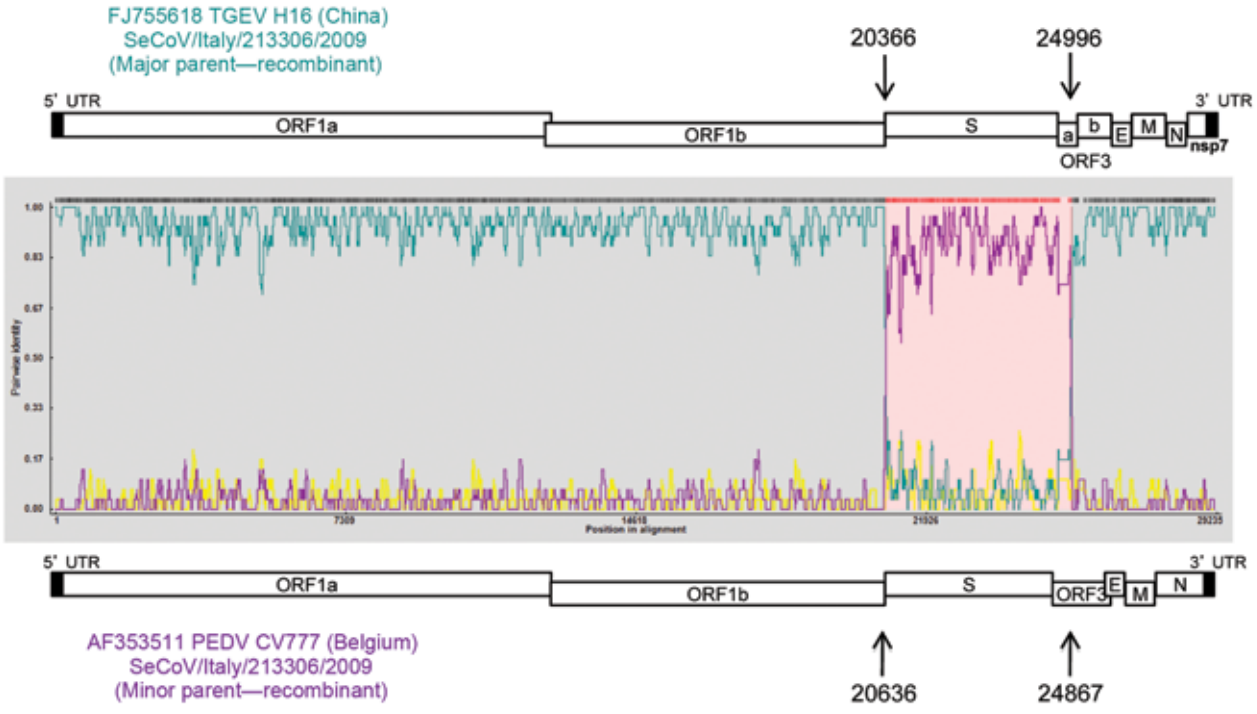

Figure 2. Potential recombination points in the SeCoV strains in study of swine enteric coronaviruses in Italy. The potential parent strains H16 (TGEV) and CV777 (PEDV) are shown in teal and purple, respectively. Arrows indicate recombinant breakpoints. UTR, untranslated region; ORF, openreading frame; S, spike; E, envelope; M, membrane; N, nucleocapsid; nsp, nonstructural protein; PEDV, porcine epidemic diarrhea virus; TGEV, transmissible gastroenteritis virus; SeCoV, swine enteric coronavirus.

of PEDV found in samples tested by ELISA, compared with the proportion found by PCR, may be explained by the number of ambiguous bases in the pan-CoV primers; the ambiguous bases severely reduce the efficiency of the reaction. The swine enteric CoV strains from Italy in our study, including the recombinant strain, were reported in pigs with mild clinical signs, indicating that PEDV and SeCoV have been circulating in Italy and likely throughout Europe for multiple years but were underestimated as a mild form of diarrhea.

To understand the evolution of PEDV in Italy, the partial RdRp, S, and M genes were sequenced from 18 samples and grouped in 3 different temporal clusters. Cluster I (2007–mid 2009) resembles the oldest PEDV strains; cluster II resembles a new TGEV and PEDV recombinant variant; and cluster III, identified from 2 pig farms in northern Italy in 2014, resembles the PEDV S-INDEL strains identified in Germany, France, Belgium, and the United States. The >99.3% nt identity of the S1 gene within cluster III and in previously identified strains could suggest the spread of the S-INDEL strain into Europe. However, directionality of spread cannot be determined because of a lack of global and temporal PEDV sequences.

Although our findings could indicate 3 introductions of PEDV in Italy, the results more likely suggest the high ability of natural recombination among CoVs and the

Table 2. Nucleotide identities of strains PEDV/Italy/7239/2009 and SeCoV/Italy/213306/2009, representative of clusters I and II, respectively, in study of swine enteric coronaviruses in Italy*

	ORF1		Spike		ORF3		Envelope		Membrane		Nucleocapsid	
Strain identification	I	II	I	II	I	II	I	II	I	II	I	II
PEDV/Belgium/CV777/1977	97.3	57.8	96.3	92.7	98.1	43.6	97.6	43.0	97.6	55.9	96.6	42.5
PEDV/South Korea/DR13 vir/2009	98.1	58.0	97.3	93.1	99.3	43.7	99.6	43.0	97.7	55.6	97.6	43.3
PEDV/USA/Colorado/2013	98.0	57.9	94.6	90.7	98.6	44.0	99.2	43.0	97.8	55.3	96.8	43.4
PEDV/USA/OH851/2014	98.1	57.9	96.9	91.9	98.7	43.8	99.2	43.0	97.7	55.5	96.7	43.4
PEDV/L00721/GER/2014	98.0	57.9	97.0	92.0	98.6	43.7	99.2	43.0	97.8	55.3	96.8	43.4
TGEV/USA/Miller M6/2006	57.9	96.8	52.0	52.5	40.0	89.1	42.6	96.4	56.1	97.1	43.1	96.3
PRCV/USA/ISU-1/2006	58.0	96.5	47.7	48.2	53.0	76.6	43.8	96.0	56.2	96.3	42.8	95.7

*Cluster I represents strains circulating from 2007 through mid-2009; cluster II represents strains circulating from mid-2009 through 2012. Ger, Germany; ORF, open reading frame; PRCV, porcine respiratory coronavirus; PEDV, porcine epidemic diarrhea virus; SeCoV, swine enteric coronavirus; TGEV, transmissible gastroenteritis virus.

continued emergence of novel CoVs with distinct pathogenic properties. Further investigation is needed to determine the ancestor of the SeCoV strain or to verify whether the recombinant virus was introduced in Italy. Recombinant SeCoV was probably generated in a country in which both PEDV and TGEV are endemic, but because the presence of these viruses in Europe is unclear and SeCoV has not been previously described, it is difficult to determine the parental strains and geographic spread of SeCoV. Future studies are required to describe the pathogenesis of SeCoV and its prevalence in other countries.

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Increase in Sexually Transmitted Infections among Men Who Have Sex with Men, England, 2014

Hamish Mohammed, Holly Mitchell, Bersabeh Sile, Stephen Duffell, Anthony Nardone, Gwenda Hughes

Surveillance data from sexual health clinics indicate recent increases in sexually transmitted infections, particularly among men who have sex with men. The largest annual increase in syphilis diagnoses in a decade was reported in 2014. Less condom use may be the primary reason for these increases.

Sexually transmitted infections (STIs) are a major public health concern; they can facilitate the transmission of HIV and are associated with severe disease. Treatment for some STIs, especially gonorrhea, has been compromised by antimicrobial drug resistance (*1*). In 2014 in England, there were 439,243 diagnoses of STIs. Although this number reflects a very small decline (0.3%) relative to 2013, numbers of diagnoses of syphilis and gonorrhea rose substantially, by 33% (from 3,236 to 4,317) and 19% (from 29,419 to 34,958), respectively (*2*). This number of syphilis diagnoses is the highest reported in England since 1949, and the number of gonorrhea diagnoses is the highest reported since 1986. These increases resulted almost entirely from increased diagnoses among men who have sex with men (MSM), among whom diagnoses of syphilis and gonorrhea increased 46% (from 2,375 to 3,477) and 32% (from 13,629 to 18,029), respectively (Figure 1), resulting in the highest number of diagnoses of these STIs since reporting among MSM began in 1994. We explored the epidemiology of these and other STIs among MSM and describe recent trends.

The Study

In England, surveillance for STIs is conducted through mandatory reporting in sexual health clinics (SHCs) (genitourinary medicine [GUM] and integrated GUM/sexual and reproductive health clinics) by using the GUM clinic activity dataset version 2 (GUMCADv2) (*3*). SHCs provide free and open access services and, since 2012, all (216 in 2014) have submitted data to Public Health England. Although STIs are not notifiable diseases in the United Kingdom, GUMCADv2 is a comprehensive, patient-level dataset of all attendances and laboratory-confirmed STIs at SHCs. Information about the sexual orientation of each attendee is collected through GUMCADv2 (completion >90% since 2011); this information was also collected by the preceding system, the KC60 aggregate return (*3*). We reviewed the most recent GUMCADv2 data, extracted on April 28, 2015, to assess trends in laboratory-confirmed gonorrhea, infectious (primary/secondary/early latent) syphilis, chlamydia, genital herpes (first episode), and genital warts (first episode). Only 1 diagnosis of each STI was counted within a 6-week period; this restriction also applies to instances of infection at multiple anatomic sites (*3*). These data represent the number of diagnoses reported, not the number of persons in whom the infections were diagnosed. Descriptive analyses by demographic characteristics and tests for linear trend were performed; p values <5% were considered significant.

Since 2013, syphilis diagnoses among MSM increased by 46% (from 2,375 to 3,477); this increase is the largest year-on-year increase in syphilis diagnoses among MSM since 2005 (Figure 1; Table 1). Relative to 2013, in 2014 the number of syphilis diagnoses among heterosexual men and men of unknown sexual orientation decreased by 0.2% (from 578 to 577), with a larger decrease in women (7.1%, from 283 to 263) (Table 1).

From 2013 to 2014, diagnoses of gonorrhea among MSM increased 32% (from 13,629 to 18,029) (Figure 1; Table 1). Although this increase was relatively large, it is consistent with the increasing trend from 2005 (3,817 diagnoses; $p = 0.015$). Compared with the increased diagnoses in MSM since 2013, in 2014 the increases in gonorrhea diagnoses among heterosexual men and men of unknown sexual orientation (5.2%, from 8,122 to 8,546) and women (9.3%, from 7,664 to 8,379) were smaller (Table 1).

Relative to 2013, in 2014, diagnoses further increased for chlamydia (25.8%), genital herpes (10.1%), and genital warts (9.5%) among MSM, consistent with the increasing trend since 2005 (Figure 1; Table 1). Diagnoses of chlamydia decreased among heterosexual men and men of unknown sexual orientation (0.4%, from 44,512 to 44,339) and increased among women (4.9%, from 48,642 to 51,045). Genital herpes diagnoses decreased by 4.8% (from 10,938 to 10,415) and 0.9% (from 20,069 to 19,883) and genital warts by 5.2% (from 37,872 to 35,893) and 4.8% (from 32,834 to 31,251) among heterosexual men and men of unknown sexual orientation and among women, respectively.

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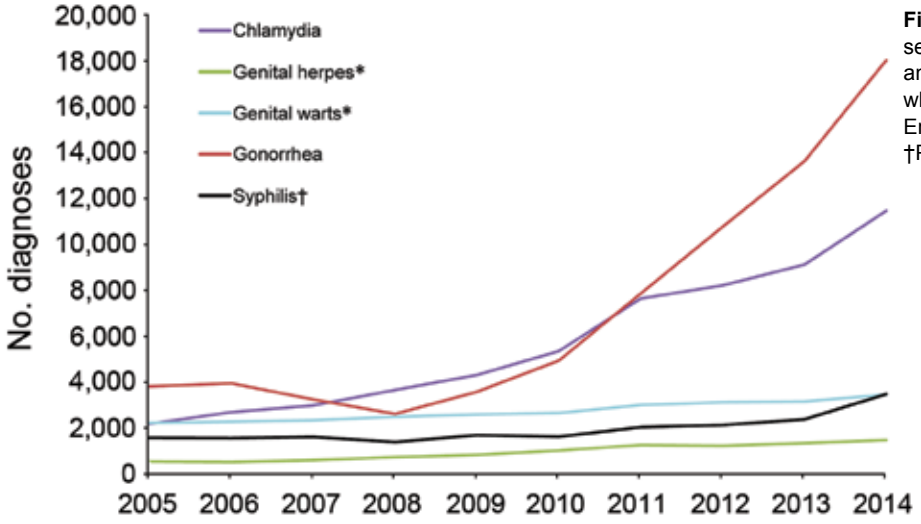

Figure 1. New diagnoses of selected sexually transmitted infections among men who have sex with men who attended sexual health clinics, England, 2005–2014. *First episode. †Primary, secondary, or early latent.

Although the number of sexual health screens (tests for chlamydia, gonorrhea, HIV, and syphilis) among MSM increased 29% from 2013 to 2014, the rates (diagnoses/1,000 screenings) of syphilis and gonorrhea increased 13.8% (from 20.9 to 23.8) and 2.9% (from 120.2 to 123.6), respectively. The rate (diagnosis/1,000 screenings) of chlamydia decreased 2.2% (from 80.4 to 78.6) (Figure 2), and the proportion of diagnoses of extragenital chlamydia and gonorrhea also decreased (Figure 2).

In 2014, the median age for MSM in whom these STIs were diagnosed ranged from 28 years (genital warts) to 36 years (syphilis) (Table 2). More than three quarters of MSM in whom STIs were diagnosed were white, 60.3%–71.4% were born in the United Kingdom, and 12.6%–18.4% were born in other European countries (Table 2). MSM in whom bacterial STIs were diagnosed were more likely to be HIV positive and live in London than were MSM in whom genital warts and herpes were diagnosed (Table 2).

Conclusions

We report the continuing increase of diagnoses of all STIs among MSM, particularly for syphilis, for which the largest number of cases was recorded since 1994. Previously reported trends (*4*) markedly worsened in 2014. Similar levels of syphilis diagnoses among men (reporting for MSM began in 1994) were last reported in the late 1970s and were followed by a precipitous decline after the emergence of HIV in the United Kingdom in the 1980s (*5*). In 2014, most MSM in whom STIs were diagnosed lived in London, and an average of 16% were born in Europe outside the United Kingdom. Given such a mobile population, the potential for spread of these infections to MSM in other major cities is clear (*6*).

Diagnoses of STIs among HIV-positive MSM since 2009 have steadily increased; the STI rate is 2–4 times that among MSM who are HIV negative or of unknown HIV status (*7*). Sex without condom use, associated with

Table 1. Number of diagnoses of selected STIs made at sexual health clinics, England*

STI	Patient category	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	% Increase, 2014 vs. 2013
Syphilis	MSM	1,569	1,560	1,610	1,387	1,692	1,618	2,036	2,129	2,375	3,477	46.4
	Non-MSM men	1,114	1,123	1,173	1,121	808	733	598	569	578	577	–0.2
	Women	503	433	424	366	345	292	291	261	283	263	–7.1
Gonorrhea	MSM	3,817	3,945	3,245	2,615	3,579	4,938	7,860	10,768	13,629	18,029	32.3
	Non-MSM men	8,707	8,142	8,340	7,205	7,250	6,696	7,221	7,815	8,122	8,546	5.2
	Women	5,108	5,104	5,534	5,165	5,299	5,198	6,007	6,992	7,664	8,379	9.3
Chlamydia	MSM	2,183	2,693	2,982	3,658	4,313	5,349	7,644	8,215	9,118	11,468	25.8
	Non-MSM men	43,122	46,363	50,584	50,892	46,169	42,669	44,405	43,327	44,512	44,339	–0.4
	Women	51,986	51,321	54,941	55,872	50,125	46,080	50,048	45,870	48,642	51,045	4.9
Genital herpes	MSM	538	515	600	735	834	1,019	1,264	1,233	1,339	1,474	10.1
	Non-MSM men	6,192	6,941	8,455	9,369	9,985	10,563	10,662	10,861	10,938	10,415	–4.8
	Women	10,649	11,798	14,432	15,990	16,604	18,101	19,226	19,770	20,069	19,883	–0.9
Genital warts	MSM	2,225	2,280	2,339	2,488	2,592	2,657	3,004	3,120	3,156	3,456	9.5
	Non-MSM men	33,750	34,741	37,384	38,606	39,308	38,238	38,596	37,272	37,872	35,893	–5.2
	Women	31,877	32,679	35,549	37,062	35,931	34,659	34,935	33,493	32,834	31,251	–4.8

*Genitourinary medicine (GUM) and integrated GUM/sexual and reproductive health clinics. In 2014 unknown sexual orientation was reported for < 4% of men. Figures for women who have sex with women are not reported here because in 2014, homosexuality was reported for <0.5% of women. MSM, men who have sex with men; non-MSM men, heterosexual men and men of unknown sexual orientation; STI, sexually transmitted infection.

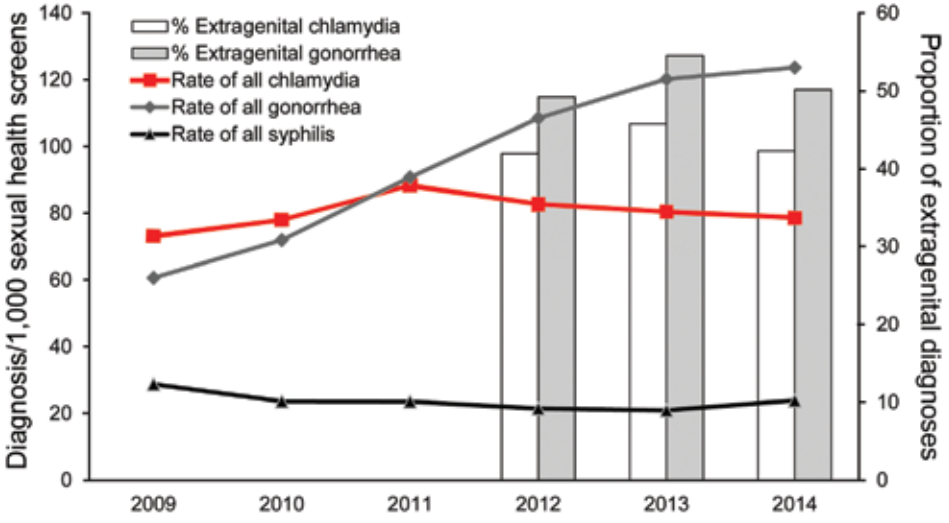

Figure 2. Rate of diagnoses (per 1,000 sexual health screens) of chlamydia, gonorrhea, and syphilis (primary, secondary, and early latent) and proportion of extragenital chlamydia and gonorrhea diagnoses among men who have sex with men who attended sexual health clinics, England, 2009–2014. Surveillance codes for extragenital infections were introduced mid-2011 and are only available for chlamydia and gonorrhea diagnoses.

increasing HIV seroadaptive behaviors (i.e., seeking out partners according to their HIV serostatus for unprotected sex) and use of geospatial social networking applications, may facilitate STI transmission in concentrated sexual networks (*8*,*9*). Given the risk for emergence of strains of *Neisseria gonorrhoeae* that are resistant to first-line antimicrobial drugs (*1*), the increase in gonorrhea diagnoses, especially among MSM, is concerning.

In response to gonorrhea testing guidance published in 2010 (*10*), use of highly sensitive nucleic acid amplification tests for screening of extragenital sites in MSM has occurred more frequently (*11*); this change may account for part of the increase in gonorrhea diagnoses in the earlier part of the decade. However, the most recent update contained no changes in syphilis testing guidelines (*12*), so recent increases are unlikely to result from changes in testing practice. Further, although more MSM were tested for STIs in 2014 compared with 2013, the rate of chlamydia and gonorrhea diagnoses remained relatively stable, while that of syphilis increased.

Given the comprehensive coverage of national STI surveillance and that SHCs in England are open access and free, most syphilis and gonorrhea cases are probably captured in GUMCADv2 (*13*). A key limitation of GUMCADv2 is that it does not collect any behavioral data; however, an enhancement to collect data on behavior, including recreational drug use and unprotected anal intercourse, is being piloted (https://www.gov.uk/guidance/genitourinary-medicine-clinic-activity-dataset-gumcadv3-pilot).

A focus on biomedical interventions, such as preexposure prophylaxis, for the control of HIV among MSM may have unintended consequences for transmission of other STIs, which highlights a need to ensure that robust STI prevention and control measures are in place (*14*). These measures should include promoting condom use; increased screening (in the United Kingdom, quarterly HIV/STI testing of MSM who engage in condomless sex with new partners is recommended); ensuring that services are easily accessible; and promoting other risk reduction strategies to improve the health and well-being of MSM (*15*).

Dr. Mohammed is a principal STI surveillance scientist at Public Health England. His team manages the national surveillance system for STIs in England, and he is the lead on a pilot of enhanced surveillance for behavioral and partner notification outcomes at sexual health clinics.

Table 2. Diagnoses of selected sexually transmitted infections among men who have sex with men attending sexual health clinics, by patient characteristics, England, 2014*

Patient characteristic	Syphilis†	Gonorrhea	Chlamydia	Genital herpes‡	Genital warts‡	No STI§
No. diagnoses	3,477	18,029	11,468	1,474	3,456	NA
Median age (interquartile range), y	36 (29–44)	31 (25–38)	32 (26–41)	31 (25–38)	28 (23–36)	34 (26–44)
% White or white British	78.9	78.8	77.4	80.1	82.6	80.4
% London residents	58.9	60.3	56.3	49.7	40.4	48.7
% Born in the United Kingdom	60.3	61.0	61.6	69.5	71.4	65.9
% Born in Europe outside the United Kingdom	18.4	18.2	16.6	12.6	12.6	14.5
% HIV positive	44.3	22.9	26.1	18.4	8.0	29.6

*Genitourinary medicine (GUM) and integrated GUM/sexual and reproductive health clinics. NA, not applicable.
†Primary, secondary, or early latent.
‡First episode.
§N = 2,549,652 attendances at SHCs where no sexually transmitted infection was diagnosed.

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Seroepidemiology of Human Enterovirus 71 Infection among Children, Cambodia

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Enterovirus 71 is reported to have emerged in Cambodia in 2012; at least 54 children with severe encephalitis died during that outbreak. We used serum samples collected during 2000–2011 to show that the virus had been widespread in the country for at least a decade before the 2012 outbreak.

In the Asia-Pacific region, human enterovirus 71 (EV71) is a widespread pathogen that causes hand, foot and mouth disease among children. Potentially fatal neurologic and systemic manifestations develop in a small proportion of patients (*1*).

In Cambodia during 2012, a disease outbreak characterized by severe encephalitis with cardiovascular collapse and pulmonary edema seized international headlines and resulted in the death of at least 54 children; EV71 subgenogroup C4 was identified as the cause (*2*). The large number of deaths during a short period was a concern for health authorities. To investigate whether EV71 had circulated in Cambodia before the 2012 outbreak, we retrospectively screened blood samples collected from children during 2000–2011.

The Study

We screened serum samples collected from inpatient children in Cambodia through routine national dengue surveillance. The study set was extracted from the Institut Pasteur in Cambodia biobank of strictly anonymized samples collected from 9,408 febrile inpatients during 2000–2011. Ethics clearance was obtained from the Cambodian National Ethics Committee for Human Research before testing commenced.

After exclusion of data entry errors, outliers in terms of year of participation, and insufficient data or samples, the database included 7,823 children 2–15 years of age for whom age, sex, and province of residence were documented. To avoid any influence from maternal antibodies, we excluded children <2 years of age from the study. Provinces were allocated to geographic quadrants and mapped by using ArcGIS 10 (Esri Co., Redlands, CA, USA) (online Technical Appendix Figure 1, http://wwwnc.cdc.gov/EID/article/22/1/15-1323-Techapp1.pdf). Random sampling was applied by using Stata 11 (StataCorp LP, College Station, TX, USA) with a representation of samples for each year. A total of 1,707 anonymized samples (1 sample/child) were selected and tested. All available samples from the sparsely populated northeastern quadrant (4% of the dataset) were included. Because the southeastern quadrant (bordering Vietnam) is the most populated quadrant, samples were selected in approximate proportion to population (46% of the dataset). Samples from the southwestern (18% of dataset) and northwestern (31% of the dataset) quadrants, each of which borders Thailand, were selected proportionally to represent a total of 48% from the quadrants bordering Thailand (Table).

The 1,707 serum samples were screened by use of a microneutralization assay to detect neutralizing antibodies against an EV71 strain (genotype C4a) isolated from an infected child during the 2012 outbreak in Cambodia. The assay was conducted on Vero E6 cells by mixing 2-fold serial dilutions (1:8 to 1:8,192) of heat-inactivated human serum samples with 100 mL (2,000 50% tissue culture infective doses/mL) of the EV71 strain. Cytopathic effect was determined visually before and after staining with 2.5% crystal violet solution. All serum samples were tested in duplicate, and positive control serum was added to each reaction plate for quality control purposes. The lowest dilution at which cytopathic effect was observed in ≥50% of wells was considered the antibody titer of the serum sample. A titer of ≥1:16 was considered the cutoff for a positive antibody response and was a more stringent cutoff than that used in previously published EV71 seroprevalence studies, in which the cutoff was usually ≥1:8 (*3*–*6*).

To reconstruct the historical annual probability of infection, we used information about the serostatus and age of the children. This reconstruction assumed that after infection, detectable antibody titers are long lasting; this method has been used to estimate the historical force of infection for other diseases, such as dengue (*7*). We estimated a separate annual probability of infection for each year from 1994 through 2011. Because no patients in our dataset had been born before 1994, we could not estimate the force of infection before this time (online Technical

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Table. Population statistics and enterovirus 71 seroprevalence among children 2–15 years of age, by geographic quadrant, Cambodia, 2000–2011

Quadrant	Population (% seroprevalence)*	No. participants (% of dataset)	Overall seroprevalence, no. (%)
Northwest	2,785,308 (20.2)	535 (31.3)	488 (91.2)
Northeast	1,445,009 (10.5)	76 (4.4)	64 (84.2)
Southwest	3,831,832 (27.8)	314 (18.4)	271 (86.3)
Southeast	5,703,047 (41.4)	782 (45.8)	693 (88.6)
Total	13,765,196 (100)	1,707 (100)	1,516 (88.8)

*All-age population in 2008 (census nearest to retrospective study midpoint). Data from the National Institute of Statistics, Directorate General for Health, Cambodia Demographic ICF Macro, 2011; General Population Census of Cambodia, 2008; and Ministry of Planning, 2009.

Appendix). Because samples included in this study were from children with a denguelike illness, rates of EV71 infection among study participants might not accurately reflect rates among all children in Cambodia. However, because an average of 87.8% of patients recruited by the National Dengue Surveillance Program had a laboratory-confirmed dengue infection (*8*), the febrile episode that triggered the hospitalization could be only slightly associated with EV71 infection and thus would have negligible influence on the EV71 neutralizing titers of the patient population.

Among children in this study, the overall seroprevalence of EV71 neutralizing antibodies was 88.8%: 1,300 (94.8%) of 1,371 (95% CI 93.5%–95.9%) among children 2–15 years of age sampled during 2006–2011 and 216 (64.3%) of 336 (95% CI 58.9%–69.4%) among those 2–7 years of age sampled during 2000–2005 (Figure 1). Seroprevalence did not vary substantially by age group. This profile across age groups remained unchanged in more stringent analyses with higher cutoff values (online Technical Appendix Figure 2) in which, despite levels of seropositivity decreasing with higher cutoff titers, the reduction was consistent across all age groups. Seroprevalence of EV71 relative to sex did not differ significantly (89.8% among girls vs. 87.7% among boys; p = 0.18).

Epidemic curves derived from the seroprevalence data show the dynamics of infection for the whole country (Figure 2) and across the 4 quadrants (online Technical Appendix Figure 3). The reconstructed curves were coherent, showing large-scale, countrywide circulation of the virus since 2002. Seroprevalence peaks every 2–3 years indicate a cyclical pattern of EV71 outbreaks. This pattern has been reported from other Asia-Pacific countries (*9*–*11*) and probably represents the time needed for establishment of a new cohort of immunologically naive patients. In countries with a larger population, such as China, infection might peak annually (*12*).

Despite our use of a more stringent cutoff value, the seroprevalence detected in our study was considerably higher

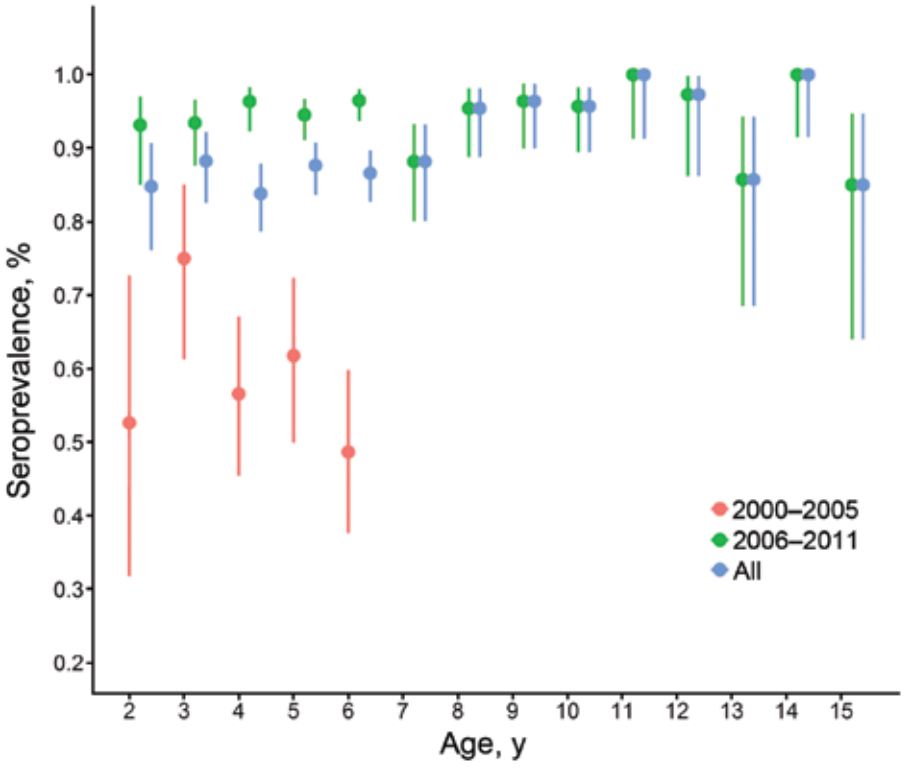

Figure 1. Age-associated seroprevalence of enterovirus 71 (EV71) infection in Cambodia, estimated by detection of EV71 seroneutralizing antibodies in inpatient children 2–15 years of age, 2000–2011. Error bars indicate 95% CIs. Serum samples were collected from routine national dengue surveillance in Cambodia.

Figure 2. Annual probability of enterovirus 71 infection (EV71) in Cambodia during 1994–2011, estimated by detection of EV71 seroneutralizing antibodies in inpatient children 2–15 years of age. Serum samples were collected from routine national dengue surveillance in Cambodia.

than that reported from previous studies in the region, during which a cutoff of 1:8 was invariably used (*3*–*6*). If we had used a neutralization titer of 1:8, seroprevalence would have been 93.1% (n = 1,590 positive samples). Intense circulation of EV71 was therefore occurring in Cambodia long before the 2012 outbreak.

In Cambodia and other Asia-Pacific countries, other enteroviruses commonly cocirculate with EV71. Some of these strains, such as coxsackieviruses A6 and A16, have also been associated with severe neurologic illnesses in children. Previous studies have established that cross-neutralization occurs among different EV71 strains and genogroups (*13*,*14*). However, there is no evidence of cross-neutralization between EV71 and other enteroviruses (*15*). Cross-neutralization at high dilutions would probably not have generated a consistent profile of seropositivity across children of different ages (online Technical Appendix Figure 2). Thus, the high level of seropositivity observed in this study is probably specific for EV71.

Conclusions

Our data support the widespread circulation of EV71 at least a decade before its reported emergence in 2012. Furthermore, reconstructed epidemic curves suggest that EV71 outbreaks occurred in a cyclical pattern in Cambodia and that the virus infected large proportions of immunologically naive children every 2–3 years. Before 2012, this circulation remained undetected, highlighting the need to further reinforce the surveillance systems in developing countries. Also needed is enhanced medical education for better identification of infectious diseases such as hand, foot and mouth disease, which, despite its association with relatively specific clinical signs, requires careful physical examination of patients.

It is still unknown why so many severe cases were detected during the 2012 EV71 outbreak in Cambodia. However, seroepidemiologic studies in other settings have also confirmed widespread circulation before outbreaks (*5*,*9*). For combatting this pathogen, developments in vaccines and antiviral drugs are urgently needed.

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Outbreak of Panton-Valentine Leukocidin–Associated Methicillin-Susceptible *Staphylococcus aureus* Infection in a Rugby Team, France, 2010–2011

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Staphylococcus aureus strains that produce Panton-Valentine leukocidin are known to cause community infections. We describe an outbreak of skin abscesses caused by Panton-Valentine leukocidin–producing methicillin-susceptible *S. aureus* (clonal complex 121) in a professional rugby team in France during July 2010–February 2011. Eight team members were carriers; 7 had skin abscesses.

Staphylococcus aureus is a leading cause of community and healthcare-associated infections, notably skin and soft-tissue infections (*1*). A strong epidemiologic link exists between community-associated *S. aureus* and Panton-Valentine leukocidin (PVL), a cytotoxin found particularly in deep primary skin infections (*2*). The prevalence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) cases seems to be low but increasing in Europe; however, heterogeneity in prevalence rates among countries occurs (*3*). Many sporadic cases and outbreaks of CA-MRSA infection have been reported in sports teams (*4*), especially among players having regular skin-to-skin contact. We describe an outbreak of recurrent PVL-positive community-associated methicillin-susceptible *Staphylococcus aureus* (MSSA) skin abscesses in a professional rugby team in France.

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The Study

On September 22, 2010, a player (patient 4) was hospitalized for a calf abscess that had spontaneously drained to the skin. Bacterial culture grew PVL-positive MSSA. Investigations identified 3 previous case-patients among the team members during the previous month (Figure). A case-patient was defined as a player on the team who developed a skin abscess. The abscesses began occurring after recruitment of a new player from Fiji (patient 1), who had untreated axillary and back abscesses when he arrived on the team in July 2010. All other case-patients had contact with him during scrimmages, suggesting that cross-transmission occurred by close physical contact. In August 2010, abscesses developed on the left wrist of patient 2 and on the arm of patient 3 (Figure).

On September 28, 2010, we screened all team members for PVL-positive *S. aureus* carriage. The team had 51 men, including 30 permanent team members; mean age was 23.6 (range 17–42) years. Screening consisted of nasal, throat, and skin-lesion swabbing. *S. aureus* was detected by bacterial culture. Gene-encoding PVL was tested by real-time PCR (*5*). DNA microarray analysis was performed by the French National Reference Center for Staphylococcal Infections (Lyon, France) and enabled detection of the *mecA* gene and genes encoding various toxins and also assisted in *agr* typing and multilocus sequence typing.

The first round of screening showed that 35 (68.6%) of the 51 team members were colonized with MSSA, and 2 (patient 1 and a player who never developed an abscess) harbored PVL-encoding genes (Figure). During the screening process, patient 1 was found to have a PVL-positive MSSA left knee abscess that spontaneously drained to the skin but was not covered.

To reduce risk of transmission, we implemented a 5-day course of *S. aureus* decontamination for all team members; decontamination consisted of mupirocin 2% nasal ointment twice daily and showering with chlorhexidine soap. We also provided information about standard hygiene measures: showering and handwashing; washing jerseys after play; regularly cleaning and disinfecting showers and shared sports equipment; avoiding sharing of personal items; protecting and disinfecting skin lesions; and treating abscesses early and appropriately. Team staff regularly checked players' adherence to the control measures.

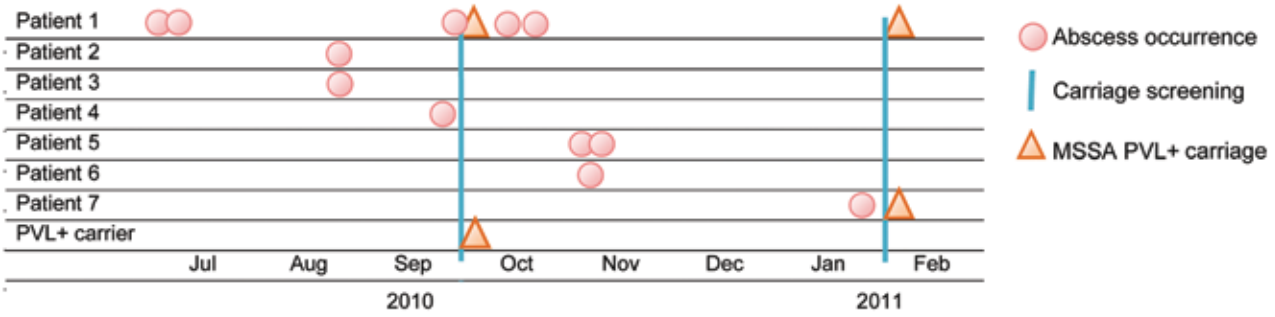

Figure. Timeline of infection and screening for PVL-positive *Staphylococcus aureus* case-patients and carriers. MSSA, methicillin-susceptible *S. aureus*; PVL, Panton-Valentin leukocidin; +, positive.

Despite these measures, 3 new skin abscesses developed in October 2010 on the chest and nape of patient 1 (Figure; isolates not available). In early November 2010, abscesses developed on the axillary and thigh of patient 5 and on the right wrist (sample not obtained) of patient 6. In January 2011, a thigh abscess developed on patient 7. On February 2, 2011, we conducted a second round of *S. aureus* carriage screening and a 10-day course of *S. aureus* decontamination for 8 players, focusing on previous PVL-positive MSSA carriers and those with abscesses. The screening showed that patients 1 and 7 carried PVL-positive MSSA. All team members were again reminded of basic hygiene measures. These infection control measures were successful: no further person-to-person transmission occurred.

Overall, 8 (15.6%) of the 51 players carried PVL-positive MSSA (n = 3) or had abscesses (n = 7); 2 players had both. All strains isolated in patients 1–7 belonged to clonal complex (CC) 121 and harbored *agr*4 allele. One player who never had an abscess carried a different PVL-positive MSSA strain in his throat (*agr*1, sequence type 152). All isolated PVL-positive MSSA strains were susceptible to all antimicrobial drugs tested except penicillin G. Except for the first 2 abscesses in patient 1, all abscesses were treated with synergistin A and B (2 grams/day for 7 days) and local disinfection.

Using a standardized questionnaire to interview the 51 rugby players, a member of the Hygiene unit at the Limoges Teaching Hospital collected epidemiologic data on demographics, sport practices, sport hygiene, and occurrence of hospitalization or abscess during the previous year. The interviews highlighted poor hygiene practices: 49% of players shared personal items, and fewer than half disinfected or protected skin lesions (Table). Occurrence of abscess during the previous year was the only significant ($p = 0.00028$, Fisher exact test) risk factor found for a PVL-positive MSSA carriage or abscess; however, given the context of this abscess outbreak, this factor was considered a bias, not a general characteristic.

Skin and soft-tissue infections are common in athletes, and the most common bacterial pathogen responsible for outbreaks is CA-MRSA, particularly the USA300 clone. The strain in the outbreak we investigated was

Table. Characteristics of rugby team members involved in outbreak of Panton-Valentine leukocidin–associated methicillin-susceptible *Staphylococcus aureus* infection, France*

Characteristic	Players' response	Players, no. (%), n = 51	*S. aureus* PVL-positive abscess or carriage,† no., n = 8	Non–*S. aureus* PVL-positive abscess or carriage,† no., n = 43	p value‡
Sharing of personal items	Yes	25 (49.0)	4	21	1.000
	No	26 (51.0)	4	22	
Disinfection of skin lesions	Yes	25 (49.0)	6	19	0.246
	No	24 (47.0)	2	22	
Skin lesion protection in daily life	Yes	18 (35.3)	3	15	0.717
	No	32 (62.7)	5	27	
Skin lesion protection during sport	Yes	24 (47.0)	3	21	0.702
	No	25 (49.0)	5	20	
Hospitalization during previous year	Yes	7 (13.7)	2	5	0.300
	No	44 (86.3)	6	38	
Skin abscess during previous year	Yes	4 (7.8)	4	0	2.8×10^{-4}
	No	47 (93.2)	4	43	
Body mass index, mean			31.40	27.80	0.057

*PVL, Panton-Valentine leukocidin.
†Values are numbers except for body mass index, which is the mean for 51 players.
‡p values used Fisher exact test except for body mass index, which used Student *t*-test. For that category, 95% CI was –0.14 to 7.51.

unrelated to USA300 but belonged to CC121. Outbreaks of PVL-positive MSSA skin infections have been described in families in Italy (*6*), schoolchildren in Switzerland (*7*), French soldiers in Côte d'Ivoire (*8*), and prison inmates in France (*9*) . As in this outbreak among rugby players, infection control measures and *S. aureus* decontamination successfully interrupted transmission in most published outbreaks.

In our study, abscess occurred in 4 players despite a round of decontamination strategies. This failure was likely because of an uncovered, untreated knee abscess in the index case-patient (patient 1) during the decontamination period. The continued occurrence of infections highlights the necessity of strict application of hygiene measures.

Nasal carriage of PVL-positive MSSA was not systematically linked to infection (e.g., 1 team member carried a PVL-positive strain in his throat but had no active skin infection). Concordance of skin and soft-tissue infection and nasal carriage is reportedly lower in MSSA than MRSA strains (*10*). Following France's guidelines for grouped cases of community-associated *S. aureus* infections (http://www.hcsp.fr/explore.cgi/avisrapportsdomaine?clefr=453), we decontaminated all athletes, even those team members not carrying PVL-positive MSSA. Decontamination temporarily reduces risk of colonization of noncarriers. Along with reinforcement of simple personal hygiene measures, our decontamination regimen sufficiently halted transmission without needing to exclude players with abscesses from the team, an important factor in professional sports.

The outbreak strain was *agr*4, PVL positive, and CC121. It belonged to a PVL-positive MSSA lineage that predominated in France during 1981–1990 (*11*). The CC121 *agr*4 lineage was also linked to furunculosis in a study in Poland (*12*). Like the strain in our study, this lineage carried no exfoliative toxin genes and expressed the *seb* superantigen. However, the strain circulating among the rugby team players was positive for *seg*, *sei*, *sem*, *sen*, *seo*, and *seu*. Superficial, deep-skin, and soft-tissue infections linked to CC121, PVL-positive MSSA strains have been reported worldwide (*13*). Similar strains have also been reported in highly lethal community-acquired pneumonia and in severe sepsis with progressive and metastatic soft-tissue infections (*14,15*).

Conclusions

We investigated an outbreak of skin abscesses caused by a PVL-producing MSSA strain and cross-transmitted through physical contact among players of a professional rugby team. A 10-day period of *S. aureus* decontamination combined with reinforcement of hygiene education and practices successfully interrupted person-to-person transmission and enabled control of the outbreak.

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Variations in Spike Glycoprotein Gene of MERS-CoV, South Korea, 2015

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An outbreak of nosocomial infections with Middle East respiratory syndrome coronavirus occurred in South Korea in May 2015. Spike glycoprotein genes of virus strains from South Korea were closely related to those of strains from Riyadh, Saudi Arabia. However, virus strains from South Korea showed strain-specific variations.

Since it was first identified in 2012, Middle East respiratory syndrome coronavirus (MERS-CoV) has emerged as a novel viral pathogen that causes severe acute respiratory illness, including fever, cough, and shortness of breath (*1*). The current outbreak of infection with this virus in South Korea, which began on May 20, 2015, has infected 186 patients and caused 36 deaths within 2 months. This developing public health concern has attracted worldwide attention as a potential cause of a global pandemic. Although extensive biologic and clinical characterization should be performed to measure the public health effect of this outbreak, currently available genetic data are informative in clarifying virus alterations that affect transmissibility.

MERS-CoV spike (S) glycoprotein binds cellular receptor dipeptidyl peptidase 4 (DPP4, CD26) for host cell entry (*2*), and a receptor-binding domain (RBD) on virus S protein mediates this interaction (*3*). In addition, S proteins expressed on the virus surface can induce host antibodies that block MERS-CoV entry (*4*). To investigate changes in the S gene associated with viral evolution and possible genetic markers of altered transmissibility, an analysis of S genes obtained from clinical specimens during the early phase of the outbreak was performed.

The Study

We identified genetic variability of MERS-CoV S genes among infected persons in South Korea. Samples from 7 patients identified as positive for MERS-CoV were sequenced. These patients were identified by using sequences upstream of the envelope protein gene and open reading frame (ORF) 1a in real-time reverse transcription PCRs (*5*) (Table 1).

Index case-patient 1 (PAT001) had traveled to Bahrain, the United Arab Emirates, and Saudi Arabia during April 24–May 4, 2015, and became symptomatic on May 11 after his return to South Korea (*6*). After he visited a local clinic, his symptoms worsened, and he was hospitalized on May 15. During his hospitalization (May 15–17), PAT001 shared a room with PAT003 and the same ward with PAT009, PAT012, PAT013, and PAT015. PAT042 was admitted to the same hospital on May 19 (Figure 1). PAT010 was the son of PAT003 and had visited his father in the hospital before traveling to China, where he became symptomatic and tested positive for MERS-CoV. PAT002 was the wife of PAT001 and cared for him during his illness.

The S gene was amplified directly from nucleic acids extracted from respiratory specimens (6 patients) or a viral isolate (1 patient) (*7*) by using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany). Reverse transcription was performed by using the Superscript III First-Strand Synthesis System (Life Technologies, Bleiswijk, the Netherlands) and virus-specific reverse primers. cDNA was amplified by using an overlapping PCR to generate products of 600–3,000 bp that covered the entire S gene.

Resulting PCR amplicons were sequenced by using Sanger sequencing with an ABI 3730 Analyzer (Applied Biosystems, Foster City, CA, USA) or next-generation sequencing. For next-generation sequencing, PCR products were pooled and fragmented to an average of 300 bp, and a sequencing library was constructed by using the Illumina TruSeq Nano DNA Sample Prep Kit (Illumina, San Diego, CA, USA). Sequencing was performed by using the Illumina MiSeq Platform (Illumina).

To explore relationships of newly isolated virus strains from South Korea with other MERS-CoV strains, 131 reference MERS-CoV S gene sequences from GenBank and MERS-CoV Sequences June 2015 (http://tinyurl.com/MERS-CoV-4Jun15) (8) and 8 strains from South Korea, including a sequence from PAT010 (ChinaGD01; Chinese Centers for Disease Control and Prevention, Beijing, China), were aligned by using MUSCLE software (9). This alignment was used for subsequent phylogenetic analysis. A phylogenetic tree was constructed by using the

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Table 1. Sequence information for Middle East respiratory syndrome coronaviruses isolated from 8 patients, South Korea, May 2015*

Patient	Sequence	Date of symptom onset/sample collection	Sample	Sequencing method	GenBank accession no.
PAT001	CoV/KOR/KNIH/001_05_2015	11/19	Sputum	Illumina,† Sanger	KT182958
PAT002	CoV/KOR/KNIH/002_05_2015	19/20	Third-passage isolate from Vero cells	Illumina	KT029139
PAT009	CoV/KOR/KNIH/009_05_2015	27/28	Sputum	Illumina	KT182953
PAT010	ChinaGD01‡	19/27	Nasopharyngeal swab	Ion torrent,§ Sanger	KT006149
PAT012	CoV/KOR/KNIH/012_05_2015	21/28	Sputum	Sanger	KT182954
PAT013	CoV/KOR/KNIH/013_05_2015	21/28	Sputum	Sanger	KT182955
PAT015	CoV/KOR/KNIH/015_05_2015	22/30	Sputum	Illumina	KT182956
PAT042	CoV/KOR/KNIH/042_05_2015	25/30	Sputum	Illumina	KT182957

*MERS-CoV, Middle East respiratory syndrome coronavirus.
†Illumina (San Diego, CA, USA).
‡Sequence obtained from the Chinese Centers for Disease Control and Prevention (Beijing, China).
§Life Technologies (Bleiswijk, the Netherlands).

maximum-likelihood method with a bootstrap value of 1,000 and RAxML software (10).

All 8 ORFs from virus isolates obtained during the outbreak in South Korea were most closely related to ORFs of the recently isolated 2015 Riyadh clade, but isolates from South Korea constituted a novel branch, which was supported by a bootstrap value of 87% (Figure 2). Phylogenetic data indicated that virus isolates from other patients originated from virus isolates from the index case-patient. These data also showed that strains detected in 2015 formed 2 groups: KSA-2466–like viruses and KKUH_0734–like viruses. Viruses from South Korea isolated in 2015 clustered with 1 sublineage of KKUH_0734–like viruses from Saudi Arabia.

Nucleotide sequence comparisons with 131 reference MERS-CoV S genes showed that the clade from South Korea had highest identity (99.68%–99.9%) with recently circulating strains from Riyadh isolated in 2015. Strains from South Korea had 8 novel nucleotide substitutions (C183G, A409C, T1586C, G1588C, T1848C, G1886A, T3177C, and C3267T) that are unique to the South Korea lineage and share nucleotide substitution T258C with some viruses from Saudi Arabia detected earlier in 2015 (Table 2). T3177C and C3267T mutations were observed only in all viruses from South Korea.

Of the 8 nucleotide substitutions, 4 (A409C, T1586C, G1588C, and G1886A) were nonsynonymous and resulted in 4 amino acid changes (S137R, I529T, V530L, and R629H) (Table 2). Among these mutations, 2 nonsynonymous variants (S137R and V530L) were identified in isolates from PAT002 after the third passage in Vero cells and were assumed to be cell culture–adaptive mutations (*11*). The I529T and V530L mutations were located in the RBD, but not at the RBD–DPP4 receptor interface (*3*). The R629H mutation was situated outside the RBD. However, on the basis of only these results, we could not determine whether these amino acid substitutions affected receptor-binding affinity between human DPP4 receptor and MERS-CoV S protein.

To understand the rate at which virus genetic diversification occurred during the outbreak in South Korea, we used the Bayesian–Markov Chain Monte Carlo method in

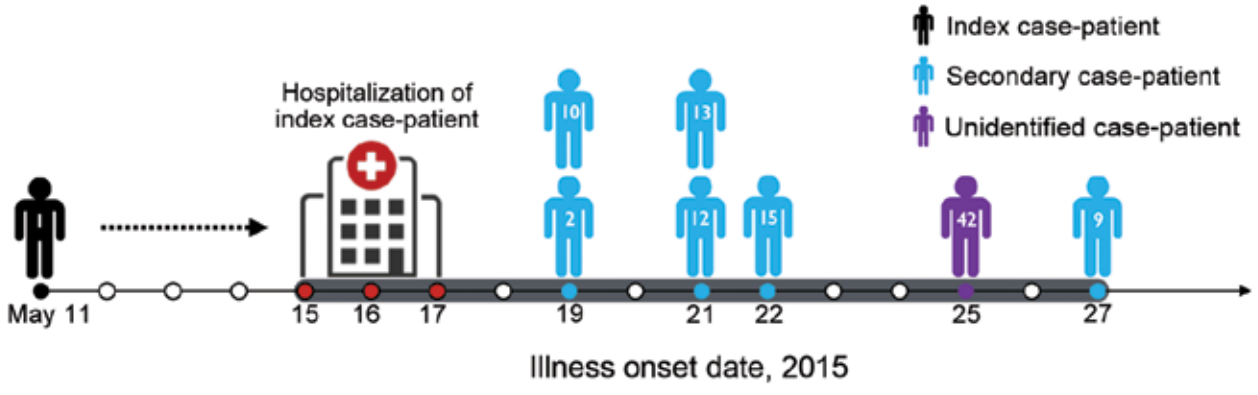

Figure 1. History of confirmed cases of Middle East respiratory syndrome coronavirus infection, South Korea, May 2015. Eight confirmed cases of human infection with this virus are shown according to date of onset of illness. The unidentified case-patient was a patient for whom the transmission source was not identified. Red circles in time line indicate hospitalization period for the index case-patient. Black, blue, and purple circles in time line indicate recorded symptom onset date for each patient. Numbers within human symbols are patient numbers.

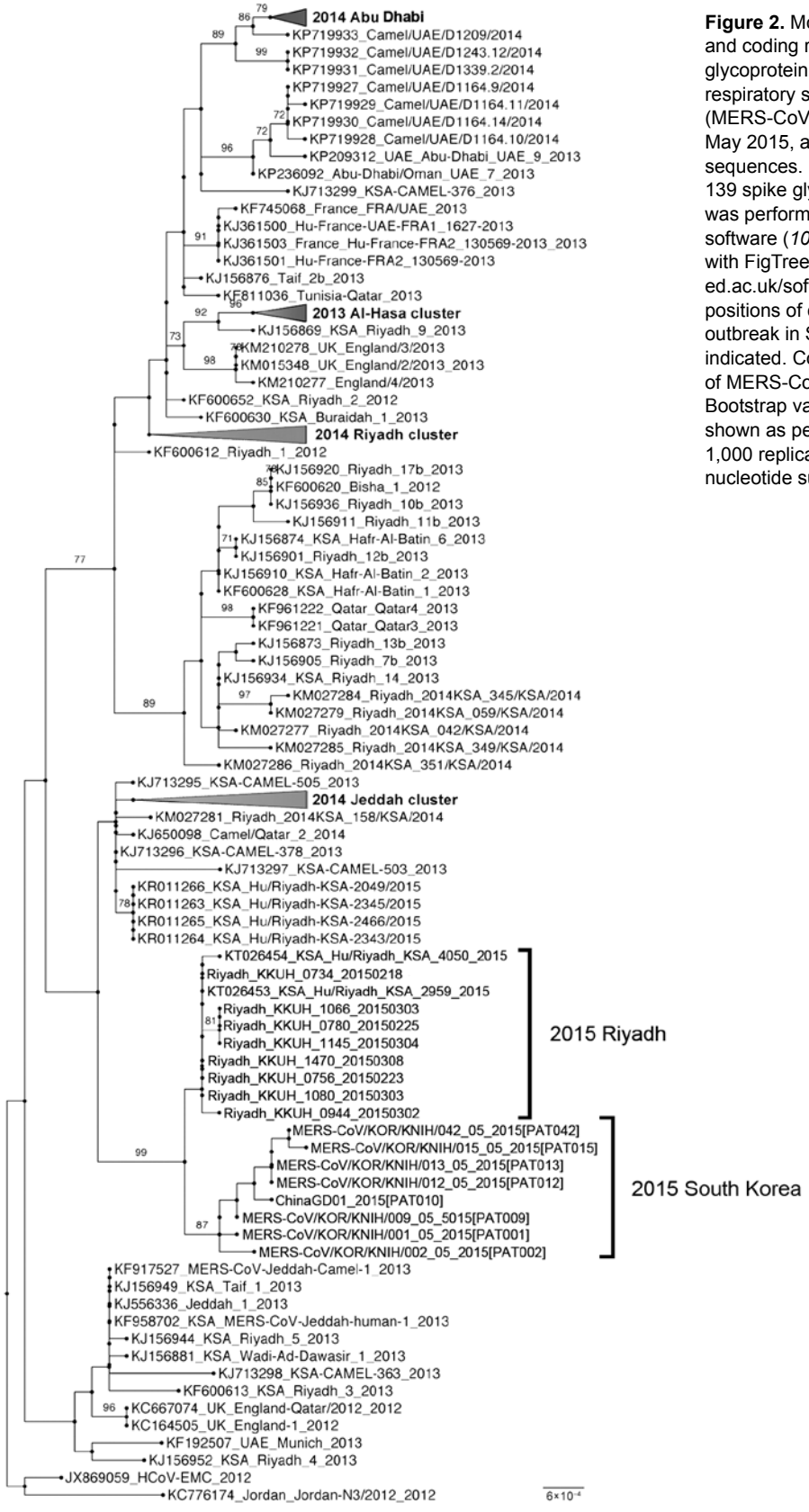

Figure 2. Molecular phylogenetic tree and coding region variants for spike glycoprotein genes of Middle East respiratory syndrome coronavirus (MERS-CoV) isolates from South Korea, May 2015, and reference MERS-CoV sequences. Phylogenetic analysis of 139 spike glycoprotein gene sequences was performed by using RAxML software (*10*). Tree was visualized with FigTree v.1.4 (http://tree.bio.ed.ac.uk/software/figtree). Taxonomic positions of circulating strains from the outbreak in South Korea and Riyadh are indicated. Compressed major clades of MERS-CoV are indicated in bold. Bootstrap values (>70%) on nodes are shown as percentages on the basis of 1,000 replicates. Scale bar indicates nucleotide substitutions per site.

Table 2. Genetic changes in spike glycoprotein gene sequences strain-specific variants of MERS-CoV from South Korea compared with those of other MERS-CoV isolates*

	Nucleotide (amino acid) positions								
	NTD			RBD		Other regions			
Virus isolate	183 (61)	258 (86)	409 (137)	1586 (529)	1588 (530)	1848 (616)	1886 (629)	3177 (1059)	3267 (1089)
JX869059_HCoV-EMC_2012	C (G)	T (V)	A (S)	T (I)	G (V)	T (V)	G (R)	T (D)	C (S)
KR011266_KSA_Hu/Riyadh-KSA-2049/2015	•	•	•	•	•	•	•	•	•
KR011263_KSA_Hu/Riyadh-KSA-2345/2015	•	•	•	•	•	•	•	•	•
KR011264_KSA_Hu/Riyadh-KSA-2343/2015	•	•	•	•	•	•	•	•	•
KR011265_KSA_Hu/Riyadh-KSA-2466/2015	•	•	•	•	•	•	•	•	•
KT026453_KSA_Hu/Riyadh_KSA-2959_2015	•	C (V)	•	•	•	•	•	•	•
Riyadh_KKUH_0734_20150218	•	C (V)	•	•	•	•	•	•	•
Riyadh_KKUH_0756_20150223	•	C (V)	•	•	•	•		•	•
Riyadh_KKUH_0780_20150225	•	C (V)	•	•	•	•	•	•	•
KT026454_KSA_Hu/Riyadh_KSA_4050_2015	•	C (V)	•	•	•	•	•	•	•
Riyadh_KKUH_0944_20150302	•	C (V)	•	•	•	•	•	•	•
Riyadh_KKUH_1066_20150303	•	C (V)	•	•	•	•	•	•	•
Riyadh_KKUH_1080_20150303	•	C (V)	•	•	•	•	•	•	•
Riyadh_KKUH_1145_20150304	•	C (V)	•	•	•	•	•	•	•
Riyadh_KKUH_1470_20150308	•	C (V)	•	•	•	•	•	•	•
MERS-CoV/KOR/KNIH/001_05_2015 [PAT001]	•	C (V)	•	C (T)	•	•	•	C (D)	C (S)
MERS-CoV/KOR/KNIH/009_05_2015 [PAT009]	•	C (V)	•	•	•	C (V)	•	C (D)	C (S)
ChinaGD01_2015 [PAT010]†	G (C)	C (V)	•	•	•	C (V)	•	C (D)	C (S)
MERS-CoV/KOR/KNIH/012_05_2015 [PAT012]	•	C (V)	•	C (T)	•	C (V)	•	C (D)	C (S)
MERS-CoV/KOR/KNIH/013_05_2015 [PAT013]	•	C (V)	•	C (T)	•	C (V)	•	C (D)	C (S)
MERS-CoV/KOR/KNIH/015_05_2015 [PAT015]	•	C (V)	•	C (T)	•	C (V)	•	C (D)	C (S)
MERS-CoV/KOR/KNIH/042_05_2015 [PAT042]	•	C (V)	•	C (T)	•	C (V)	A (H)	C (D)	T (S)
MERS-CoV/KOR/KNIH/002_05_2015 [PAT002]‡	•	C (V)	C (R)	•	C (L)	•	•	C (D)	T (S)

*Alphanumeric codes in brackets indicate patient (PAT) number. Dots indicate no change in sequence identity. MERS-CoV, Middle East respiratory syndrome coronavirus; NTD, N-terminal domain; RBD, receptor-binding domain. Dots indicate sequence identity.
†MERS-CoV strain ChinaGD01 (KT006149).
‡Third-passage isolate from Vero cells.

BEAST version 2.1.3 (http://beast2.org/) for 8 S genes. The Hasegawa, Kishino, and Yano substitution model was selected under uncorrelated lognormal molecular clock and a birth–death coalescent.

The S gene was estimated to evolve at mean rate of 6.72×10^{-3} substitutions/site/year (95% highest posterior density [HPD] $5.59–6.93 \times 10^{-3}$ substitutions/site/year). This mutation rate for the S gene was higher than that for complete MERV-CoV genomes in other studies: 1.12×10^{-3} substitutions/site/year (95% HPD 8.76×10^{-4}–1.37×10^{-3} substitutions/site/year) (*12*) and 9.29×10^{-4} substitutions/site/year (95% HPD 7.19×10^{-4}–1.15×10^{-3} substitutions/site/year) (*13*). However, more data are required to demonstrate the pattern of MERS-CoV evolution during the outbreak in South Korea because results are limited by a relatively low number of sequences, short selected time points, examination of only the S gene region, and different sequencing methods.

Conclusions

Accurate genome sequencing can identify spatiotemporal patterns that help understand dynamics of rapid spread of MERS-CoV infection. We report S glycoprotein gene sequences of MERS-CoV from 8 patients and a strain cultured in Vero cells. Genetic information obtained is useful for understanding the evolutionary history of MERS-CoV.

On the basis of our phylogenetic analyses, virus sequences of strains isolated in South Korea in 2015 form a unique clade. Genetic variations elucidated in this study show an unreported sequence in the RBD, which suggests that MERS-CoV circulating in South Korea during the outbreak in 2015 has higher genetic variability and mutation rates. However, we cannot conclude that deleterious effects promoting spread of infection will occur because of these mutations. Additional genetic information will resolve precise characteristics of the MERS-CoV obtained during the outbreak in South Korea.

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Effectiveness of Ring Vaccination as Control Strategy for Ebola Virus Disease

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Using an Ebola virus disease transmission model, we found that addition of ring vaccination at the outset of the West Africa epidemic might not have led to containment of this disease. However, in later stages of the epidemic or in outbreaks with less intense transmission or more effective control, this strategy could help eliminate the disease.

During 2014–2015, trials of candidate vaccines for Ebola virus disease (EVD) were fast tracked in response to the unprecedented EVD epidemic in West Africa (*1*). In March 2015, a phase 3 ring vaccination trial of a recombinant vesicular stomatitis virus–Zaire Ebola virus vaccine began in Guinea (*2*). Interim trial results suggested that the vaccine could have a high level of efficacy in humans (*3*). Ring vaccination has also been used for disease control, notably in the final stages of the smallpox eradication program (*4*). Furthermore, a recent modeling study calibrated by using population-level EVD data from Sierra Leone and Liberia (*5*) suggested that ring vaccination could supplement case isolation and contact tracing in reducing transmission. However, it remains unclear whether prompt ring vaccination, as opposed to large-scale mass vaccination, could have contained the EVD epidemic in West Africa, and under what circumstances it could be effective in controlling future outbreaks.

The Study

We developed a stochastic model of EVD transmission (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-1410-Techapp1.pdf) using individual-level transmission data from Guinea to inform our model structure. Transmission chains during March–August 2014 suggest substantial variation in the number of secondary cases generated (*6,7*). In particular, index cases, defined as those that could not be linked to an already known transmission chain, had a reproduction number of $R_m = 7$, where m indicates missed cases and R_m denotes the average number of secondary cases generated, whereas cases within known transmission chains (w) had a reproduction number of $R_w = 0.66$ (online Technical Appendix, Figure 1).

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In the model, transmission followed a branching process (*8*), and secondary cases were generated from a negative binomial distribution to include potential for superspreading events (*6,9*). Each cluster started with an index case, which generated an average of $R_m = 7$ secondary cases. Many EVD cases reported in Guinea were not part of already known transmission chains (online Technical Appendix, Figure 2). We therefore assumed there was a probability ρ that a secondary case would missed and go on to seed an independent transmission cluster as an index case with $R_m = 7$. Otherwise, the case would remain within the known chain of transmission (with probability $1 – \rho$); these cases would then generate an average of $R_w = 0.66$ secondary cases. The simulated outbreaks ended when, by chance, no secondary cases were generated by active cases. Distributions of incubation period, duration of infectiousness, and time to reporting were obtained from reported values for Guinea in 2014 (*10*). Model simulations produced similar patterns to those observed in 2014 (Figure 1). When half of the cases were missed, the overall reproduction number, defined as the mean number of secondary cases generated across all infectious persons, was ≈1.5, which was similar to values observed in early 2014 in West Africa (*11*) and in the initial stages of other outbreaks (online Technical Appendix Table 1).

We simulated ring vaccination by using a protocol similar to that used in Guinea trial (*3*). We defined a ring as all persons who could potentially form part of the known chain of transmission (i.e., traceable contacts of infected persons within a transmission cluster and their contacts). Once the index case was reported, we assumed it took 2 days to vaccinate a ring and that protective immunity developed 7 days after vaccination. In the model, we assumed that vaccine efficacy was 80% and that 70% of the ring received vaccination (online Technical Appendix). The reproduction number within a ring was therefore reduced by a factor of $1 –(0.8 \times 0.7) = 0.44$ once the vaccine became effective (online Technical Appendix Figure 3).

To estimate the effect of ring vaccination, we simulated multiple outbreaks and calculated the proportion of these outbreaks that became large (i.e., >500 clusters). We found that if more than a few cases were missed, large outbreaks could occur under ring vaccination (Figure 2, panel A). This event could occur because missed cases, which had a higher reproduction number, would not be inside the ring when vaccination was introduced. Although ring vaccination failed to contain the outbreak in this scenario, it still reduced disease transmission (online Technical Appendix

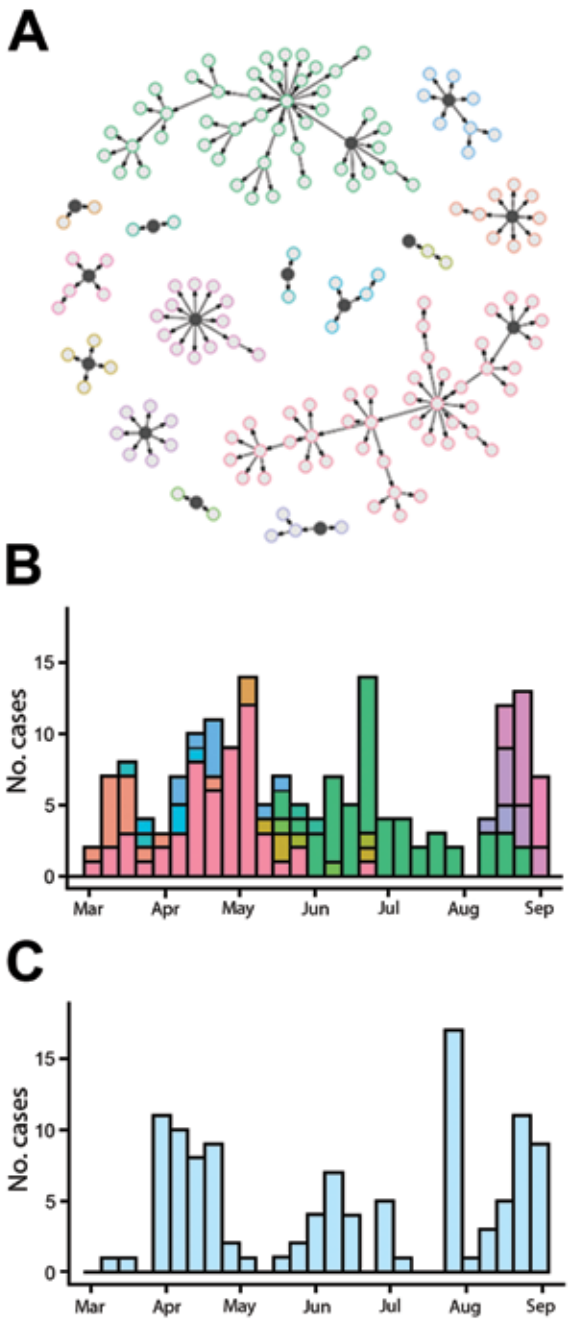

Figure 1. Outbreak dynamics in a model of transmission of Ebola virus disease. A) Chains of transmission generated in a simulated outbreak starting with 2 infected persons on March 1, 2014. Black circles indicate the index case within each cluster, and arrows indicate routes of transmission. Within each cluster, we assumed that there was a 15% probability that a secondary case would be missed and would instead seed a new cluster (these missed links are not shown). B) New cases per week, by date of symptom onset, for the chains of transmission shown in panel A. Colors of clusters in panel A match colors of bars in panel B. C) Observed weekly confirmed and probable cases reported in Conakry Prefecture, Guinea, during March–September 2014. Data were obtained from the Guinea Ministry of Health and World Health Organization Situation Reports (*11*).

Figure 4). We also considered the effect of preemptive mass vaccination, which reduced the reproduction number for all cases by a factor of 0.44, regardless of whether cases were in the cluster or missed. This strategy was more effective in containing outbreaks, even if many cases were missed (Figure 2, panel A). Similar qualitative patterns were observed when vaccine efficacy was 95% (online Technical Appendix Figure 5).

In the later stages of the EVD epidemic in West Africa, behavior changes and improved control measures led to less transmission from burials and in hospital settings than in early 2014 (*12*). Similar reductions were observed in other Ebola outbreaks (e.g., in 1976 in Yambuku, Zaire) (*13*). We therefore also explored a partial control scenario. We omitted index cases in the 2014 Guinea transmission chains that were involved in funeral or hospital transmission, which resulted in $R_m = 2.5$ for missed cases (online Technical Appendix Figure 6). We also assumed a shorter duration of infectiousness and time to reporting on the basis of data for 2015 (*3,10*) (online Technical Appendix Table 2).

In this partial control scenario, outbreaks could be controlled with ring vaccination, even if 40% of cases were missed (Figure 2, panel B). Our results suggest that ring vaccination could substantially reduce the potential size and duration of outbreaks if other control measures are also in place (Table). We also estimated how many vaccine doses would be required for ring vaccination (online Technical Appendix); in the partial control scenario, several thousand doses might be needed (online Technical Appendix Table 3). We could not estimate doses required for mass vaccination, and thus could not perform an economic analysis of different strategies, because this would depend on the potential for long-distance transmission events and populations in different areas. However, implementing mass vaccination for even a single district in West Africa could require >100,000 doses.

Our analysis has some limitations. In the early 2014 transmission scenario, we assumed that missed cases had a much higher reproduction number than cases within clusters. However, if an effective vaccine became available, persons at risk might be more likely to engage with public health efforts. The high reproduction number for index cases might also be caused in part by ascertainment bias: cases that generate many secondary infections are more likely to be designated as index cases. We also assumed that mass vaccination would target 70% of the population at random; in practice, there could be clustering effects. Furthermore, we assumed that chains of transmission were independent and that the reproduction number remained unchanged over time. In reality, missed cases might have shared contacts and behavior might change during outbreak, which could reduce transmission. Our estimates are therefore likely to represent a reasonable worst-case scenario.

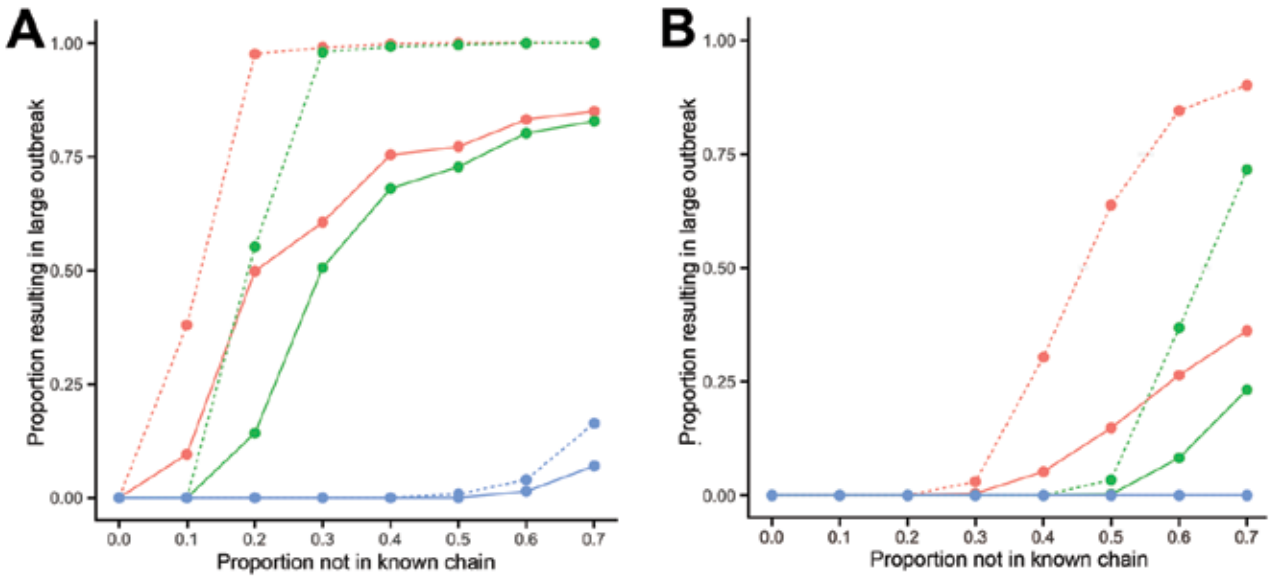

Figure 2. Effectiveness of vaccination strategies for Ebola virus disease under different transmission scenarios. A) Proportion of simulations that led to a large outbreak (defined as >500 clusters) in the early 2014 Guinea transmission scenario. Red lines indicate no vaccination, green lines indicate ring vaccination, blue lines indicate mass vaccination; solid lines indicate outbreaks that started with 1 index case, and dashed lines indicate outbreaks that started with 5 index cases. We simulated 1,000 outbreaks and calculated the proportion that resulted in >500 clusters. When the space between the red and green lines is large, the model suggests that ring vaccination would provide substantial additional value over standard public health control measures alone. B) Proportion of simulations that led to a large outbreak in partial control scenario.

Conclusions

Ring vaccination enhances standard public health measures of contact tracing, isolation, and community engagement (*14*) and could be effective when such measures are in place. However, if standard measures are not working because many cases are not in known transmission chains, as in West Africa in early 2014, ring vaccination might be insufficient to contain the outbreak. If an EVD vaccine is shown to be efficacious, our results suggest that mass vaccination, or hybrid strategies involving mass and ring vaccinations, might need to be considered alongside ring vaccination when planning for future outbreaks.

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Table. Estimated total cases and outbreak duration in partial control scenario with 5 index cases initially by using the model of Ebola virus transmission*

Probability of case missed	No vaccination	Ring vaccination	Mass vaccination
Median no. cases (95% CI)			
10%	42 (14–235)	30 (13–79)	13 (7–60)
20%	63 (15–551)	39 (14–131)	13 (7–57)
30%	104 (17–2,660)	53 (15–229)	13 (6–48)
40%	296 (20–2,410)	78 (18–452)	13 (6–46)
Duration of outbreak, d (95% CI)			
10%	87 (28–278)	62 (26–145)	41 (12–139)
20%	123 (33–480)	83 (31–214)	43 (11–149)
30%	185 (43–1,020)	110 (36–319)	47 (11–142)
40%	364 (51–1,150)	149 (45–486)	47 (9–147)

*Model assumes 80% vaccine efficacy.

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Autochthonous *Nocardia cerradoensis* Infection in Humans, Spain, 2011 and 2014

Maria Ercibengoa, Emilio Pérez-Trallero, José Maria Marimón

Nocardia cerradoensis was first isolated in 2003 in the El Cerrado region of Brazil; since then, only 2 human infections, in France and Spain, have been reported. We describe 3 autochthonous cases in residents of Spain during 2011 and 2014. Together these cases support the idea of an emerging global pathogenic microorganism.

Nocardia cerradoensis was first described in 2003 from a soil sample collected from a cultivated field in the Brazilian Cerrado, a broad region of woodland savannah (*1*). However, the first human case of disseminated infection was not reported until 2015; the patient was an immunosuppressed woman in Rennes, France (*2*). Later in 2015, a case of human skin infection with *N. cerradoensis* was described in Spain (*3*).

Little is known about the transmission of *Nocardia* species, although it is assumed that the bacteria enter the body mainly by inhalation of contaminated dust. In fact, pulmonary nocardiosis, which mostly affects immunocompromised patients, is the most frequent clinical manifestation of *Nocardia* infection (*4*). We report 3 autochthonous cases of *N. cerradoensis* infection in humans in Spain and discuss the source of infection with this recently described human pathogen.

The Study

The study was performed at Donostia University Hospital in San Sebastián-Donostia, the capital city of the province of Gipuzkoa in northern Spain, during 1992–2014. A total of 253 isolates of *Nocardia* species were obtained from 179 patients. Species identification was performed by sequencing 1,188-bp and 401-bp fragments of the 16S rRNA (*5*) and *hsp65* (65-kDa heat shock protein) (*6*) genes, respectively. A total of 3 *N. cerradoensis* isolates were identified; for these and other infrequently found species, we also analyzed 400-bp and 445-bp fragments of the *rpoB* (RNA polymerase B) (*7*) and *secA1* (essential secretory protein SecA1) (*8*) genes. We then identified similar gene sequences in GenBank by using blastn (http://www.ncbi.nlm.nih.gov/blast).

For the 3 *N. cerradoensis* strains, sequences of the 16S rRNA, *rpoB*, and *secA1* gene fragments had the best GenBank matches (≥99% similarity) with corresponding genes of *N. cerradoensis* strains W9747 (accession no. NR117400) and DSM44546 (accession nos. JN215712 and JN042082). Sequences of the *hsp65* genes from the 3 isolates shared 99.9% similarity with each other but only 85.0% similarity with the *hsp65* gene sequence of *N. cerradoensis* strain DSM44546 (accession no. AY756519). Sequences from our study were submitted to GenBank (accession nos. KT749656– KT749667).

Antimicrobial susceptibility was determined by broth microdilution (Sensititer microtiter trays; Trek Diagnostics Systems, East Grinstead, UK), and results were interpreted according to Clinical and Laboratory Standards Institute guidelines (*9*). Pulsed-field gel electrophoresis (PFGE) was performed as described (*10*), except that restriction enzyme digestion was performed with *Xba*I.

The first *N. cerradoensis* strain isolated in Gipuzkoa was from a morbidly obese 67-year-old woman with stable, but severe, chronic obstructive pulmonary disease (COPD) that was being treated with corticosteroids and bronchodilators. On January 18, 2011, the woman had a routine follow-up visit with her general practitioner, during which a sputum sample was obtained. *Haemophilus influenzae* and *N. cerradoensis* were isolated from the sample, which was of good microbiologic quality (>25 leukocytes and no squamous epithelial cells per 10× magnification field). The patient's COPD status was stable, so she was not given antimicrobial drugs for treatment of either of the 2 pathogens. However, on February 11, 2011, she was hospitalized in the study hospital's pulmonary department because of an episode of respiratory failure in the context of an acute COPD exacerbation. No clinical specimens were obtained for microbiologic testing, but because *N. cerradoensis* was previously isolated from her sputum, the patient was given supportive care, inhaled tobramycin, and oral trimethoprim/sulfamethoxazole (160/800 mg) for 1 month, and the symptoms resolved. Sputum samples collected on February 14 and March 30 were negative for *Nocardia* species. Of note, the daughter of this patient had lived in El Cerrado for a number of years; however, the patient had not visited her daughter in Brazil before the isolation of *N. cerradoensis*, and the daughter had not returned to Spain in the year preceding isolation of *N. cerradoensis* from her mother.

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The second *N. cerradoensis* strain was isolated on October 11, 2011, from a sputum sample of a 64-year-old man with a history of moderate COPD. He was a heavy smoker and had been using inhaled, short-acting bronchodilators and corticosteroids since 2001. The patient sought outpatient consultation at the study hospital because of cough and hemoptysis; he was afebrile. Tuberculin test results and cultures for mycobacteria were negative. The patient was empirically treated with amoxicillin/clavulanic acid. Sputum samples collected on October 29 and 30 were negative for *N. cerradoensis* and other potential pathogens.

The third *N. cerradoensis* strain was isolated from the sputum sample of an 82-year-old man with pulmonary emphysema. On October 16, 2014, the man sought care in the study hospital's emergency department for increasing dyspnea of a few days' duration that had not improved despite treatment with corticosteroids and levofloxacin (500 mg 1×/d for 3 days). The patient had frequent coughing with yellowish expectoration but no fever; he received a diagnosis of respiratory infection and alteration (worsening) of his COPD status. Thoracic scan results showed cylindrical bronchiectasis and subpleural consolidations in both lungs; culture of a sputum sample obtained on October 22 was positive for *N. cerradoensis* and *Aspergillus fumigatus*. Because of the patient's clinical status, the *A. fumigatus* was considered a colonizer (i.e., present without causing active disease). He was hospitalized and given trimethoprim/sulfamethoxazole (160/800 mg 2×/d for 30 days) for treatment of the *N. cerradoensis* infection. Cultures of sputum samples collected on December 3 and 16 were negative for *Nocardia* species.

The 3 *N. cerradoensis* isolates showed the same antimicrobial susceptibility pattern: nonsusceptibility to amoxicillin/clavulanic acid, minocycline, and ciprofloxacin (MICs >64/32 μg/mL, >4 μg/mL, and >8 μg/mL respectively) and susceptibility to ceftriaxone, imipenem, linezolid, amikacin, and trimethoprim/sulfamethoxazole (MICs ≤8 μg/mL, ≤0.25 μg/mL, 1 μg/mL, ≤0.25 μg/mL, and ≤0.5/9.5 μg/mL, respectively). After *Xba*I digestion, isolates from the first 2 patients had indistinguishable PFGE patterns that differed from that of the third patient's isolate (Figure). However, no epidemiologic relationship could be established between the first and second patients: they lived in different villages (≈50 km apart) and had not been hospitalized nor been in the hospitals outpatient clinic or emergency department at the same time.

Because of the environmental origin of *Nocardia* species, their isolation, particularly in respiratory samples, might indicate colonization rather than clinical infection. In these cases, *N. cerradoensis* was isolated from the bronchial secretions of 3 patients who were at high risk for development of *Nocardia* clinical infection because they were immunosuppressed as a consequence of long-term corticosteroid therapy for COPD. The first and third patients received a diagnosis of pulmonary nocardiosis and were specifically treated with trimethoprim/sulfamethoxazole, with symptom resolution. However, the physicians in charge considered the presence of *N. cerradoensis* in the second patient to be a colonization rather than active disease. This diagnosis was reinforced by the quick disappearance of the pathogen from respiratory samples without specific treatment (the patient received only a short course of amoxicillin/clavulanic acid, to which *N. cerradoensis* was resistant).

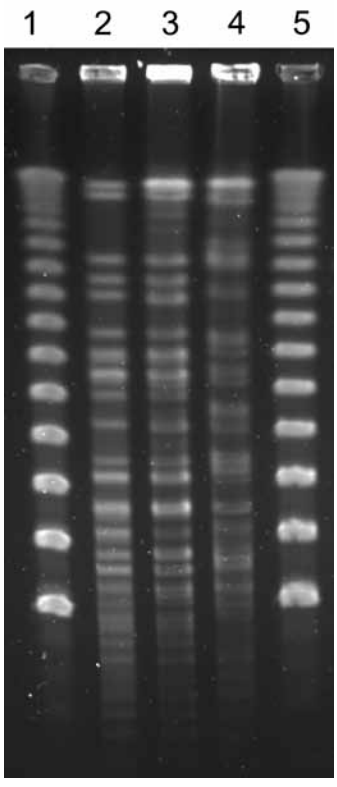

Figure. Pulsed-field gel electrophoresis patterns of *Nocardia cerradoensis* isolates (after *Xba*I restriction enzyme digestion) from 3 chronic obstructive pulmonary disease patients in Gipuzkoa, northern Spain. Lanes 1 and 5, DNA molecular weight marker (50-kbp ladder). Lanes 2, 3, and 4, isolates corresponding to patients 1, 2, and 3, respectively.

Conclusions

N. cerradoensis has not been isolated from environmental samples from any part of Europe. The first *N. cerradoensis* isolate detected in Gipuzkoa was from a patient whose daughter lived in the El Cerrado region of Brazil. However, 2 facts point to local acquisition of the pathogen: the patient and her daughter had not had personal contact during the year preceding the isolation of *N. cerradoensis*, and PFGE patterns were identical for the isolates from this patient and the second patient, with whom she had no contact. Our finding of human infections with *N. cerradoensis*, together with the reported cases from France and from a different city in Spain (*2,3*) support the idea of an emerging global pathogenic microorganism.

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Dr. Ercibengoa is a biologist and investigator in the Microbiology Department of the University Hospital Donostia, San Sebastián, Spain. Her research interests are in the area of respiratory infections, especially infections caused by *Streptococcus pneumoniae* and *Nocardia* species.

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Asymptomatic Lymphogranuloma Venereum in Men who Have Sex with Men, United Kingdom

Cara Saxon, Gwenda Hughes, Catherine Ison, for the UK LGV Case-Finding Group[1]

We investigated prevalence of lymphogranuloma venereum (LGV) among men who have sex with men who were tested for chlamydia at 12 clinics in the United Kingdom during 10 weeks in 2012. Of 713 men positive for *Chlamydia trachomatis*, 66 (9%) had LGV serovars; 15 (27%) of 55 for whom data were available were asymptomatic.

Lymphogranuloma venereum (LGV) is a sexually transmitted infection (STI) caused by the L1, L2, and L3 serovars of *Chlamydia trachomatis* (CT). An LGV outbreak among men who have sex with men (MSM) first reported in the Netherlands in 2003 has since spread across other industrialized countries (*1*). Cases are typically seen among white, HIV-positive MSM who report unprotected anal intercourse, other high-risk behaviors, and STI co-infection and who commonly have symptoms of proctitis (i.e., rectal pain, rectal discharge, bloody stools, constipation, and tenesmus) (*2*).

The United Kingdom now has the largest documented outbreak of LGV among MSM worldwide (*3,4*). Infection control in England has relied on CT DNA typing and treatment of symptomatic MSM who have CT-positive rectal infections and their contacts, as well as health promotion. These measures were supported by a large prospective study in the United Kingdom during 2006–2007 that reported <6% of LGV CT infections were asymptomatic (*5*). However, studies in the Netherlands and Germany, and a smaller UK study, have reported higher proportions (17%–53%) of asymptomatic infection (*6–8*). We reinvestigated the prevalence of asymptomatic LGV CT infection among MSM in the United Kingdom to assess whether it may be sustaining the current epidemic.

The Study

In the UK, STI clinics are open access and provide free testing and treatment. Regular STI and HIV screening is encouraged for sexually active MSM with or without symptoms (*9*). A full medical and sexual history are recorded for all patients, and a physical examination is done for those with symptoms.

Twelve UK STI clinics participated; all serve cities with large MSM populations and routinely screen MSM for CT by examining urine or swab samples of the pharynx, urethra, and rectum (either clinician-obtained or self-taken) according to UK guidelines (*10*). All MSM tested for CT during September 24–December 7, 2012, were included except those who had received antibiotic drugs during the previous 6 weeks.

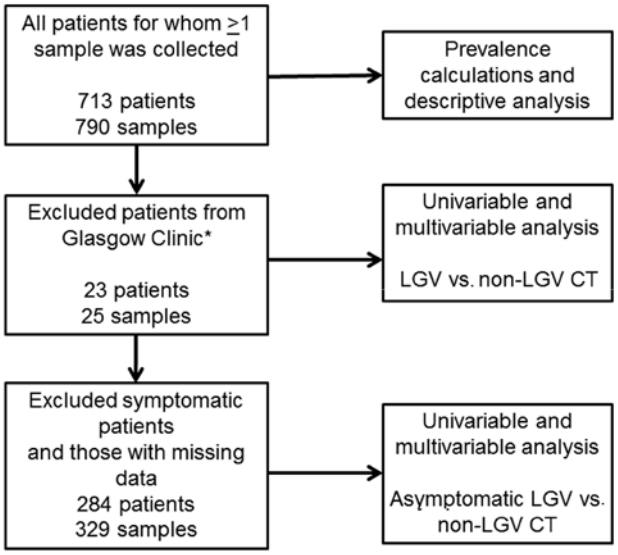

Figure. Data analysis flowchart for univariable and multivariable analyses of symptomatic lymphogranuloma venereum (LGV) versus non-LGV *Chlamydia trachomatis* (CT) infection (Table 1) and asymptomatic LGV versus non-LGV CTinfection (Table 2) in men who have sex with men, United Kingdom. *Patients from Glasgow were excluded from risk factor analyses because they do not routinely report to the Genitourinary Medicine Clinic Activity Dataset.

More than 10,000 CT tests were performed during the study period. Local laboratories performed routine testing for CT and referred all positive specimens from study participants to the Sexually Transmitted Bacteria Reference Unit of the national reference laboratory in London to test for LGV. At the reference laboratory, all specimens underwent extraction by using the Roche MagNA Pure LC extractor (Roche Diagnostics, Indianapolis, IN, USA), then CT confirmation by using a plasmid targeted real-time PCR and an LGV-specific real-time PCR targeting the *pmpH* deletion on the RotorGene (QIAGEN, Valencia, CA, USA). Details of the LGV reference service were previously published (*11*).

Clinical data for symptoms were submitted for all study participants to Public Health England (PHE) through a

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[1]Additional members of the UK LGV Case-Finding Group who contributed to data collection, analysis, and manuscript review are listed at the end of this article.

secure web portal (online Technical Appendix Figure, http://wwwnc.cdc.gov/EID/article/22/1/14-1867-Techapp1.pdf). Patients reporting symptoms at first medical examination or follow-up were defined as symptomatic. Those with no symptoms at first examination or follow-up were defined as asymptomatic. Additional clinical data were available from the national anonymized patient-level electronic surveillance system (the Genitourinary Medicine Clinic Activity Dataset [GUMCADv2]), which records all tests and diagnoses in STI clinics in England (*12*).

PHE has authority to collect anonymized patient-level data for public health monitoring and infection control. The study was reviewed in PHE's research and development office and deemed to fit this criterion.

We used univariable and multivariable logistic regression modeling in STATA version 13.1 (StataCorp LP, College Station, TX, USA) to investigate risk factors associated with LGV versus non-LGV CT infection and asymptomatic versus symptomatic LGV. A clinic in Glasgow, Scotland, was excluded from risk factor analyses because it does not report GUMCADv2 (Figure).

During the study period, 921 eligible specimens were received for DNA typing. On confirmatory testing, 90 (10%) specimens were CT negative, 36 (4%) inhibitory,

Table 1. Descriptive, univariable, and multivariable analysis of all patients with LGV versus non-LGV CT infection among men who have sex with men, by demographic and behavioral characteristics, United Kingdom*

Patient characteristics	No. (%) or median [IQR]			Univariable analysis		Multivariable analysis†	
	All CT	Non-LGV CT	LGV CT	OR (95% CI)	p value	OR (95% CI)	p value
All patients	713 (100)	647 (90.7)	66 (9.3)	ND	ND	ND	ND
Clinic location, n = 713							
London	563 (79.0)	512 (79.1)	51 (77.3)	1	0.73		
Manchester	85 (11.9)	75 (11.6)	10 (15.2)	1.34 (0.65–2.75)			
Brighton	42 (5.9)	38 (5.9)	4 (6.1)	1.06 (0.36–3.08)			
Glasgow‡	23 (3.2)	22 (3.4)	1 (1.5)				
Infection site, n = 710							
Nonrectal	221 (31.1)	217 (33.7)	4 (6.1)	1		1	
Rectal	489 (68.9)	427 (66.3)	62 (93.9)	7.83 (2.81–21.82)	<0.001	10.08 (3.37–30.17)	<0.001
Multiple infection sites, n = 713							
No	641 (89.9)	581 (89.8)	60 (90.9)	1			
Yes	72 (10.1)	66 (10.2)	6 (9.1)	0.89 (0.37–2.15)	0.8		
Symptoms present, n = 650							
No	453 (69.7)	438 (73.6)	15 (27.3)	1		1	
Yes	197 (30.3)	157 (26.4)	40 (72.7)	7.93 (4.20–14.99)	<0.001	13.33 (6.53–27.21)	<0.001
Age, y, n = 710	33 [27–42]	33 [27–42]	39 [33–46]				
18–24	108 (15.2)	106 (16.5)	2 (3.0)	1	0.002		
25–34	276 (38.9)	258 (40.1)	18 (27.3)	3.72 (0.85–16.31)			
35–44	192 (27.0)	166 (25.8)	26 (39.4)	7.92 (1.84–34.15)			
≥44	134 (18.9)	114 (17.7)	20 (30.3)	9.11 (2.08–39.93)			
Ethnicity, n = 603							
White	480 (79.6)	432 (79.3)	48 (82.8)	1	0.17		
Black or Black British	28 (4.6)	27 (5)	1 (1.7)	0.33 (0.44–2.51)			
Mixed	27 (4.5)	27 (5)	0	NP			
Asian and Asian British	21 (3.5)	21 (3.9)	0	NP			
Other ethnic groups	23 (3.8)	19 (3.5)	4 (6.9)	1.89 (0.62–5.80)			
Unknown	24 (4)	19 (3.5)	5 (8.6)	2.37 (0.85–6.63)			
HIV status, n = 603							
Negative	367 (60.9)	350 (64.2)	17 (29.3)	1		1	
Positive	236 (39.1)	195 (35.8)	41 (70.7)	4.33 (2.40–7.82)	<0.001	3.63 (1.80–7.32)	<0.001
No. sexual partners in previous 3 mo, n = 635							
No. partners	3 [1–5]	3 [1–5]	3 [1–8]				
0–1	169 (26.6)	153 (26.3)	16 (29.6)	1	0.26		
2–5	321 (50.6)	299 (51.5)	22 (40.7)	0.66 (0.34–1.31)			
≥6	145 (22.8)	129 (22.2)	16 (29.6)	1.13 (0.54–2.36)			
Concurrent sexually transmitted infection, n = 631							
No	451 (71.5)	406 (71)	45 (76.3)	1			
Yes	180 (28.5)	166 (29)	14 (23.7)	0.76 (0.41–1.42)	0.39		
STI within previous 12 mo, n = 594							
No	469 (79)	435 (80.3)	34 (65.4)	1			
Yes	125 (21)	107 (19.7)	18 (34.6)	2.15 (1.17–3.96)	0.01		

*n values indicate number of patients for which data were available in each category. CT, *Chlamydia trachomatis*; LGV, lymphogranuloma venereum; IQR, interquartile range; OR, odds ratio; ND, no data were available; NP, not performed. Blank cells indicate not applicable.
†Multivariable analysis adjusted for age and location.
‡Glasgow patients were excluded from all univariable and multivariable analyses because they do not routinely report to the Genitourinary Medicine Clinic Activity Dataset.

Table 2. Descriptive, univariable and multivariable analysis of patients in whom asymptomatic LGV CT or asymptomatic non-LGV CT infection was diagnosed, by demographic and behavioral characteristics, United Kingdom*

Patient characteristics	No. (%) or median [IQR] All asymptomatic CT	Asymptomatic non-LGV CT	Asymptomatic LGV	Univariable analysis OR (95% CI)	p value	Multivariable analysis† OR (95% CI)	p value
All patients	429 (100.0)	414 (96.5)	15 (3.5)				
Clinic location, n = 429							
London	333 (77.6)	323 (78.0)	10 (66.7)	1	0.47		
Manchester	54 (12.6)	52 (12.6)	2 (13.3)	1.24 (0.26–5.83)			
Brighton	26 (6.1)	24 (5.8)	2 (13.3)	2.69 (0.56–12.99)			
Glasgow‡	16 (3.7)	15 (3.6)	1 (6.7)				
Infection site, n = 427							
Nonrectal	129 (30.2)	126 (30.6)	3 (20.0)	1			
Rectal	298 (69.8)	286 (69.4)	12 (80.0)	1.67 (0.46–6.08)	0.44		
Multiple infection sites, n = 429							
No	397 (92.5)	383 (92.5)	14 (93.3)	1			
Yes	32 (7.5)	31 (7.5)	1 (6.7)	0.95 (0.12–7.48)	0.96		
Age, y, n = 429	33 [27–42]	33 [26–42]	38 [29–44]				
18–24	73 (17.0)	72 (17.4)	1 (6.7)	1	0.64		
25–34	172 (40.1)	167 (40.3)	5 (33.3)	2.19 (0.25–19.07)			
35–44	105 (24.5)	99 (23.9)	6 (40.0)	3.72 (0.43–32.59)			
≥44	79 (18.4)	76 (18.4)	3 (20.0)	2.8 (0.28–27.55)			
Ethnicity, n = 378							
White	302 (79.9)	290 (79.5)	12 (92.3)	1	0.32		
Black or Black British	18 (4.8)	18 (4.9)	0	NP			
Mixed	19 (5.0)	19 (5.2)	0	NP			
Asian and Asian British	17 (4.5)	17 (4.7)	0	NP			
Other ethnic groups	13 (3.4)	13 (3.6)	0	NP			
Unknown	9 (2.4)	8 (2.2)	1 (7.7)	3.02 (0.35–26.13)			
HIV status, n = 378							
Negative	235 (62.2)	231 (63.3)	4 (30.8)	1			
Positive	143 (37.8)	134 (36.7)	9 (69.2)	3.88 (1.17–12.84)	0.03	3.91 (0.92–16.66)	0.06
No. sexual partners in previous 3 mo, n = 401							
No. partners	3 [1–5]	3 [1–5]	3 [2–5]				
0–1	106 (26.4)	103 (26.7)	3 (20.0)	1	0.87		
2–5	207 (51.6)	198 (51.3)	9 (60.0)	1.41 (0.37–5.44)			
≥6	88 (21.9)	85 (22.0)	3 (20.0)	1.16 (0.23–5.90)			
Concurrent sexually transmitted infections, n = 398							
No	295 (74.1)	283 (73.7)	12 (85.7)	1			
Yes	103 (25.9)	101 (26.3)	2 (14.3)	0.47 (0.10–2.12)	0.32		
STI during previous 12 mo, n = 374							
No	297 (79.4)	291 (80.4)	6 (50.0)	1			
Yes	77 (20.6)	71 (19.6)	6 (50.0)	4.1 (1.28–13.09)	0.02	3.1 (0.87–10.99)	0.08

*n values indicate number of patients for which data were available in each category. CT, *Chlamydia trachomatis*; LGV, lymphogranuloma venereum; No., number; IQR, interquartile range; CI, confidence interval; OR, odds ratio; NP, not performed. Blank cells indicate not applicable.
†Multivariable analysis adjusted for age and location
‡Glasgow patients were excluded from all univariable and multivariable analyses because they do not routinely report to the Genitourinary Medicine Clinic Activity Dataset.

and 1 (0.1%) equivocal; 4 (0.4%) were not tested. CT infection was confirmed in 790 specimens from 713 patients; these specimens then underwent DNA typing. Overall, we found 69 (9%) LGV CT–positive specimens from 66 (9%) patients and 721 (91%) non-LGV CT–positive specimens from 647 (91%) patients (Table 1). Co-infection with LGV CT at 1 anatomic site and non-LGV CT at a different site was found in 4/713 (0.6%) patients. Clinical data coordinating with the symptom checklist and GUMCADv2 were available for 95% (680/713) and 87% (603/690) of CT-positive patients, respectively. During the study period, GUMCADv2 recorded 1,097 CT diagnoses among 10,143 MSM screened at the 11 STI clinics in England, showing an estimated CT prevalence of 10.8%.

Compared to those positive for non-LGV CT, patients with LGV CT infection were older and more likely to be symptomatic, to be HIV-positive, to have rectal infection, and to have had a previous STI diagnosis. In adjusted logistic regression analysis, symptomatic infection (adjusted odds ratio [aOR] 13.33; p<0.001), rectal infection (aOR 10.08; p<0.001) and being HIV-positive (aOR 3.63; p<0.001) remained statistically significant (Table 1).

Of those with LGV for whom data were available, 27% (15/55) overall and 22% (12/54) with rectal-only infection

were asymptomatic. Study prevalence of asymptomatic LGV was 2.3% (15/650) overall and 3.8% (9/236) in HIV-positive MSM (Tables 1, 2).

Of the 15 patients with asymptomatic LGV, 12 (80%) had rectal, 2 (13%) urethral, and 1 (7%) pharyngeal infections. All cases of asymptomatic LGV were from patients with single-site infection.

Among asymptomatic patients, those with LGV were more likely to be HIV-positive (69% vs. 31%; odds ratio 3.88; p = 0.03) and to have had an STI in the past 12 months (50% vs. 20%; odds ratio 4.1; p = 0.02) than those infected with non-LGV CT. These characteristics were only weakly associated in the adjusted analysis (aOR 3.91, p = 0.06, and aOR 3.1, p = 0.08, respectively).

Conclusions

This large multicenter case-finding study found a higher rate of asymptomatic LGV (27%) than previously reported in the United Kingdom, in agreement with studies done in Germany and the Netherlands. LGV case-patients were typically older, white, HIV-positive MSM who had a concurrent or recent STI diagnosis. Most infections were rectal; few urethral and pharyngeal infections were detected.

The number of CT infections confirmed at the reference laboratory (713) was lower than those reported to national surveillance (1,097), possibly related to differences in test sensitivity, degradation of CT DNA during transportation, or incorrect surveillance coding. No patients were excluded because of study restrictions; therefore, it is likely the study case-patients were representative of all MSM with diagnoses of CT infection in the United Kingdom.

More than one quarter of LGV cases in the United Kingdom may go undiagnosed if those who have asymptomatic chlamydial infection are not tested, as is the current strategy. Recommending that all CT-positive specimens from MSM be DNA tested for LGV serovars is unlikely to be cost-effective or feasible. However, because 3.8% of asymptomatic HIV-positive MSM had LGV (i.e., in excess of the recommended 3% prevalence threshold for CT screening [*13*]), inclusion of these patients in the testing algorithm, as is done in Scotland (*14*), may be warranted.

Whether LGV symptomatology in the United Kingdom has changed or asymptomatic cases were previously missed is unclear. Changes in screening practice or selection pressure for asymptomatic infection after treatment of persons with symptomatic infection may have contributed. Most asymptomatic patients will be treated for non-LGV CT infection, but if treatment is suboptimal, it may not prevent onward transmission (*15*).

An undiagnosed reservoir of CT infection is unlikely to be the sole cause of the current epidemic. High-risk sexual behavior remains a substantive challenge for control of LGV and related epidemics among MSM (*3*). Future public health strategies will require a combined strategy of increased testing, prompt treatment, and continued promotion of safer sexual behavior among MSM.

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Increased Risk for ESBL-Producing Bacteria from Co-administration of Loperamide and Antimicrobial Drugs for Travelers' Diarrhea[1]

Anu Kantele, Sointu Mero, Juha Kirveskari, Tinja Lääveri

Antimicrobial drug treatment of travelers' diarrhea is known to increase the risk for colonization with extended-spectrum b-lactamase-producing *Enterobacteriaceae*. Among 288 travelers with travelers' diarrhea, the colonization rate without medications was 21%. For treatment with loperamide only, the rate was 20%; with antimicrobial drugs alone, 40%; and with loperamide and antimicrobial drugs, 71%.

Resistance to antimicrobial drugs (AMDs) is predisposed in areas with poor hygiene and weak or nonexistent antimicrobial policy. Travelers visiting these areas presumably have a central role as transporters of multidrug-resistant intestinal bacteria across the globe (*1*), because a significant proportion of travelers (20%–70%) to high-prevalence areas become colonized with extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) (*2*–*7*). Clinical infections do not develop in most travelers (*6*), and colonization is transient, waning within months (*7*). However, as evidenced by intrahousehold transmission from colonized patients after hospitalization, the bacteria may spread to household members (*8*,*9*) and eventually to local healthcare settings in the home countries of the travelers.

Several factors have been identified to increase the risk for ESBL-E colonization: travel destination (*2*–*7*), travelers' diarrhea (TD) (*2*,*6*,*7*), use of AMDs (*5*–*7*), and age (*2*,*6*). In a recent study, we found that ESBL-E was contracted by 11% of travelers who did not have TD and did not take AMDs (TD–AMD–), 21% of those with TD who did not take AMDs (TD+AMD–), and 37% of those with TD who took AMDs (TD+AMD+) (*6*). Our conclusion that mild or moderate diarrhea should not be treated with AMDs raised questions about safe alternatives (*10*). In our previous study, probiotics appeared not to affect colonization (*6*). We found no studies that assessed possible risks posed by non-AMD antidiarrheal medications for treating TD, such as loperamide.

Loperamide, a drug with both antisecretory and antimotility effects (*11*), is widely used by travelers (*12*). Although mostly used alone, loperamide is sometimes used with AMDs; the combination stops symptoms faster than AMDs alone during the first 2 days of TD. After that, the combination no longer appears advantageous, probably because symptoms resolve naturally (*11*). Using loperamide with AMDs is presented as a safe option in general guidelines published by the US Centers for Disease Control and Prevention (*13*). However, the effects of co-administration on the risk for ESBL-E acquisition have not been addressed.

Some researchers have posed the question as to whether the antimotility effect of loperamide, involving prolonged passage through the gastrointestinal tract, would, in fact, increase the risk for colonization (data not shown). Such speculations prompted us to revisit our recent data (*6*) to compare loperamide, AMDs, and their combination in the treatment of TD with regard to the risk for contracting travel-acquired ESBL-E.

The Study

We reviewed our recent data on ESBL-E acquisition among 430 travelers from Finland (*6*), selecting those with TD for separate analysis (Figure). All the volunteers provided fecal samples and completed questionnaires before and after travel. Symptoms of TD and use of medications, such as loperamide and AMDs, were included in the post-travel questionnaires. The countries visited were grouped as described (Table 1; *6*); processing of fecal specimens and identification of ESBL-E were detailed in our previous study (*6*). TD was defined by the World Health Organization criteria: passing ≥3 loose/liquid stools per 24 hours, or more frequently than normal (*14*).

Study participants were divided into 4 groups by treatment of TD: those taking no loperamide or AMDs (LO–AMD–); only loperamide (LO+AMD–); only AMDs (LO–AMD+); or loperamide plus AMDs (LO+AMD+). Those having taken AMDs for non-TD indications were categorized in groups with those with TD who took AMDs.

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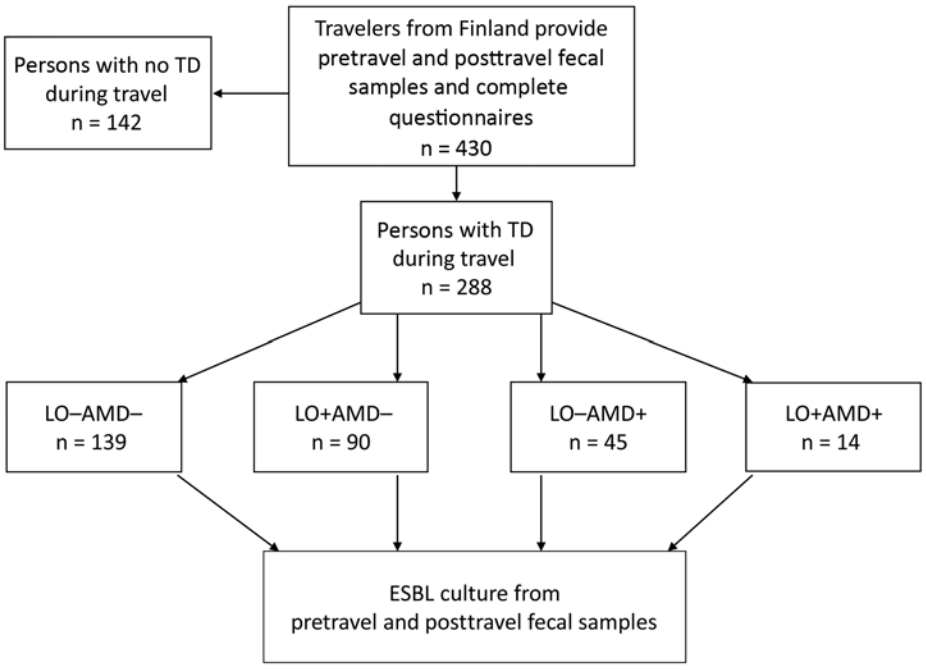

Figure. Study protocol for investigating risk for contracting ESBL-producing *Enterobacteriaceae* among travelers from Finland with TD. LO–AMD–, not treated with medication; LO+AMD–, treated with LO alone; LO–AMD+, treated with AMDs alone; LO+AMD+, treated with a combination of both drugs. AMD, antimicrobial drugs; ESBL, extended-spectrum β-lactamase; LO, loperamide; TD, travelers' diarrhea.

We used a multivariable binary logistic regression model to test our main hypotheses. Loperamide, AMDs, and their interaction (effect modification) were included in the model, along with risk factors that showed a p value <0.2 in univariate analysis in our previous study (*6*): sex, travel destination, use of AMDs, meals with residents of the location, contact with local healthcare, sites of meals, accommodations, duration of travel, age, and use of alcohol. Variables were eliminated to the final model by using backward selection of factors by Akaike Information Criteria, except for loperamide and the use of AMDs and their interaction, which were forced to the final model. Missing values were taken into account by multiple imputations, to reduce possible biases and efficiency loss, assuming that data were missing at random. We analyzed statistics using SPSS statistical software version 21 (IBM Corporation, Armonk, NY, USA).

Of all travelers in the previous study (*6*), a total of 288 of 430 (67%) who reported TD constituted the final study group (Table 1). ESBL-E was contracted by 26% of the subjects: 21% in the LO–AMD– group; 20% in the LO+AMD– group (adjusted odds ratio [aOR] 0.8, 95% CI 0.4–1.7); 40% in the AMD+LO– group (aOR 2.9, 95% CI 1.2–7.4); and 71% in the LO+AMD+ group (aOR 7.4, 95% CI 1.7–32.6) (Table 2). aOR for the interaction term of loperamide and AMDs was 3.1 (95% CI 0.6–16.6). Travel destination remained an independent risk factor, and sharing meals with locals appeared protective (Table 2).

Studies showing AMD treatment of patients with TD to be an independent risk factor for contracting ESBL-E (*5*–*7*) have evoked the question of less harmful treatments. The recommendation to restrict AMDs to severe cases (*5*,*6*,*15*) seems reasonable, as TD generally remains

Table 1. Characteristics of and co-administered treatments for 288 travelers with travelers' diarrhea*

Characteristics	Total no. (%)	LO–AMD– no. (%)	LO+AMD– no. (%)	LO–AMD+ no. (%)	LO+AMD+ no. (%)
Total	288	139 (48)	90 (31)	45 (16)	14 (5)
Sex					
F	180 (62)	86 (62)	54 (60)	32 (71)	8 (57)
M	108 (38)	53 (38)	36 (40)	13 (29)	6 (43)
Age, y, median (IQR)	34 (25)	34 (26)	34 (23)	35 (24)	31 (38)
Geographic region					
South Asia	46 (16)	19 (14)	17 (19)	5 (11)	5 (36)
Southeast Asia	78 (27)	41 (29)	24 (27)	10 (22)	3 (21)
East Asia	4 (1)	1 (1)	1 (1)	2 (4)	0 (0)
Sub-Saharan Africa	130 (45)	62 (45)	39 (43)	23 (51)	6 (43)
North Africa and Middle East	5 (2)	3 (2)	1 (1)	1 (2)	0 (0)
South and Central America and the Caribbean	23 (8)	12 (9)	7 (8)	4 (9)	0 (0)
Europe and North America	2 (1)	1 (1)	1 (1)	0 (0)	0 (0)

*LO, loperamide; AMD, antimicrobial drugs.

Table 2. Multivariable analysis of acquisition of extended-spectrum β-lactamase-producing *Enterobacteriaceae* by 288 travelers on the basis of administration of treatments for travelers' diarrhea*†

Characteristics	Total, no. (%)	ESBL neg, no. (%)	ESBL pos, no. (%)	Univariate analysis		Multivariable analysis with imputation	
				p value	OR (95% CI)	p value	aOR (95% CI)
Total	288 (100)	213 (74)	75 (26)	NA	NA	NA	NA
Study groups							
LO–AMD–	139 (48)	110 (79)	29 (21)	NA	1.0	NA	1.0
LO+AMD–	90 (31)	72 (80)	18 (20)	0.874	0.9 (0.5–1.8)	0.583	0.8 (0.4–1.7)
LO–AMD+‡	45 (16)	27 (60)	18 (40)	0.012	2.5 (1.2–5.2)	0.022	2.9 (1.2–7.4)
LO+AMD+	14 (5)	4 (29)	10 (71)	<0.001	9.5 (2.8–32.4)	0.008	7.4 (1.7–32.6)§
Travel destination							
South Asia	46 (16)	21 (46)	25 (54)	NA	1.0	NA	1.0
Southeast Asia	78 (27)	48 (62)	30 (38)	0.087	0.5 (0.3–1.1)	0.186	0.6 (0.3–1.3)
East Asia	4 (1)	2 (50)	2 (50)	0.867	0.8 (0.1–6.5)	0.989	1.0 (0.1–12.3)
Sub-Saharan Africa	130 (45)	114 (88)	16 (12)	<0.001	0.1 (0.1–0.3)	<0.001	0.1 (0.05–0.3)
North Africa and Middle East	5 (2)	3 (60)	2 (40)	0.546	0.6 (0.1–6.7)	0.536	0.5 (0.1–3.8)
South and Central America and the Caribbean	23 (8)	23 (100)	0	NA	NA	NA	NA
Europe and North America	2 (1)	2 (100)	0	NA	NA	NA	NA
Other factors							
Sharing meals with locals¶	52 (19)	46 (88)	6 (12)	0.01	0.3 (0.1–0.8)	0.017	0.3 (0.1–0.8)
Contact with local healthcare	32 (11)	18 (56)	14 (44)	<0.001	2.5 (1.2–5.3)	0.314	1.7 (0.6–4.7)

*ESBL, extended-spectrum β-lactamase; OR, odds ratio; aOR, adjusted odds ratio; LO, loperamide; AMD, antimicrobial drugs; LO–AMD–,not treated with medication; LO+AMD–, treated with LO alone; LO–AMD+, treated with AMDs alone; LO+AMD+, treated with a combination of both drugs; NA, not applicable; pos, positive; neg, negative.
†Values represent proportions with a given risk factor, aOR and p values in univariate and multivariable analysis. By using backward selection of factors by Akaike Information Criteria, the following factors were eliminated of the variables in the final model: age, duration of travel, sex, alcohol, site of meals, and type of accommodation.
‡Includes 7 travelers having taken antimicrobial drugs for indications other than TD.
§aOR for interaction term of loperamide and AMDs is 3.1 (95% CI 0.6–16.6).
¶Information missing for 18 travelers.

mild or moderate and resolves spontaneously (*12*,*15*). If symptoms require medical treatment, loperamide appears to be a sensible alternative for travelers who have no fever or bloody stools. However, because of its antimotility effect, its safety against contracting resistant intestinal bacteria has been questioned. Among studies that explored risk factors for ESBL carriage, we found none that showed data on the use of loperamide alone or in combination with AMDs.

Consistent with our previous analysis (*6*), we found AMD treatment of TD was an independent risk factor for colonization with ESBL-E; the rate increased from 21% (LO–AB–) to 40% (LO–AB+) (aOR 2.9, 95% CI 1.2–7.4). When used alone, loperamide did not add to the risk (20% colonized in the LO+AB– group).

In the group taking both loperamide and AMDs, the colonization rate was strikingly high, increasing from 21% (LO–AB–) to 71% (LO+AB+). The rate also appeared to exceed the risk for using AMDs alone (40%), yet the interaction term of loperamide and AMDs did not reach statistical significance (aOR 3.1, 95% CI 0.6–16.6), and the small subject number resulted in wide CIs. Theoretically, an additional increase in the risk seen in the combination group could be brought about by loperamide: because of its antimotility effect, contact time of the AMD to the gut lumen is increased, and the selection pressure posed by the AMD may be prolonged, thus intensifying its unfavorable effects.

Our study design was limited by not including a randomized allocation of therapy and the varied use of loperamide according to symptoms. However, no association was seen between the severity of symptoms and acquisition of ESBL-E (data not shown).

Studies are needed to compare the relative risk posed by various AMD classes. Exploration of the influence of nonantimicrobial antidiarrheal agents with only antisecretory effect, such as racecadotril, as monotherapy and in combination with AMDs would also be beneficial.

Conclusions

Our results show that loperamide alone offers a safe alternative to AMDs for TD treatment, because it does not add to the risk for acquiring drug-resistant intestinal bacteria. In contrast, combining loperamide with AMDs predisposes to ESBL-E colonization and may add to the substantial risk posed by AMDs alone. Our data dispute the safety of this combination.

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Hemagglutinin Gene Clade 3C.2a Influenza A(H3N2) Viruses, Alachua County, Florida, USA, 2014–15

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Influenza A(H3N2) strains isolated during 2014–15 in Alachua County, Florida, USA, belonged to hemagglutinin gene clade 3C.2a. High rates of influenza-like illness and confirmed influenza cases in children were associated with a decrease in estimated vaccine effectiveness. Illnesses were milder than in 2013–14; severe cases were concentrated in elderly patients with underlying diseases.

Influenza vaccines are less effective when antigenic drift occurs (i.e., when amino acids change at the antigenic epitopes of the viruses) (*1*,*2*). The influenza A(H3N2) virus for the 2014–15 vaccine was an A/Texas/50/2012 (H3N2)–like virus, which is a hemagglutinin (HA) gene clade 3C.1 virus (*3*–*5*). We identified an HA gene clade 3C.2a virus as the predominant strain in Gainesville, Florida, USA, during the 2014–15 influenza season and assessed vaccine effectiveness and clinical outcomes associated with this strain. All work was approved by the University of Florida (Gainesville) Institutional Review Board.

The Study

Influenza in Gainesville and the surrounding Alachua County began in September 2014. Multiplex PCR (eSensor respiratory viral panel; GenMark Dx, Carlsbad, CA, USA) for patients seen at a major teaching hospital in Alachua County indicated that >95% of illnesses were caused by H3N2 strains. Influenza A(H3N2) virus HA, neuraminidase (NA), and matrix (M) gene sequences from 5 specimens collected from patients in November 2014 were directly sequenced from the specimens, and corresponding first-passage viruses were isolated in MDCK cells by using previously described primers (*6*,*7*). These viruses were designated as A/Gainesville (H3N2) isolates 5–9 from 2014 (e.g., A/GNVL/05/2014) (Table 1). For all, the consensus viral genomic sequences determined directly from clinical specimens and their matched virus isolates were identical.

The deduced HA major antigenic epitopes A and B1 of the 5 viruses isolated had some amino acids not found in the HA protein of vaccine strain A/Texas/50/2012 (H3N2) (Table 1). No changes were observed for major antigenic epitopes B2, C1, C2, D, and E. Changes at the major antigenic epitopes included N144S, N145S, F159Y, K160T, N225D, and at other epitopes L3I and Q311H; those changes are characteristic of HA gene clade 3C.2a viruses (*4*). Substitutions at residues 144 and 160 have been associated with the loss and the gain, respectively, of possible N-linked glycosylation sites of the HA protein (*8*,*9*). An H3N2 virus isolated in February 2014 in Gainesville, designated A/GNVL/01/2014 (H3N2), was from a different HA gene clade (Table 1), suggesting that the clade 3C.2a viruses were introduced locally later. The NA proteins of the 5 viruses from November 2014 also differed from those of the Northern Hemisphere H3N2 vaccine strain at 3 amino acids, whereas the M proteins were identical.

Data on frequency of medically attended influenza-like illness (ILI) for the 2014–15 influenza season were obtained from Florida's Electronic Surveillance System for the Early Notification of Community-based Epidemics (ESSENCE) for Alachua County; the 13-county Florida Department of Health Region 3 (2010 population: 2.2 million), which includes Alachua County; and the state of Florida (*10*,*11*). ILI-associated emergency department (ED) visits were defined as visits for which a chief complaint contained the word "influenza" or "flu" or the word "fever" plus "cough" and/or "sore throat." Region 3 and Florida are comparison areas, so they exclude Alachua County data. For the 2014–15 influenza season (September 28, 2014–May 23, 2015), the rate of reported ILI for Alachua County residents was 499 cases per 100,000 population. Sixteen percent of cases were among children ≤17 years of age, a significant increase over 2013–14, when 11% of cases were among children in this age group ($p = 0.001$, Pearson χ^2 test, 2-tailed). The pattern for laboratory-confirmed influenza cases in our hospital ED was similar; although the rate of confirmed influenza cases among adult ED patients did not increase, the rate of confirmed cases among pediatric ED patients increased significantly from 6/1,000 unique ED patients in 2013–14 to 43/1,000 in 2014–15 ($p<0.0001$, χ^2).

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Table 1. Amino acid sequences at hemagglutinin major immunogenic epitopes A and B1 of influenza A(H3N2) viruses, Gainesville, Florida, USA, November 2014

Strain	Epitope A, aa 121–146																									Epitope B1, aa 155–163								
Consensus sequence*	N	N	E	S	F	N	W	T	G	V	T	Q	N	G	T	S	S	A	C	K	R	R	S	N	N	S	T	H	L	K	F	K	Y	P	A
A/Texas/50/2012†								N												I										N					
A/GNVL/01/2014‡								A												I		G				S				N					
A/GNVL/05/2014§																				I				S	S					N		Y		T	
A/GNVL/06/2014§																				I				S	S					N		Y		T	
A/GNVL/07/2014§																				I				S	S					N		Y		T	
A/GNVL/08/2014§																				I				S	S					N		Y		T	
A/GNVL/09/2014¶																				I				S	S					N		Y		T	

*Yamashita et al. (*9*).
†Virus in 2013–14 and 2014–15 vaccines, Northern Hemisphere; GenBank accession no. KC892952.1.
‡GenBank accession no. KJ439217.
§Viruses in nasopharyngeal swabs collected in November 2014.
¶Virus in sputum collected in November 2014.

Alachua County has a nationally recognized school-based influenza vaccination program that administers live attenuated influenza vaccine (FluMist; AstraZeneca, Wilmington, DE, USA); parenteral influenza vaccine is administered by private physicians (*12*). Since 2010, the average overall influenza vaccination rate for children 0–17 years of age in Alachua County has been approximately 45%. As previously reported (*11*), this rate has correlated with a reduction in rates of ED visits for ILI (as reported through the Florida ESSENCE surveillance system) among children in Alachua County, compared with those in Florida Region 3 and the state of Florida. Corresponding estimates of vaccine effectiveness (relative reductions in risk) in Alachua County, by year, are shown in Table 2. Accompanying the increase in cases among children for 2014–15 was a small but significant decrease in estimated effectiveness of vaccination for children in Florida Region 3 (Table 2).

As a marker of disease severity, we identified the number of patients at our hospital admitted to the medical intensive care unit (MICU) because of influenza by querying the University of Florida Health Integrated Data Repository for patients with International Classification of Diseases, Ninth Revision, diagnostic codes for influenza (487) or novel influenza (488) listed in the first 10 diagnoses. In the 2014–15 influenza season, 1.8% of MICU patients carried the diagnosis of influenza, a significant reduction from the 4.8% of MICU admissions in 2013–14, when an H1N1 dominated ($p<0.0001$, Fisher exact test, 2-tailed). The mean age for persons admitted to MICU with influenza in 2014–15 was 60 years, compared with 50 years for 2013–14 ($p = 0.006$, Mann-Whitney U test, 2-tailed). Seven of these MICU patients died; all were >50 years of age (mean 70 years). Patient ages were significantly higher than for persons with fatal cases in 2013–14 (mean 48 years) ($p = 0.04$, Mann-Whitney U test, 2-tailed), with a significantly higher Charleston co-morbidity index (4.9 vs. 2.4, $p = 0.04$, Mann-Whitney U test, 2-tailed).

Table 2. Estimated influenza vaccine effectiveness for children in Alachua County, Florida, USA, as compared with Florida Region 3, and Florida as a whole*

Age group, y	Influenza season	Region 3, % (95% CI)	Florida, % (95% CI)
5–17	2012–13	71 (67–76)	73 (68–78)
	2013–14	73 (68–79)	77 (72–82)
	2014–15	63 (58–69)	73 (68–78)
0–4	2012–13	82 (79–86)	84 (80–87)
	2013–14	85 (82–89)	88 (85–91)
	2014–15	80 (77–84)	86 (83–89)

*Estimates are based on reports of influenza-like illness from emergency departments reported through Florida's Electronic Surveillance System for the Early Notification of Community-based Epidemics. See Tran et al. (*11*) for detailed methods. Region 3 and Florida are comparison areas, so they exclude Alachua County data.

Conclusions

The predominant influenza strain in this community in 2013–14 was an H1N1 variant bearing the D225G polymorphism, which was associated with more severe disease outside of elderly age groups (*10*). Rates of illness severe enough to warrant MICU admission with the H3N2 clade 3C.2a strain circulating in 2014–15 were significantly lower than those with H1N1 in 2013–14 and occurred in a significantly older age group with a higher rate of co-morbidities, more closely matched observations from in the 2012–13 H3N2 influenza season. These findings highlight the substantial strain-related differences in virulence that occur from year to year and the importance of ongoing genetic monitoring of strains circulating within communities.

We noted an increase in influenza incidence in children ≤17 years of age in Alachua County during 2014–15. This increase was probably due, at least in part, to a decrease in vaccine effectiveness with the clade 3C.2a strain in this highly vaccinated population of children.

Our hospital-based ED and MICU data use laboratory-confirmed cases, whereas the regional and state data and vaccine effectiveness calculations are based on ILI data from the Florida ESSENCE system, which are potentially less reliable. Although we found data trends in the 2 systems to be consistent, our findings underscore the need for expanded surveillance of laboratory-confirmed cases statewide, particularly with the increasingly widespread distribution of clade 3C.2a strains in the Americas and Europe (*13*). These issues might be particularly relevant in light of the recent World Health Organization recommendation to substitute the HA gene clade 3C.3a virus A/Switzerland/9715293/2013 (H3N2) (rather than a clade 3C.2a virus) for A/Texas/50/2012 (H3N2) in the 2015 vaccine for the Northern Hemisphere.

The HA, NA, and M genomic sequences have been deposited in GenBank and are available as follows: HA gene sequences, KP153349–KP153353: NA gene sequences, KP153354–KP153358; M gene sequences, KP126913–KP126917.

All authors contributed to preparation of the manuscript, and approved the final version. This work was conceived by J.A.L. and J.G.M., Jr. In addition, J.A.L. performed virus isolations and virus gene–specific reverse transcription PCR, sequenced the PCR amplicons, and analyzed the nucleotide sequence data. J.G.M. Jr., J.B., and J.D.S. monitor the epidemiology and vaccine effectiveness of influenza virus strains, in conjunction with the work of the University of Florida Emerging Pathogens Institute. N.M.I. recognized early 2014–15 season cases of influenza in Gainesville, and she and K.H.R. assisted with case identification and sample collection. The viruses were first identified as H3N2 strains by using the GenMark DX eSensor respiratory viral panel instrument in the clinical microbiology and virology laboratory directed by K.H.R.

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Factors Related to Fetal Death in Pregnant Women with Cholera, Haiti, 2011–2014

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We assessed risk factors for fetal death during cholera infection and effect of treatment changes on these deaths. Third trimester gestation, younger maternal age, severe dehydration, and vomiting were risk factors. Changes in treatment had limited effects on fetal death, highlighting the need for prevention and evidence-based treatment.

Cholera infections during pregnancy are associated with high rates of fetal death, especially when women are severely dehydrated (*1*–*7*). In Haiti in 2011, pregnant women with clinical signs of cholera who sought treatment from Médecins Sans Frontières (MSF) in Port-au-Prince were sent to a general cholera treatment center (CTC). In April 2012, MSF established a CTC to improve fetal outcomes in pregnant women by facilitating intensive follow-up for dehydration and rapid access to obstetric and neonatal services. In June 2013, a more aggressive rehydration protocol was implemented (online Technical Appendix Table 1, http://wwwnc.cdc.gov/EID/article/22/1/15-1078-Techapp1.pdf). To assess the effects of cholera infection, establishment of a specialized CTC, and the new rehydration protocol, we conducted a retrospective cohort analysis of pregnant women with suspected cholera admitted to MSF's CTCs during September 1, 2011-December 31, 2014.

The Study

This study was approved by the National Bioethical Committee of the Ministry of Public Health and Population of Haiti. A cholera case-patient was defined as someone who passed ≥3 liquid stools with or without vomiting or dehydration in the previous 24 hours or within 6 hours of seeking treatment. Women of childbearing age were asked whether they were pregnant. Urine dipstick tests were conducted in cases of uncertainty or early stages of pregnancy. Gestational age was determined by date of the woman's last menstruation, uterine height, or ultrasound. Fetal status was assessed at admission and hourly by using fetal stethoscope or ultrasonic fetal Doppler. Women who had a miscarriage at home and were not bleeding at admission were not classified as pregnant. Dehydration status was determined according to World Health Organization categories (online Technical Appendix Table 1) (*8*). We assigned women into 3 treatment groups (TGs) according to whether they were treated in the general or specialized CTC and whether they were given the original or new protocol (online Technical Appendix Table 1).

We analyzed fetal outcome for all pregnant women by initial signs and symptoms, TG, and clinical evolution. Multiple logistic regression modeling was used for adjusted analyses. All analyses used Stata software version 12.0 (StataCorp LP, College Station, TX, USA).

During September 1, 2011–December 31, 2014, a total of 936 pregnant women were admitted. Thirty-six were excluded from analysis: 33 (0.35%) lacked fetal outcome data, and 3 (0.03%) died (1 each during second and third trimester; for 1, trimester was unknown). Of the remaining 900, mean age was 26.7 years (range 15–48, median 26 years); 168 (18.9%) were in their first trimester, 303 (34.2%) second trimester, and 416 (46.9%) third trimester. Trimester was unknown for 13. A total of 444 (49.3%) sought treatment within 24 hours of symptom development.

Fetal death occurred in 141 (15.7%) of the 900 analyzed pregnancies, more often in women <20 years of age (odds ratio [OR] 2.0, 95% CI 1.2–3.2), in their third trimester (OR 2.4, 95% CI 1.4–4.3), seeking treatment >24 hours after symptom onset (OR 1.5, 95% CI 1.0–2.2), with severe dehydration (OR 2.2, 95% CI 1.2–3.8), or who vomited (OR 2.1, 95% CI 1.3–3.5) (Table 1). A total of 64 (45.4%) fetal deaths occurred before admission.

Women who experienced preadmission or postadmission fetal death did not differ by age or clinical presentation (online Technical Appendix Table 2). However, preadmission fetal death was more likely in women who arrived >24 hours after symptom onset (OR 1.9, 95% CI 0.9–4.1) or were severely dehydrated (OR 2.0, 95% CI 0.7–5.7). In unadjusted analysis, postadmission fetal death was associated with moderate dehydration (OR 2.1, 95% CI 1.2–3.6) and vomiting (OR 2.1, 95% CI 1.2–3.8; Table 2). Of 836 women with a viable fetus at admission, 120 were in TG1, 399 in TG2, and 317 in TG3.

There was no modification effect of TG on postadmission fetal death. Weak evidence of a difference in effect of

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Table 1. Characteristics of pregnant cholera patients by pregnancy outcome and risk factors, Haiti, 2011–2014*

Characteristic	No. (%) fetal deaths, n = 141	Unadjusted analysis		Adjusted analysis†	
		OR (95% CI)‡	p value§	OR (95% CI)‡	p value§
Age group, y					
<20	30 (21.3)	2.08 (1.30–3.33)	0.01	1.98 (1.21–3.24)	0.02
20–34	96 (68.1)	Referent		Referent	
≥35	15 (10.6)	1.02 (0.57–1.83)		1.00 (0.55–1.84)	
Missing	0				
Time from onset to admission, h					
≤24	59 (41.8)	Referent	0.04	Referent	0.04
>24	82 (58.2)	1.47 (1.02–2.12)		1.49 (1.02–2.17)	
Missing	0				
Trimester of pregnancy					
First	17 (12.1)	Referent	0.02	Referent	0.001
Second	41 (29.1)	1.39 (0.76–2.53)		1.37 (0.74–2.53)	
Third	79 (56.0)	2.08 (1.19–3.64)		2.43 (1.37–4.31)	
Missing	4 (2.8)				
Dehydration level					
None	37 (26.2)	Referent	0.001	Referent	0.02
Moderate	73 (51.8)	1.83 (0.20–2.79)		1.54 (0.99–2.41)	
Severe	31 (22.0)	2.62 (1.55–4.44)		2.17 (1.24–3.81)	
Missing	0				
Vomiting					
Yes	116 (82.3)	2.25 (1.42–3.59)	0.001	2.10 (1.27–3.47)	0.001
No	24 (17.0)	Referent		Referent	
Missing	1 (0.7)				

*Of 900 pregnancies analyzed. OR, odds ratio.
†Adjusted for all list characteristics.
‡From logistic regression model with fetal death as the outcome and list characteristics as exposure.
§From Wald tests of parameters of logistic regression model.

severe dehydration on postadmission fetal death between TGs (p = 0.09) (Table 2) was potentially due to a lower rate among severely dehydrated women in TG2 (OR 0.4. 95% CI 0.1–1.7; data not shown). However, there was insufficient power to detect these differences, and the final model did not require adjustment. Although the proportion of postadmission fetal deaths within a TG decreased with each protocol change, the proportion in TG3 (8.5%, 27/317) was not different from TG1 (10.0%, 12/120) or TG2 (9.5%, 38/399).

Conclusions

Fetal death occurred in 141 (16%) of 900 pregnancies. Risk factors were third trimester, younger maternal age, severe dehydration, and vomiting. Treatment in a specialized CTC and aggressive rehydration did not prevent fetal death though the trend was toward improved outcomes.

Severe dehydration at admission increased risk for fetal death (*2,5-7*). Fetal death may occur due to fetal hypoxia and acidosis resulting from excessive maternal dehydration (*2,4,9*). The proportion of fetal deaths we found was higher than that previously recorded in Haiti (8%, 21/263) (*2*) but close to that of the 2006 Senegal cholera outbreak (12%, 6/52) (*3*). Earlier studies in India (*4*), Nigeria (*1,7*), and Pakistan (*6*) found higher proportions.

Women <20 years of age were twice as likely as older women to experience fetal death. Although the relationship between fetal death and maternal age during cholera has not been documented, younger age is associated with increased risk for other adverse pregnancy outcomes (*10*). The risk for fetal death was highest in the third trimester, even after controlling for maternal age, dehydration level, and vomiting. The relationship between fetal death and trimester of pregnancy is unclear (*1,3,6*).

Determination of dehydration status of pregnant women is difficult in later stages of pregnancy (*2,11*). Misclassification of dehydration status could affect fetal outcome due to placement of patients under the wrong treatment protocol. In addition, increased placental blood flow with gestational age may increase the effect of dehydration (*12*). Even after we controlled for dehydration level, we determined that fetal death was twice as likely in women experiencing vomiting, potentially due to electrolyte changes in amniotic fluid (*2,7,13–15*).

Lack of effect of a specialized CTC on fetal death could result from a detection bias in that establishment of the specialized CTC led to an increased likelihood of detection of fetal deaths. In addition, 45% of fetal deaths occurred before women sought treatment. Fetal death may occur early in a pregnant woman's illness with cholera (*6*), and more than half the women sought treatment >24 hours after symptom onset, likely contributing to poor fetal outcomes. Likewise, the effect of the new treatment protocol may have been limited by fetal death occurring before the women sought treatment or by women being assigned the incorrect protocol due to difficulty in determining dehydration status.

Limitations include lack of laboratory-confirmed diagnoses. Data were collected for programmatic rather than research purposes and lack electrolyte levels,

Table 2. Characteristics of pregnant cholera patients by pregnancy outcome and treatment group, Haiti, 2011–2014*

Characteristic	No. (%) postadmission fetal deaths, n = 77	Unadjusted analysis		Adjusted analysis†	
		OR (95%)‡	p value§	OR (95% CI)†	p value§
Treatment group					
1	12 (15.6)	1.19 (0.58–2.44)	0.85	1.39 (0.66–2.91)	0.68
2	38 (49.4)	1.13 (0.67–1.90)		1.15 (0.67–1.96)	
3	27 (35.1)	Referent		Referent	
Age group, y					
<20	14 (18.2)	1.73 (0.92–3.24)	0.23	1.63 (0.85–3.12)	0.33
20–34	54 (70.1)	Referent		Referent	
≥35	9 (11.7)	1.09 (0.52–2.28)		1.07 (0.50–2.27)	
Missing	0				
Time from onset to admission, h					
≤24	37 (48.1)	Referent	0.57	Referent	0.57
>24	40 (51.9)	1.15 (0.72–1.83)		1.15 (0.71–1.86)	
Missing	0				
Trimester of pregnancy					
First	10 (13.0)	Referent	0.23	Referent	0.06
Second	25 (32.5)	1.44 (0.67–3.08)		1.50 (0.69–3.24)	
Third	41 (53.2)	1.84 (0.90–3.76)		2.28 (1.09–4.76)	
Missing	1 (1.3)				
Dehydration level					
None	20 (26.0)	Referent	0.03	Referent	0.09
Moderate	45 (58.4)	2.08 (1.20–3.61)		1.89 (1.07–3.33)	
Severe	12 (15.6)	1.88 (0.89–3.98)		1.56 (0.71–3.41)	
Missing	0				
Vomiting					
Yes	63 (81.8)	2.10 (1.15–3.82)	0.02	2.05 (1.08–3.90)	0.03
No	14 (18.2)	Referent		Referent	
Missing	0				

*Of 836 women with a viable fetus at admission. OR, odds ratio.
†Adjusted for all list characteristics.
‡From logistic regression model with fetal death as the outcome and list characteristics as exposure.
§From Wald tests of parameters of logistic regression model.

amniotic fluid composition, maternal blood group, and fetal cause of death. Some first-trimester pregnancies may have been missed. Pregnancies in women who completed miscarriage at home were not counted, potentially underestimating overall risk for fetal death. Because there was no follow-up of women after discharge, some early-term fetal deaths might have been missed. In addition, the long-term effect of treatment on fetal well-being could not be determined. TG outcomes also may have been affected by differences in factors such as women's access to health services over time.

Although the implementation of a specialized CTC did not decrease fetal deaths, specialized CTCs play a vital role in preserving patients' dignity and providing patient-centered care. Determining the mechanism of fetal death in cholera infection would enable development of evidence-based treatment protocols. Because many fetal deaths occurred before women sought treatment, the importance of cholera prevention and the risk for poor fetal outcomes should be emphasized.

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E.S. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. E.S. and C.A. analyzed the data, and E.S. drafted the manuscript. A.L. supervised the epidemiologic analysis, and L.B. and R.S.D. coordinated the data collection. All authors contributed to the conception and design of the study and to the interpretation of the data. All authors critically revised the manuscript for intellectual content and approved the final draft for submission. The study design, data collection, analysis, decision to publish, and preparation of the manuscript was approved by Médecins Sans Frontières and the Haitian Ministry of Health.

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Rift Valley Fever Virus among Wild Ruminants, Etosha National Park, Namibia, 2011

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After a May 2011 outbreak of Rift Valley fever among livestock northeast of Etosha National Park, Namibia, wild ruminants in the park were tested for the virus. Antibodies were detected in springbok, wildebeest, and black-faced impala, and viral RNA was detected in springbok. Seroprevalence was high, and immune response was long lasting.

Rift Valley fever virus (RVFV) is a zoonotic mosquito-borne virus that infects humans and a wide range of domestic and wild ruminants. RVFV is a phlebovirus within the family *Bunyaviridae*. The RVFV genome comprises 3 single-stranded RNA segments that encode structural and nonstructural proteins (*1*). Mosquitoes, mainly from the genera *Aedes*, *Culex*, and *Anopheles*, spread the virus between animals and humans (*1*); direct transmission through aerosol spread of infected biological fluids plays a major role in human infection (*2*). RVFV was first isolated in the Rift Valley of Kenya in the early 1930s; since then, multiple epizootics and epidemics among animals and humans have occurred in Africa, Madagascar, the Comoros Archipelago, and the Arabian Peninsula (*3*). Although outbreaks are often underreported because of surveillance deficiencies, RVF is considered endemic to many African countries, where outbreaks occur at irregular intervals, usually after exceptionally heavy rains and floods (*4*).

During May 2011, an outbreak of RVF occurred among livestock in the Oshikoto region of Namibia, northeast of Etosha National Park (*5*). The ongoing sampling of wildlife as part of the surveillance program for Emerging Infectious Diseases and Transboundary Animal Diseases in Etosha National Park provided an opportunity to investigate the role played by wildlife in RVF epidemiology. We report detection of RVFV in springbok (*Antidorcas marsupialis*), wildebeest (*Connochaetes taurinus*), and black-faced impala (*Aepyceros melampus petersi*) in Etosha National Park.

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The Study

To maximize the chances of detecting RVFV circulation in a potentially infected area with extensive mixing of susceptible animals, widespread distribution of vectors, and consequently no particular factors that would lead to sampling bias, we chose to collect samples from animal species with a long life expectancy and widespread distribution. In collaboration with local staff and veterinary authorities, the first phase of sampling was conducted during May–July 2011. During the first phase, 200 springbok and 50 wildebeest were randomly selected, immobilized by darting, and fitted with radio collars for identification. During the second phase (December 2011), 45 springbok, 7 wildebeest, and 8 black-faced impala were sampled. Of these, 15 springbok and 4 wildebeest that had been sampled in phase 1 were recaptured. During both phases, blood samples were collected from each animal for serologic and virologic investigations.

We investigated the presence of antibodies against RVFV in serum samples by using 2 ELISAs (both from ID Vet, Grabels, France): 1) the ID Screen Rift Valley Fever Competition Multi-species Kit, to detect total antibody activity; and 2) the ID Screen Rift Valley Fever IgM Kit, to detect IgM against RVFV. RNA was purified from serum by using the High Pure Viral Nucleic Acid extraction kit (Roche Diagnostics, Mannheim, Germany) in accordance with the manufacturer's instructions. The presence of RVFV RNA was determined by specific real-time reverse transcription PCR (rRT-PCR) as previously described (6) by using primers and probe targeting the large segment of RVFV genome.

During phase 1, antibody activity was detected in 70 (35%) of 200 springbok (95% CI 28.73–41.85) and in 12 (24%) of 50 wildebeest (95% CI 14.33–37.49). IgM was detected in 30 (15%) of 200 springbok (95% CI 10.73–20.62) (Table 1). Viral RNA was detected in 18 (9%) of 200 springbok (95% CI 5.79–13.78). Of these 18 springbok, 7 were seropositive for RVFV and 4 were positive for IgM only (Table 1). Antibodies against RVFV were not detected in the remaining 11 springbok with positive rRT-PCR results. During phase 2, antibody activity was detected in 25 (56%) of 45 springbok (95% CI 41.11–69.10), in 1 (14%) of 7 wildebeest (95% CI 3.19–52.65), and in 5 (63%) of

Table 1. Results of serologic and virologic testing of wild ruminants for Rift Valley fever virus, Etosha National Park, Namibia, 2011*

Animal and time of sampling	rRT-PCR	IgM	Total antibodies other than IgM	No. positive/no. tested (% positive)
Springbok (*Antidorcas marsupialis*), n = 230				
May–Jul	–	–	–	119/200 (59.5)
	–	–	+	37/200 (18.5)
	–	+	–	26/200 (13.0)
	+	–	–	11/200 (5.5)
	+	+	–	4/200 (2.0)
	+	–	+	3/200 (1.5)
Dec	–	–	–	20/45 (44.4)†
	–	–	+	25/45 (55.6)†
Wildebeest (*Connochaetes taurinus*), n = 53				
May–Jul	–	–	–	38/50 (76.0)
	–	–	+	12/50 (24.0)
Dec	–	–	–	6/7 (85.7)†
	–	–	+	1/7 (14.3)†
Black-faced impala (*Aepyceros melampus petersi*), n = 8				
Dec	–	–	–	3/8 (37.5)
	–	–	+	5/8 (62.5)

*rRT-PCR, real-time reverse transcription PCR.
†15 springbok and 4 wildebeest were recaptured during the second phase of sampling (December 2011).

8 black-faced impalas (95% CI 29.9–86.30) (Table 1). In December 2011, no rRT-PCR or IgM results were positive for any animal, but among the 15 springbok and 4 wildebeest recaptured, RVF antibody activity was detected for 11 animals (10 springbok and 1 wildebeest). In particular, 2 of the 18 viremic springbok that were positive by rRT-PCR during phase 1 showed seroconversion, and a persistent immune response was detected in the 6 of the 70 seropositive animals after resampling 6 months apart (Table 2).

Conclusions

After the 2010 epidemic of RVF in Namibia (*7*), 3 outbreaks affecting domestic livestock were reported in the Oshikoto region of northern Namibia (*5*). Aware that ELISA results rely on the use of commercial tests not validated for wildlife testing, we supported our serologic findings by the detection of RVFV genome in the serum of springbok and seroconversion of viremic animals 6 months later. Furthermore, the contemporaneous detection of RVFV genome and antibody activity in springbok serum are consistent with data generated by experimental infection of livestock (*2*,*8*,*9*). Our findings also confirmed wildlife capability to develop a long-lasting immune response after RVFV infection (*2,10*).

The high seroprevalence among wild ruminants suggests intense RVFV activity in the area during May–July. Active virus circulation was not detected in December 2011, when only antibody activity was detected. It can be assumed that the onset of the dry season, which suppressed vector activity, associated with the high seroprevalence in susceptible hosts, mainly contributed to the abatement of virus circulation.

Although antibodies against RVFV have been detected in many species of wildlife, including springbok, wildebeest, and impala (*11–13*), the epidemiologic role of wildlife is far from elucidated. Wildlife are suspected to play a role during interepidemic periods, but their involvement during epidemics and the existence of sylvatic cycles involving wildlife and mosquitoes in maintenance and perpetuation of RVFV has rarely been investigated (*12*). More likely, RVFV persistence results from a balance of transmission between numerous susceptible vertebrates and mosquitoes, including vertical transmission in vectors (*14*). Further investigation of the duration and the extent of RVFV viremia in wild ruminants could therefore clarify the potential role of wildlife in maintaining the disease in the environment. This information could lead to hypotheses of the origin of the virus that circulated in Etosha

Table 2. Evolution of Rift Valley fever virologic and immune status of recaptured animals, Etosha National Park, Namibia, 2011*

	May–Jul			Dec			
Animal	rRT-PCR	IgM	Total antibody	rRT-PCR	IgM	Total antibody	No. (%) positive/no. tested (% positive)
Springbok (*Antidorcas marsupialis*)	–	–	–	–	–	–	5/15 (33.3)
	–	–	–	–	–	+	2/15 (13.3)
	+	–	–	–	–	+	2/15 (13.3)
	–	+	–	–	–	+	1/15 (6.7)
	–	–	+	–	–	+	5/15 (33.3)
Wildebeest (*Connochaetes taurinus*)	–	–	–	–	–	–	3/4 (75.0)
	–	–	–	–	–	+	1/4 (25.0)

*rRT-PCR, real-time reverse transcription PCR.

National Park during 2011 and whether it originated from neighboring areas or arose from an adaptation to wildlife from cryptic endemic foci.

Whatever the source of infection, RVFV in wildlife has only recently (2008–2011) been associated with severe disease in South Africa, where outbreaks began with abortions in buffaloes and the death of a waterbuck, but infection of livestock was not reported (*10,15*). The large number of predators within Etosha National Park may have prevented detection of clinical cases and may explain why no abortions, hemorrhagic disease, or deaths were reported during this study. The lack of human cases in the park or surrounding areas does not imply a lack of threat to public health. Whether the differences in prevalence of viremic animals and in serologic results may be associated with higher resistance of wildebeest to infection with RVFV or to differences in vector exposure should be investigated. More studies are warranted to gain a better understanding of the role of wildlife in the spread of RVFV and the interepidemic maintenance of the virus and to explore the potential use of wild ruminants in Africa as sentinels for monitoring RVFV activity.

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Legionnaires' Disease in South Africa, 2012–2014

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During June 2012–September 2014, we tested patients with severe respiratory illness for *Legionella* spp. infection and conducted a retrospective epidemiologic investigation. Of 1,805 patients tested, *Legionella* was detected in samples of 21 (1.2%); most were adults who had HIV or tuberculosis infections and were inappropriately treated for *Legionella*.

Data are limited regarding prevalence of *Legionella* spp. bacteria that cause community-acquired pneumonia (CAP) in Africa (*1*), despite the high prevalence of HIV-infected adults in many African countries, including South Africa (*2*). Legionellosis is a notifiable disease in South Africa but is rarely reported. We sought to determine the prevalence of *Legionella* spp. infections in South Africa and describe epidemiologic characteristics of patients with Legionnaires' disease (LD).

The Study

During June 2012–September 2014, we conducted a prospective, hospital-based, observational study as part of the severe respiratory illness (SRI) surveillance at 2 sites in South Africa: Klerksdorp-Tshepong Hospital Complex, Klerksdorp, North West Province; and Edendale Hospital, Pietermaritzburg, KwaZulu-Natal Province. A patient with SRI was defined as a person hospitalized with lower respiratory tract infection of any duration. We used a standardized questionnaire to collect demographic and clinical information. Nasopharyngeal specimens and induced sputum samples were tested for *Legionella* spp. infections by using a real-time PCR assay, as previously described (*3*). Specimens that were *Legionella* positive were also tested by real-time PCR assays to identify *L. pneumophila* and *L. longbeachae*. In addition, patients' specimens were tested for other respiratory pathogens and for HIV. Of the 22 *Legionella*-positive patients, we could trace 17 with whom we conducted a retrospective epidemiologic investigation, which included interviews (detailed study methods in the online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-0972-Techapp.pdf).

During June 2012–September 2014, a total of 4,525 SRI patients were enrolled; induced sputum specimens, the recommended specimen type for *Legionella* spp. detection, were collected from 1,805 (40%). Of 1,803 patients with sputum specimens for which data were available, 885 (49%) were male, and 324 (18%) were children <5 years of age. HIV prevalence was 64% (1,025 of 1,594 patients with sputum specimens and known HIV status), and prevalence of active tuberculosis (TB) infection was 24% (421 of 1,758 patients with sputum specimens and known TB status). Of 1,720 patients with sputum specimens and known survival status, 142 (8%) patients died.

Among the 1,805 patients with sputum samples, 21 (1.2%, 95% CI 0.7%–1.7%) tested positive for *Legionella* spp. by real-time PCR. For 1 patient (designated E1 in the online Technical Appendix Table) from whom sputum could not be collected, *Legionella* spp. infection was detected in the nasopharyngeal specimen, so 22 patients with *Legionella* spp. infections were detected in total. Among the 21 patients whose sputum tested positive for *Legionella* spp. infections, median age was 40 years (range 19–59 years; Figure 1), and 11 (52%) were males.

A cluster of case-patients (15/21 [71%]) was observed during July–December 2012 (Figure 2), including all 6 from Edendale Hospital and 10 (10/16, 63%) from Klerksdorp-Tshepong Hospital Complex. These sites are geographically distant (≈600 km) from one another, so the respective clusters or outbreaks are unlikely to be related. We did not culture samples with *Legionella* spp. infection, so we were unable to perform strain typing to confirm whether the clusters were caused by related strains. The remaining 6 patients from Klerksdorp-Tshepong Hospital Complex appeared to have sporadic infections.

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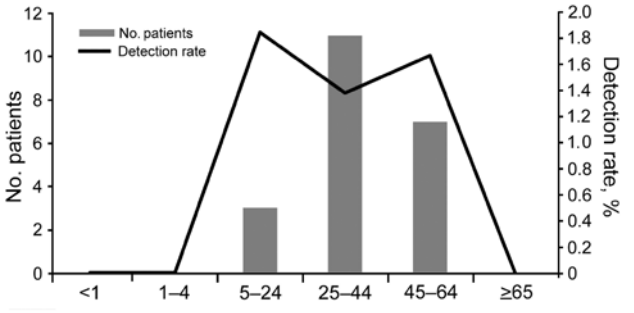

Figure 1. Number of case-patients and detection rate for *Legionella* spp. infections, by age group, South Africa, June 2012–September 2014 (N = 1,803).

Legionella patients resided in different areas or communities within the cities of Pietermaritzburg and Klerksdorp. Epidemiologic investigation revealed exposure to several potential sources of infection, such as waste management, air conditioners, plumbing, mining, and swimming pools; however, no common exposure could be identified, so environmental sampling and testing were not performed.

Fifteen (75%) of 20 *Legionella* patients with known HIV status were infected with HIV, and 9 (43%) of the 21 patients tested positive for TB at the admission during which *Legionella* infection was detected. HIV or TB infection, or both, was detected in 18 (90%) of 20 patients with known HIV and TB status. A history of active TB before the admission during which *Legionella* was detected was reported for 14 (82%) of 17 patients. For 17 *Legionella* spp.–infected patients for whom information was available, additional LD-associated factors included regular alcohol consumption (10 [59%]), cigarette smoking (9 [53%]), asthma (2 [12%]), and heart disease (2 [12%]).

Eighteen (86%) of 21 patients had symptoms >7 days before hospital admission, a delay possibly occurring because many patients were chronically ill (75% were HIV infected and ≥43% had TB). Median duration of hospitalization for *Legionella* patients was 4 days (range 1–35 days), and 1 (9%) patient was admitted to intensive care and survived the illness; 4 (20%) patients died. Antimicrobial drug treatment (in-hospital and discharge medication) was known for 21 patients and included amoxicillin/clavulanic acid (16 [76%]), anti-TB medications (15 [71%]), cotrimoxazole (7 [33%]), cefuroxime/ceftriaxone (5 [24%]), and erythromycin (5 [24%]).

Legionella spp. isolates were identified for 2 patients as *L. pneumophila* serogroup 1 and *L. longbeachae*. Species could not be determined for 19 patients because of low bacterial loads in their specimens. Of the 21 patients with *Legionella*-positive sputum specimens, co-infections were detected in 14 (67%). Co-infecting pathogens were *Mycobacterium tuberculosis* (9 [43%]), rhinovirus (6 [29%]), respiratory syncytial virus (2 [10%]), adenovirus (2 [10%]), *Bordetella pertussis* (1 [5%]), and *Streptococcus pneumoniae* (1 [5%]).

Legionella spp. detection rates in this study were similar to those described in other countries (*4*). However, age distribution tended toward younger adults, not the elderly, the population previously reported as most affected (*4*). Men and women were evenly distributed in our study, although a substantial male predominance is common for LD (*2,4*). Differences in age and gender distributions, compared with distributions in other studies, likely result from high HIV and TB prevalence among younger adults in our study population. LD is typically associated with summer because warm and wet conditions promote bacterial replication (*2,4*). Longer periods of surveillance are needed to establish seasonality of LD in South Africa.

Clinically, patients with LD in this study were likely to be HIV-infected, chronically ill persons with suspected or confirmed TB and were therefore usually treated for TB infection and discharged. HIV-induced immune suppression and lung damage because of biologic or chemical agents likely increased their susceptibility to *Legionella* infections. Cases of LD and TB occurring simultaneously have been previously described (*5–7*). *Legionella* infection in populations with HIV or TB co-infections may cause acute exacerbation of respiratory symptoms, prompting patients to seek hospital care.

In South Africa, treatment for CAP is usually penicillin or ampicillin for adults <65 years of age and amoxicillin/clavunate or cefuroxime for elderly or HIV-infected adults (*8*). However, treatment for LD should include a macrolide or fluoroquinolone (*4*). Only one fourth of *Legionella* patients in this study received appropriate treatment, likely because of clinical inability to distinguish LD from other forms of pneumonia and because clinicians rarely consider *Legionella* when they lack access to diagnostic testing and local prevalence data. This problem is further compounded by the high prevalence of HIV and TB in South Africa. Anti-TB treatment, which was

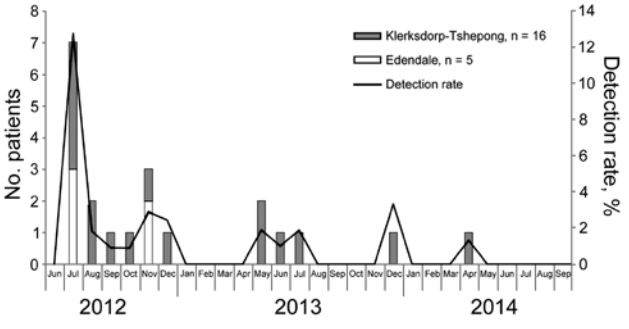

Figure 2. Number of case-patients and detection rate of *Legionella* spp. infections, by month and year, for Edendale Hospital and Klerksdorp-Tshepong Hospital Complex, South Africa, June 2012–September 2014 (N = 1,805).

administered to more than two thirds of the *Legionella* patients, would have had therapeutic benefits; rifampin has been shown to have activity against *Legionella* spp. (*9,10*). However, suboptimal treatment of *Legionella* patients with co-infections likely contributed to a case-fatality ratio (20%) more than twice that for all SRI patients (8%) (*4,11*). Lack of appropriate treatment of patients with CAP in South Africa for atypical pathogens has been described ((*12*).

Conclusions

In South Africa, patients with LD often have chronic illness caused by co-infections such as HIV and TB at time of admission. *Legionella* infections in most patients were undiagnosed, and patients were suboptimally treated for TB or more typical causes of CAP. Increased awareness and improved diagnostic testing could result in earlier diagnosis, appropriate treatment, and improved outcomes for these patients. In addition to routine diagnostics, surveillance for LD should be performed on an ongoing basis for rapid identification and response to outbreaks.

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Severe Community-Acquired Bloodstream Infection with *Acinetobacter ursingii* in Person who Injects Drugs

Helmut J.F. Salzer, Thierry Rolling, Stefan Schmiedel, Eva-Maria Klupp, Christoph Lange, Harald Seifert

We report a community-acquired bloodstream infection with *Acinteobacter ursingii* in an HIV-negative woman who injected drugs. The infection was successfully treated with meropenem. Species identification was performed by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Improved identification of *Acinetobacter* spp. by using this method will help identify clinical effects of this underdiagnosed pathogen.

Actinetobacter spp. are a diverse group of gram-negative, aerobic coccobacilli. Currently, >50 *Acinetobacter* spp. are recognized; most are not pathogenic to humans. Most human infections are nosocomial and predominantly caused by *A. baumannii* and other members of the *A. baumannii* group, including *A. nosocomialis*, *A. pittii*, and *A. seifertii* (*1*). Novel species, such as *A. ursingii* and *A. schindleri*, which have the potential to cause severe opportunistic infections, are emerging (*2–8*). Community-acquired *Acinetobacter* spp. infections are reported mainly from tropical regions. Most patients infected have community-acquired pneumonia caused by *A. baumannii* (*1*). We report a case of community-acquired bloodstream infection with *A. ursingii* in an HIV-negative woman who injected drugs.

The Case-Patient

In March 2014, a 47-year-old woman was hospitalized at the Bernhard Nocht Clinic (Hamburg, Germany) with chills, nausea, and fever (temperature ≤40°C). The patient had a history of intravenous drug use (cocaine and benzodiazepines) and was currently enrolled in a methadone substitution program. She also had chronic hepatitis C virus infection that was successfully treated with pegylated interferon-α, ribavirin, and boceprevir. The patient had a sustained virologic response and negative results for hepatitis C virus RNA at follow-up without progression to liver fibrosis or cirrhosis.

At admission, her peripheral blood leukocyte count was 6.6 × 10^3 cells/μL, and her C-reactive protein level was increased (29 mg/L [reference value <5 mg/L]). Other routine serum and urine chemical test results were unremarkable. HIV-1/2 infection was not detected. Chest radiograph showed no abnormalities. Soft tissue abscess was not observed, but the patient admitted having injected cocaine and unknown crushed tablets intravenously the day before admission. Toxicologic screening showed highly increased levels of benzodiazepine (>5,000 ng/mL) and cocaine (>1,000 ng/mL) (reference values <20 ng/mL for both substances). Three blood cultures were collected before empiric antimicrobial therapy with intravenous ceftriaxone (2 g 1×/d) was given on day 1.

The next day, the patient's condition deteriorated. Her leukocyte count increased to 27.6 ×10^3 cells/μL, and C-reactive protein level increased to 112 mg/L. She was highly febrile and became hypotensive and tachycardic, which are compatible with severe sepsis. The patient responded rapidly to fluid substitution, became hemodynamically stable, and did not need vasopressors. Antimicrobial therapy was changed empirically to meropenem (1,000 mg 3×/d) on day 2.

After 18 h of culture incubation, microbial growth was detected in 3 aerobic blood culture bottles inoculated at admission. Gram staining showed gram-negative rods, and matrix-assisted laser desorption ionization/time-of-flight mass spectrometry fingerprinting with direct sample deposition without extraction (Bruker Daltonim GmbH, Bremen, Germany) identified *A. ursingii* (score 2.19). A BLAST search (http://www.ncbi.nlm.nih.gov/BLAST) of partial 16S rRNA gene sequence was performed by using a taxonomy browser (http://www.ncbi.nlm.nih.gov) and showed 99% identity with the *A. ursingii* type strain NBRC110605 (GenBank accession no. LC014147.1).

Antimicrobial susceptibility testing was performed by using Vitek 2 (bioMérieux, Nürtingen, Germany). Results showed susceptibility to imipenem (MIC ≤0.25 mg/mL), meropenem (≤0.25 mg/mL), gentamicin (≤1 mg/mL), and ciprofloxacin (≤0.25 mg/mL) and resistance to ceftriaxone (32 mg/mL).

The patient became afebrile the next day, her clinical symptoms improved, and laboratory parameters of

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inflammation returned to reference values. Follow-up cultures remained negative. A transesophageal echocardiogram was unremarkable, without any evidence of infective endocarditis. Antimicrobial drug therapy was continued for 10 days before discharge. At follow-up 2 months later, the patient had no symptoms and was attending group-counseling sessions to maintain drug abstinence.

Conclusions

After the first description of *A. ursingii* as a new species (*2*), for which 13 of 29 *A. ursingii* isolates were obtained from patients with nosocomial bloodstream infections, few cases of *A. ursingii* bloodstream infections have been reported (Table). We were not able to identify cases of community-acquired *A. ursingii* bloodstream infections in the literature. Loubinoux et al. reported a case of *A. ursingii* bloodstream infection in a severely immunocompromised patient with pulmonary adenocarcinoma who received chemotherapy and corticosteroids (*3*). In that study, *A. ursingii* was associated with an implanted port device and considered of low pathogenic potential.

However, in the past few years, there have been reports of serious *A. ursingii* bloodstream infections in immunocompromised and immunocompetent patients. Horii et al. reported nosocomial *A. ursingii* bloodstream infections in 2 pregnant women who had no concurrent illnesses (*4*), but the route of transmission was not elucidated. The authors hypothesized that infections might have been acquired from intravenous catheters from the ward shower bath, where *A. ursingii* and 2 different strains of *A. junii*

Table. Characteristics of 16 patients with *Acinetobacter ursingii* bloodstream infections*

Patient, age†/sex	Immune status	Clinical manifestation	Acquisition	Treatment	Outcome	Reference
63 y/M	Compromised	Catheter-related bactemia	Nosocomial	Imipenem, amikacin, rifampin	Survived	(*3*)
99 y/M	Competent	Bacteremia, cholangitis	Nosocomial	NR	NR	(*8*)
67 y/F	Competent	Bacteremia, septic shock	Nosocomial	NR	NR	(*8*)
38 y/F	Compromised	Bacteremia	Nosocomial	NR	NR	(*8*)
38 wk/M (newborn)	NR	Wet lung, bacteremia	Nosocomial	Meropenem, amikacin	Survived	(*5*)
23 wk/M (newborn)	NR	Premature, ARDS, bacteremia	Nosocomial	Meropenem, amikacin	Died	(*5*)
37 wk/F (newborn)	NR	Hypoxic ischemic encephalopathy, bacteremia	Nosocomial	Meropenem, amikacin	Survived	(*5*)
32 wk/F (newborn)	NR	Premature, ARDS, bacteremia	Nosocomial	Meropenem, amikacin	Survived	(*5*)
25 wk/M (newborn)	NR	Premature, ARDS, intracranial hemorrhage, bacteremia	Nosocomial	Meropenem, amikacin	Died	(*5*)
36 wk/M (newborn)	NR	Premature, necrotizing enterocolitis, bacteremia	Nosocomial	Ceftazidime, amikacin, immunoglobulin	Survived	(*6*)
29 wk/F (newborn)	NR	Premature, necrotizing enterocolitis, severe hypoxia, bacteremia	Nosocomial	Ciprofloxacin, piperacillin	Died	(*6*)
31 wk/F (newborn)	NR	Premature, ARDS, necrotizing enterocolitis, bacteremia	Nosocomial	Netilmicin, clindamycin;‡ teicoplanin, cefotaxim, clindamycin;§ tobramycin, ciprofloxacin;¶ piperacillin/ tazobactam, vancomycin#	Survived	(*6*)
26/F**	Competent	Bacteremia	Nosocomial	Piperacillin	Survived	(*4*)
30/F**	Competent	Bacteremia	Nosocomial	Cefepime	Survived	(*4*)
NR/NR	Compromised	Bacteremia	Nosocomial	Ciprofloxacin††	Survived	(*9*)
47/F	Competent	Bacteremia, severe sepsis	Community	Meropenem	Survived	This study

*NR, not reported; ARDS, acute respiratory distress syndrome.
†Age for the 8 newborns is gestational age.
‡First treatment.
§Second treatment.
¶Third treatment.
#Fourth treatment.
**These women were pregnant.
††Infected with a carbapenem-resistant strain.

were found. *A. ursingii* was also found associated with nosocomial bloodstream infections among newborns, as demonstrated by 2 outbreaks in neonatal intensive care units that had high mortality rates. In both outbreaks, the source of infection could not be identified. In outbreaks reported by Kilic et al. (*5*) and Máder et al. (*6*), catheter-related infections were assumed. Kilic et al. (*5*) identified the organisms as *A. septicus* sp. nov., which were later shown by Nemec et al. (*7*) to be identical to *A. ursingii*. Dortet et al. (*8*) reported 10 *A. ursingii* infections, which included 3 cases of nosocomial bloodstream infections, but they did not provide clinical details.

Although severe infections with *A. ursingii* are rare, identification of *A. ursingii* by molecular methods, such as 16S rRNA gene sequencing, *rpoB* gene sequence cluster analysis, and amplified fragment length polymorphism fingerprinting, indicates a relatively high prevalence of *A. ursingii* (range 2.6%–4.5%) among clinical *Acinetobacter* spp. isolates (*10*–*13*). In a study from the United Kingdom, *A. ursingii* was identified in 28 (4%) of 690 clinical *Acinetobacter* spp. isolates collected over a 20-month period during 2008–2009; a total of 17 (71%) of 24 *A. ursingii* isolates were recovered from blood cultures, but clinical details were not reported (*12*). Karah et al. (*13*) reported 113 case-patients with *Acinetobacter* spp. bloodstream infections in Norway; 3 (2.6%) were with *A. ursingii*, but clinical details of these patients were not provided. This relatively high incidence is consistent with results from the Netherlands (*10*) and Northern Ireland (*11*).

In most reports, *A. ursingii* isolates were more susceptible to antimicrobial drugs than *A. baumannii* isolates (*4*,*6*,*8*,*12*,*13*). However, Endo et al. (*9*) reported an IMP-1–producing, carbapenem-resistant *A. ursingii* isolate from a patient in Japan with a nosocomial bloodstream infection.

A. ursingii isolates are usually resistant to cephalosporins (*3*,*5*,*8*), as was the isolate from the patient we report, which explains the progression from severe sepsis when she was empirically given ceftriaxone to rapid clinical improvement after administration of meropenem. Máder et al. (*6*) reported a premature infant with an *A. ursingii* bloodstream infection also given a cephalosporin (ceftazidime). However, the infant received a combination with amikacin and immunoglobulins for 3 weeks. The infant survived and was discharged.

These findings suggest that *A. ursingii* is an emerging and serious clinical pathogen that is often involved in nosocomial bloodstream infections and associated with use of intravascular catheters. Its natural habitat remains unclear, but so far, it has only been isolated from human clinical sources. The frequency and clinical role of *A. ursingii* might have been underestimated because species identification by phenotypic and semiautomated identification methods is unreliable. For example, Vitek 2 has misidentified *A. ursingii* as *Bordetella bronchiseptica* (*6*,*8*). The most recent software version for Vitek 2 enabled unambiguous identification of *A. ursingii* (H. Seifert, pers. comm.).

We report a case of community-acquired bloodstream infection and severe sepsis caused by *A. ursingii* that was probably associated with intravenous drug use in an immunocompetent patient. This report also highlights the usefulness of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for identification of *Acinetobacter* spp. We believe that this technology might improve correct species identification of *Acinetobacter* spp., including *A. ursingii*, in routine clinical practice and help elucidate the prevalence and clinical role of this emerging pathogen.

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Highly Pathogenic Avian Influenza Virus, Midwestern United States

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To the Editor: Novel highly pathogenic avian influenza (HPAI) viruses of subtypes H5N2, H5N8, and H5N1 have recently caused numerous outbreaks in commercial poultry farms in the United States and Canada (*1*). Risk for zoonotic transmission is low; humans are affected primarily from the extensive economic repercussions of suspending poultry-farming activities (*1*).

Large-scale research is under way, including case-control studies of infections on poultry farms and modeling studies to investigate the spread of virus in waterfowl (*1*,*2*). The US Department of Agriculture has published a report that summarizes various biosecurity measures of affected farms, results of airborne pathogen testing, and geospatial analyses correlating wind speed and direction to outbreaks (*1*). These studies found insufficient evidence to support any particular modes of virus spread and suggest that farms are contaminated from infected migrating waterfowl and/or unauthorized movements (e.g., of vehicles, equipment, persons, or animals) between farms and that unusually high wind speeds are the likely mechanism of spread (*1*). The spread from farm to farm, but not from barn to barn within a single farm (*3*), further adds to the puzzle of how infection has been transmitted.

To better understand the outbreak behavior, we used publicly available sources (*4*–*6*) to create maps of outbreaks of HPAI virus, subtype H5, infections in relation to poultry distribution and wild bird migratory patterns (Figure; online Technical Appendix Figures 1, 2, http://wwwnc.cdc.gov/EID/article/22/1/15-1053-Techapp1.pdf; Video, http://wwwnc.cdc.gov/EID/article/22/1/15-1053-V1.htm). From November 30, 2014, through June 17, 2015, a total of 280 outbreaks caused by HPAI virus subtype H5 in Canada and the United States were reported to the World Organisation for Animal Health (*4*). Most outbreaks occurred during April (n = 116) in commercial turkey farms (n = 154) and were caused by HPAI virus subtype H5N2 (n = 256) (online Technical Appendix Figure 3). Related reassortant HPAI subtypes H5N8 and H5N1 were also found among infected poultry; however, these appeared infrequently. Subtype H5N1 appeared in 4 of 21 outbreaks in backyard and commercial farms and was found in 1 of 3 infections in a backyard farm. Backyard farms generally contain flocks for local consumption and implement fewer biosecurity measures (*4*).

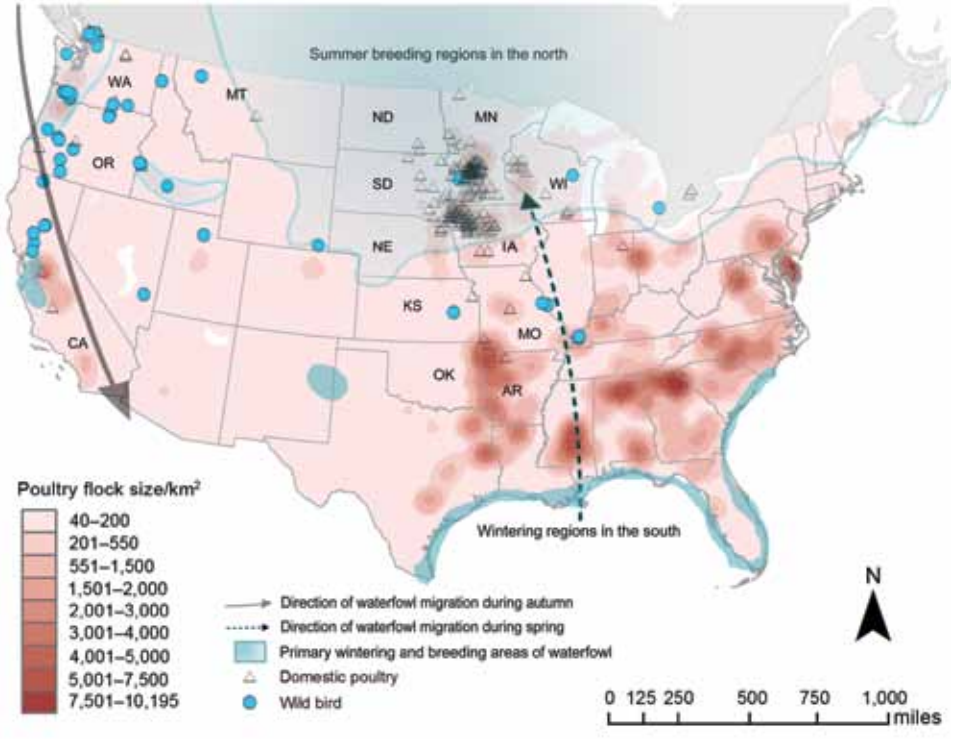

Figure. Distribution of highly pathogenic avian influenza (HPAI) H5 outbreaks in domestic poultry compared with domestic poultry flock density and direction of wild waterfowl migration, United States. Triangles represent HPAI H5 domestic poultry outbreaks; circles represent HPAI H5 wild bird outbreaks. Solid gray shading indicates migratory waterfowl wintering and breeding regions, and arrows represent general direction of seasonal movements. Gradient gray shading indicates density of domestic poultry holdings, with darker shades representing areas where flock densities are higher. A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/22/1/15-1053-F1.htm).

Initial outbreaks on poultry farms that began in November 2014, near the British Columbia–Washington State border, have been associated with timing of waterfowl migration and reported infection in migratory waterfowl (*7*,*8*). Subsequent surveillance of avian influenza virus in wild birds in the Pacific flyway has also shown sporadic infections caused by HPAI virus subtype H5, primarily in waterfowl species of the family Anseriformes (*4*) (online Technical Appendix Table 1).

In late February 2015, however, HPAI virus subtype H5, emerged in US midwestern states, leading to a substantial number of outbreaks in commercial poultry farms in the region. The spread from west to east does not correlate with the direction of typical waterfowl migration, in which movement occurs from south to north. Unlike the earlier outbreaks in poultry in Canada, in the outbreaks in midwestern states, corresponding high numbers of virus were not detected in samples of wild birds in surrounding regions (despite increased surveillance). Of 3,300 samples tested, 1 sample tested positive for HPAI virus subtype H5 (*4*,*9*). In addition, most poultry farms were affected in April, and migratory waterfowl typically appear in Minnesota in March and April (online Technical Appendix Figure 1). This February introduction of virus to Minnesota may be explained by an earlier-than-usual spring (*10*).

Minnesota and Iowa lie within regions where migrating waterfowl spend their breeding season, and waterfowl densities on commercial poultry farms are particularly high (online Technical Appendix Figure 2). In southern parts of the United States, where poultry density is also high, isolated outbreaks of HPAI have occurred in poultry, although the introduction of virus into these regions did not result in a surge of outbreaks. The timing of waterfowl migration enables the mixing of highly dense populations of wild waterfowl and poultry, which likely plays a key role in spreading virus onto farms.

Of particular note, outbreaks in poultry were densely concentrated within Minnesota and Iowa in a spatial pattern inconsistent with the much more geographically dispersed spread of infection in wild birds. The magnitude and clustered distribution of poultry outbreaks are suggestive of local spread, rather than multiple introductions from passing migratory waterfowl. Genetic analyses have similarly shown evidence for concurrent multiple introductions as well as common source exposures, and surveys of affected farms have shown that local spread could be facilitated by the sharing of equipment by multiple farms or through animals entering barns (*1*).

The combination of high poultry densities and timing of waterfowl migration have likely predisposed Minnesota and Iowa to outbreaks of avian influenza among poultry flocks. However, consistent with US Department of Agriculture findings, local factors have likely also contributed to the large number of outbreaks in these states. We suggest that network modeling analyses would be valuable in exploring how virus may spread from farm to farm.

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Widespread Bat White-Nose Syndrome Fungus, Northeastern China

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To the Editor: Emerging infectious diseases have caused catastrophic declines in wildlife populations, and the introductions of many pathogen have been linked to increases in global trade and travel (*1*). Mapping the distribution of pathogens is necessary to identify species and populations at risk and identify sources of pathogen spillover and introduction. Once pathogen distributions are known, management actions can be taken to reduce the risk for future global spread (*2*).

Bats with symptoms of white-nose syndrome (WNS) were first detected in the United States in 2006, and the disease has subsequently caused precipitous declines in temperate bat populations across eastern North America (*3*,*4*). *Pseudogymnoascus destructans*, the causative agent of WNS, is a cold-growing fungus that infects bats' skin during hibernation, leading to more frequent arousals from torpor and death (*3*). *P. destructans* is widespread throughout Europe (*5*), but, to our knowledge, its presence in Asia has not been documented.

We sampled bats and hibernacula surfaces (cave walls and ceilings) across northeastern China during 2 visits (June–July 2014 and March 2015) using a previously described swab-sampling technique (*6*). Bats were captured inside caves and at their entrances. DNA was extracted from samples by using a modified QIAGEN DNeasy blood and tissue kit (QIAGEN, Valencia, CA, USA) and tested in duplicate for the presence of *P. destructans* with a quantitative real-time PCR (qPCR) (*6*,*7*).

In the summer of 2014 and winter of 2015, we collected 385 samples from hibernacula surfaces at 12 sites in 3 provinces and 1 municipality (Figure, panel A) and 215 samples from 9 species of bats at 10 sites (summer: *Rhinolophus ferrumequinum, Rhinolophus pusillus, Myotis adversus, Myotis macrodactylus, Myotis pilosus, Myotis chinensis, Murina usseriensis*; winter: *R. ferrumequinum, Murina leucogaster, Myotis petax*). During the summer, *P. destructans* was widely distributed across the study region with positive samples (determined on the basis of qPCR results) obtained from cave surfaces at 9 of 12 sites and from bats at 2 of the 9 sites where bats were sampled (Figure, panel A).

Prevalence of *P. destructans* was low during summer in the environment (mean prevalence across sites 0.06 ± 0.03) and in bats. Bats of 3 species tested positive for *P. destructans* in the summer: *M. macrodactylus* (1/10), *M. chinensis* (1/1), and *M. ussuriensis* (1/1). *P. destructans* was not detected in bats of 4 other species, of which >20 individual animals of each species were sampled (*R. ferrumequinum*, *R. pusillus*, *M. pilosus*, and *M. adversus*). The low prevalence of *P. destructans* in bats and on hibernacula surfaces in China during the summer was similar to comparable results from studies in North America (*6*).

In winter, prevalence at the 2 sites we revisited was much higher; 75% of 85 samples from 3 species tested positive, including samples from 16/17 *M. petax* bats. We also detected *P. destructans* in bats from 2 additional species (*R. ferrumequinum* [11/19 bats] and *M. leucogaster* [11/16 bats]).

In addition, during March 2015, we observed visual evidence of *P. destructans* in bats (*M. petax*; Figure, panel C) and obtained 2 fungal cultures from swab specimens taken from these bats. To isolate *P. destructans* from these samples, we plated swab specimens from visibly infected bats on Sabouraud dextrose agar at 10°C. We identified potential *P. destructans* isolates on the basis of morphologic characteristics. DNA was then extracted from 2 suspected fungal cultures and tested for *P. destructans* by qPCR, as previously described.

To further confirm the presence of *P. destructans*, we prepared the fungal isolates for Sanger sequencing (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-1314-Techapp1.pdf). The 600-nt amplification products from these 2 isolates were sequenced and found to be 100% identical to the *P. destructans* rRNA gene region targeted for amplification. In addition, using BLAST (http://www.ncbi.nlm.nih.gov/blast.cgi), we found that sequences were a 100% match with isolates from Europe (GenBank accession no. GQ489024) and North America (GenBank accession no. EU884924). This result confirms that the same species of fungus occurs on all 3 continents. We also obtained wing biopsy punches from these bats and found lesions characteristic of WNS by histopathologic examination (Figure, panel B; online Technical Appendix).

The occurrence of *P. destructans* at most sites sampled indicates that this pathogen is widespread in eastern

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Asia (Figure, panel A). The presence of *P. destructans* in bats from 6 species in China and on bats in 13 species in Europe (*8*) confirms the generalist nature of this fungus and suggests that it may occur throughout Eurasia (Figure, panel D).

Decontamination and restrictions on the use of equipment that has been used in caves in Asia would help reduce the probability of introducing *P. destructans* to uninfected bat populations (e.g., western North America, New Zealand, southern Australia, and temperate areas of South

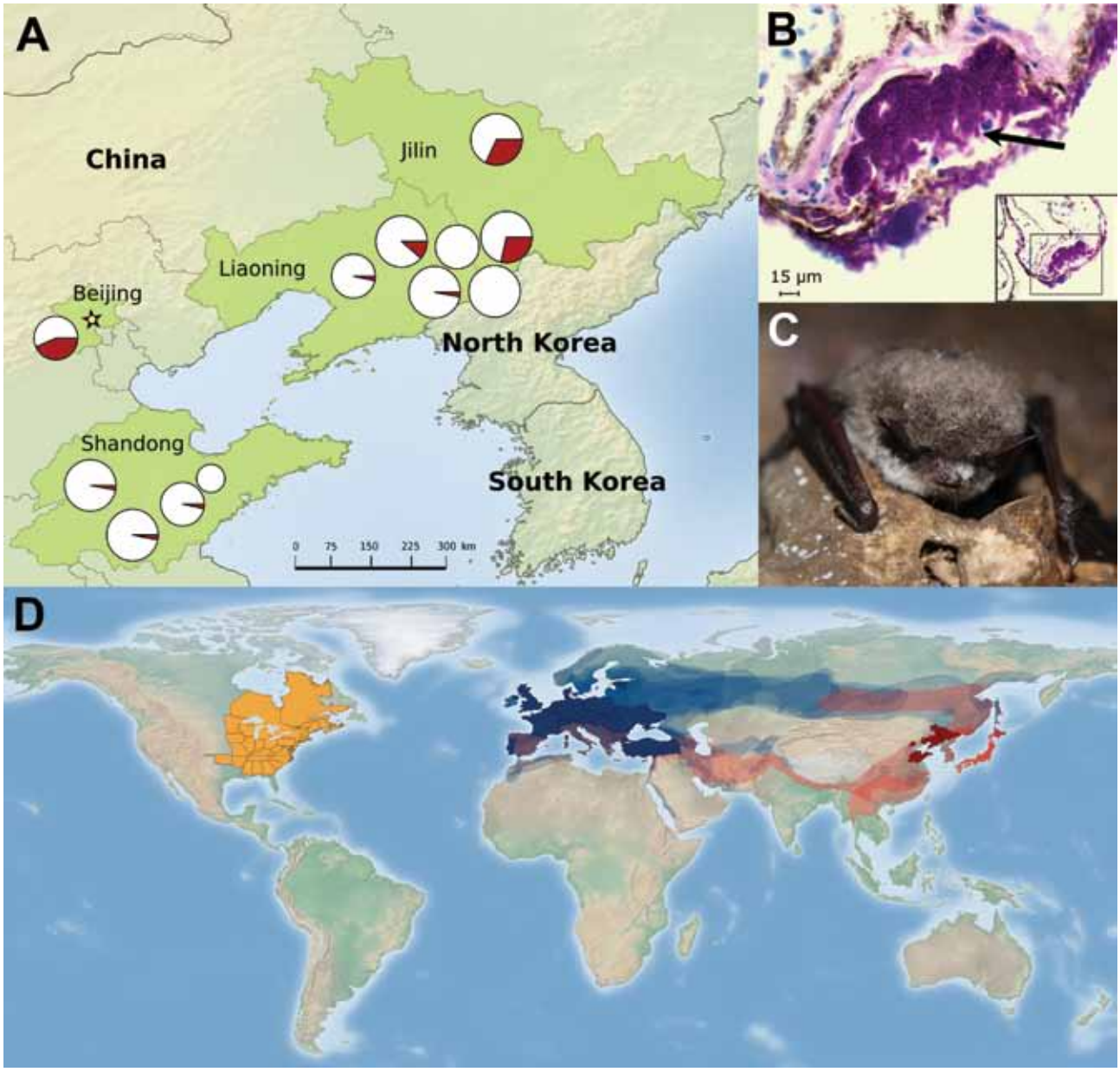

Figure. A) Distribution of *Pseudogymnoascus destructans* in cave environments during summer at 9 sites in northeastern China. Pie charts show the prevalence of *P. destructans,* and the size of pie graphs indicates the number of samples taken at each site (range 10–35). B) Histologic wing cross-section from *Myotis petax* bat collected in March 2015 with cup-like lesion (arrow) diagnostic of white-nose syndrome (periodic acid–Schiff staining). C) *M. petax* bat found in a cave in Jilin, China, showing visible signs of white-nose syndrome, March 2015. D) Documented global distribution of *P. destructans*. Areas in solid black represent the provinces and countries in China and Europe, respectively, where *P. destructans* was detected in this study and from previous research (*5*). Semitransparent regions show the species ranges (range data taken from http://www.iucnredlist.org/) for the bat species detected with *P. destructans* in Asia (n = 6) and Europe (n = 13) (*8*) and possible distribution of *P. destructans*. The solid black region in North America shows the extent of *P. destructans* spread as of May 15, 2015 (https://www.whitenosesyndrome.org/resources/map). A color version of this figure is available online (http://wwwnc.cdc.gov/EID/article/22/1/15-1314-F1.htm).

America). These measures would also reduce the risk of introducing new strains of *P. destructans* to regions where bats are already infected (e.g., eastern North America and Europe). These measures are necessary to prevent the devastating effects this pathogen has had on bats in North America and would help maintain the ecosystem services that bats provide (*9,10*).

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New Clinical Strain of *Neisseria gonorrhoeae* with Decreased Susceptibility to Ceftriaxone, Japan

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To the Editor: In 2009, 2010, and 2013, *Neisseria gonorrhoeae* strains H041 (ceftriaxone MIC of 2 mg/L), F89 (ceftriaxone MIC of 1 mg/L), and A8806 (ceftriaxone MIC of 0.5 mg/L) were isolated from samples from patients in Japan (*1*), France (*2*) and Australia (*3*), respectively. In Japan, no other clinical *N. gonorrhoeae* strains with decreased susceptibility to ceftriaxone were reported until 2014, when clinical strain GU140106 (ceftriaxone MIC of 0.5 mg/L) was isolated from a man in in Nagoya, Japan. We report details of this case and sequencing results of the *penA* gene for the strain. The study was approved by the Institutional Review Board of the Graduate School of Medicine, Gifu University, Japan.

N. gonorrhoeae strain GU140106 was isolated from a urethral swab sample from a man with acute urethritis. The man had received fellatio, without condom use, from a female sex worker in Nagoya in December 2013. He visited our clinic in January 2014 for urethral discharge. Culture of a urethral swab sample was positive for *N. gonorrhoeae*. We used the Cobas 4800 CT/NG Test (Roche Molecular Systems Inc., Pleasanton, CA, USA) to test a first-voided urine sample; results were positive for *N. gonorrhoeae* but negative for *Chlamydia trachomatis*. The infection was treated with a single-dose regimen of ceftriaxone (1 g) administered by intravenous drip infusion. Two weeks later,

[1]These authors contributed equally to this article.

the man reported no symptoms, and his first-voided urine sample was negative for leukocytes. The test-of-cure for *N. gonorrhoeae* was not performed. The female sex worker could not be examined for the presence of *N. gonorrhoeae* strain GU140106 in her pharynx.

The strain was confirmed to be a gonococcal species by testing with Gonochek-II (TCS Biosciences Ltd, Buckingham, UK), the HN-20 Rapid system identification test (Nissui, Tokyo, Japan), and the Aptima Combo 2 assay for CT/NG (Hologic, Inc., Bedford, MA, USA) and by 16S rRNA gene sequencing and *porA* pseudogene PCR (*4*). MICs of antimicrobial drugs for GU140106 were as follows, as determined by using the agar dilution method: 2.0 mg/L for penicillin G, 1.0 mg/L for tetracycline, 2.0 mg/L for cefixime, 0.5 mg/L for ceftriaxone, 8.0 mg/L for levofloxacin, 0.5 mg/L for azithromycin, and 32.0 mg/L spectinomycin. The strain was determined to be resistant to penicillin G, tetracycline, cefixime, ceftriaxone, and levofloxacin, according to criteria of the European Committee on Antimicrobial Susceptibility Testing (*5*).

The *penA* gene of strain GU140106 was sequenced as previously described (*6*); results showed the presence of a novel mosaic penicillin-binding protein 2 (PBP2; GenBank accession no. LC056026) (Figure). Multilocus sequence typing (MLST) and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) of GU140106 were performed as previously reported (*8*,*9*). MLST assigned strain GU140106 to sequence type 7363, the same as strains H041 and A8806 (*1*,*2*). NG-MAST assigned strain GU140106 to sequence type 6543. MLST and NG-MAST results for GU140106 differed from those for F89 (*3*).

Since the naming of the mosaic PBP2 associated with decreased susceptibilities to oral cephalosporins as pattern X (*6*), various PBP2 mosaic structures have been discovered. Mosaic PBP2 structures are basically composed of fragments analogous to PBP2s in *Neisseria* species. Before strain H041 emerged, strains harboring mosaic PBP2s had been resistant to oral cephalosporins but susceptible to ceftriaxone. H041 (ceftriaxone MIC of 2 mg/L) had additional novel amino acid changes, including A311V, V316P, and T483S, in its mosaic PBP2. The presence of substitutions A311V, V316P, and T483S was reported to be responsible for resistance to ceftriaxone (*7*). Like strain H041, strains GU140106 and A8806 (ceftriaxone MICs of 0.5 mg/L) had substitutions A311V and T483S, but instead of substitution V316P, they had substitution V316T. In addition, GU140106 had several changes in positions 227–281 that were not present in other strains. These alterations might also contribute to the decreased susceptibility to ceftriaxone.

On the basis of pharmacodynamic analyses (*10*), a 1-g dose of ceftriaxone (the recommended first-line treatment for gonorrhea in Japan) would be effective against genital gonorrhea caused by strains exhibiting decreased susceptibility to ceftriaxone (e.g., strains GU140106 and A8806). However, such strains could be resistant to lower-dose regimens, including 250-mg and 500-mg doses of ceftriaxone.

This *N. gonorrhoeae* strain, GU140106, was isolated from the urethra of a man who received fellatio from a

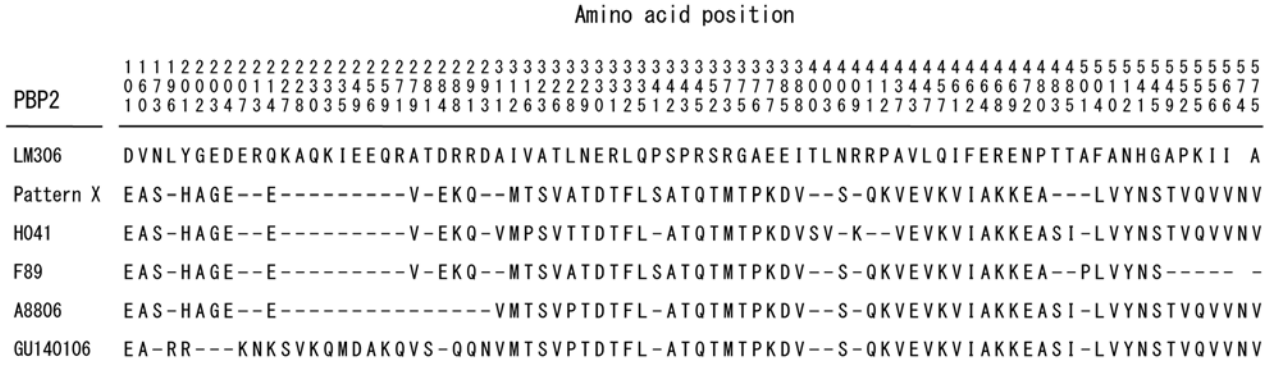

Figure. Sequences of altered amino acids in penicillin-binding protein 2 (PBP2) of *Neisseria gonorrhoeae* strains with decreased susceptibility to oral cephalosporins and strains with resistance to ceftriaxone. Strain GU140106 was isolated from a urethral swab sample from a man in in Nagoya, Japan, who had received fellatio, without condom use, from a female sex worker. Sequences are aligned with wild-type PBP2 derived from nucleic acid sequence of the *penA* gene of penicillin-susceptible *N. gonorrhoeae* strain LM306 (GenBank accession no. M32091). The PBP2 pattern X of strains with decreased susceptibility to oral cephalosporins is quoted from our previous study (*6*). The PBP2s of ceftriaxone-resistant strains H041 and F89 are derived from their *penA* genes (GenBank accession nos. AB546858 and JQ073701, respectively). The PBP2 of strain A8806, which has decreased susceptibility to ceftriaxone, is derived from the nucleic acid sequence of the *penA* gene (David M. Whiley, pers. comm., 2015). In strain H041, the concurrent presence of substitutions A311V, V316P, and T483S was reported to be responsible for conferring resistance to ceftriaxone (*7*). The PBP2 of strain GU140106 is derived from the nucleic acid sequence of the *penA* gene (GenBank accession no. LC056026); the strain has the same A311V and T483S substitutions as strain H041, but it has substitution V316T instead of V316P. The PBP2s of strains GU140106 and A8806 have the same amino acid substitutions at A311, V316, and T483. In addition, PBP2 of strain GU140106 has several amino acid changes in positions 227–281 that were not observed in other strains.

female sex worker; thus, the bacteria could have derived from her pharynx. *N. gonorrhoeae* strain H041 was previously isolated from the pharynx of a female sex worker (*1*). To prevent the emergence and spread of ceftriaxone-resistant *N. gonorrhoeae*, pharyngeal gonorrhea must be treated. It is uncertain whether a 1-g dose of ceftriaxone would be effective against pharyngeal gonorrhea caused by strains with decreased susceptibility to ceftriaxone, and this regimen might facilitate the selection of such strains from oral cephalosporin-resistant strains in the pharynx. The emergence of *N. gonorrhoeae* GU140106 in Japan suggests that new strategies (not just increased ceftriaxone doses), including combination treatment with ceftriaxone and another class of antimicrobial drugs and multiple dose regimens of ceftriaxone, might be required to treat pharyngeal gonorrhea.

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Measles Outbreak among Adults, Northeastern China, 2014

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To the Editor: In 2005, the World Health Organization (WHO) proposed to eliminate measles in the Western Pacific Region by 2012, and in 2006, China began a 6-year measles elimination campaign. The strategy included a routine 2-dose measles-containing vaccine (MCV) for children 8 months and 18–23 months of age, supplemented by nationwide vaccination activities in 2010 for children born during 1996–2010 (*1*). As a result, China's measles incidence rate has dropped sharply since 2008 and reached its lowest level (0.46 cases/100,000 population) in 2012 (*2*). However, the rate has risen again since 2012; in 2014, incidence was 3.88 cases/100,000 population (*3*). Shenyang, a hub city in northeastern China, experienced a massive measles outbreak in 2014, and we analyzed the causes and characteristics of this outbreak.

Shenyang Center of Disease Control reported 2,058 confirmed measles cases (1,447 laboratory diagnosed, 611 clinically diagnosed) in 2014 (25.02 cases/100,000 population), much higher than that reported in Shenyang in 2013 (2.33/100,000). Most cases occurred in children 0–1 years of age (487 cases; 1,145.77/100,000), followed by persons 25–30 (227 cases; 28.57/100,000), 30–35 (203 cases; 32.42/100,000), and 35–40 (203 cases; 35.02/100,000) years of age. Among all 2,058 confirmed cases, 438 patients were hospitalized because of measles complications; no deaths were reported.

[1]These authors contributed equally to this article.

Within Shenyang, Kangping district had the highest confirmed measles incidence rate (80.59 cases/100,000 persons), followed by Tiexi (38.08/100,000) and Faku (32.2/100,000) districts. The remaining confirmed cases occurred in other districts.

Of the 1,207 adults with confirmed measles, migrant workers (640 cases) and farmers (234 cases) accounted for 72.4% of total cases. All confirmed measles-infected adults were surveyed by questionnaire; 93.0% did not recall receiving MCV or had no history of MCV. All 44 measles virus samples genotyped were genotype H1a.

The most notable characteristic of this outbreak was that adults accounted for more than half of reported cases (Figure). Shenyang conducted citywide supplementary vaccination activities in 2009 directed toward children born during 1995–2009, and among these cohorts (now 5–19 years of age), the incidence rate was lower in this outbreak, proving the efficiency of the supplementary vaccination activities. However, for patients >20 years of age, who were not included in the supplementary vaccination activities, the efficacy of their previous 2-dose vaccines also should have offered protection. Thus, other potential risk factors must exist.

One risk factor is the limited vaccine coverage. China started the measles vaccine plan in the 1960s, but from 1960 until the 1980s, local vaccination coverage was poor for suburban populations. In recent years, the national reported coverage of both 1-dose and 2-dose MCV have increased from <85% to >98.5% for 2-year-olds (*2*,*4*). However, a door-to-door measles questionnaire survey during an outbreak in Henan province in 2013 reported vaccine coverage of only 80%–90% (*5*). The reason may be that, currently, China calculates vaccine coverage using the number of vaccinated children as the numerator and the number of clinic-registered children as the denominator. This method excludes those who did not register at a community clinic (e.g., because the family breached the 1-child policy and therefore refused registration or because of lack of medical insurance) and thus resulted in higher reported coverage rates. Unvaccinated persons who missed supplementary vaccination activities also possibly became susceptible to measles.

The second characteristic was the higher incidence rates in the suburban than urban districts (Figure). In fact, the 3 districts (Kangping, Tiexi, Faku) reporting the highest incidence rates were all suburban and industrial districts. The underlying reason was the aggregation of migrant workers in these districts. Shenyang is a hub city in northeastern China where workers from the surrounding rural regions come for job opportunities. These labor workers gather at suburban and industrial districts, and ≈20% of them lack proper vaccination because of limited healthcare access during childhood. Eventually, the aggregation of these susceptible persons caused the adult epidemic in this outbreak.

Although measles incidence in China has decreased sharply since 2010, multiregion epidemics have again been reported, especially among adults, in recent years. The underlying reasons for the Shenyang outbreak in 2014 are limited vaccine coverage and aggregation of susceptible persons. This adult-centered epidemic should serve as a reminder that preventing measles in adults might play an increasing role in future measles elimination efforts.

World Health Assembly and global vaccination partners endorsed the Global Vaccine Action Plan in 2012, and WHO now aims to eliminate measles in 5 of the 6 WHO regions by 2020 (*6*,*7*); the United States first achieved this goal in 2000. However, multiple measles outbreaks were reported in recent years in countries where elimination has been achieved, such as the United States (*8*) and Australia (*9*), mainly because of transmission resulting from international travel and low vaccine coverage in some populations (*10*). China is the most populous country in the world, and eliminating measles in China would help prevent future global transmission events.

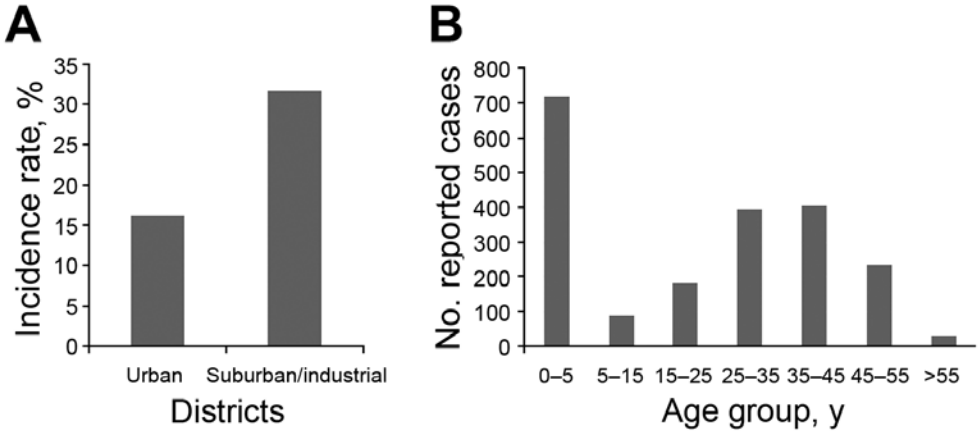

Figure. Geographic (A) and age (B) distributions of measles patients, Shenyang, China, 2014.

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Objective Determination of End of MERS Outbreak, South Korea, 2015

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To the Editor: After not finding any additional cases of Middle East respiratory syndrome (MERS) for several weeks in South Korea, in July 2015, the South Korean government and the World Health Organization (WHO) discussed the appropriate time to declare the end of the outbreak in July 2015 (*1*). This declaration would enable allocation of human resources to healthcare facilities to return to normal and would help restore international travel to the country. A widely acknowledged criteria of WHO to determine the end of an epidemic has been twice the length of the incubation period since the most recently diagnosed case (*2*). For MERS, the longest incubation period is 14 days. Thus, adopting 28 days as the waiting period, and counting days from diagnosis of the most recent case on July 4, 2015, the earliest date the South Korean government could have declared the end of outbreak was August 2 if it adhered to WHO criteria (*1*). However, to emphasize safety to the nation and to international travelers at an earlier time, the South Korean government originally decided to announce the end of the MERS outbreak on July 27, the date the last quarantined MERS patient was released from movement restriction. Because we are concerned about the validity of strict adherence to the WHO criteria, we objectively calculated the probability of observing additional cases at a given time and compared that probability with the WHO criteria.

To clearly define the end of the outbreak, we excluded reintroduction of imported cases and cases of MERS coronavirus infection resulting from a zoonotic reservoir. We defined the end of the outbreak as the end of continued chains of transmission. The probability of observing additional cases was derived by using the serial interval; that is, the time from illness onset in the primary case-patient to illness onset in a secondary case-patient, and the transmissibility of MERS (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-1383-Techapp1.pdf). Both of these epidemiologic variables were estimated by using case data in South Korea (*3*,*4*). As practiced in the determination of the length of quarantine (*5*), the end of outbreak can be declared if that probability is <5%, a threshold value.

Our analysis showed that the first date on which the posterior median probability decreased to <5% was July 21 (Figure, panel A). The first date on which the posterior median decreased to 1% was July 23. Compared with August 2 as calculated from the WHO criteria, the end of the outbreak could have been declared 11 and 9 days earlier, respectively. Because the choice of 5% or 1% as the threshold probability is arbitrary (as practiced in determining the p value in any hypothesis testing) and because of the need to account for parameter uncertainties, we also measured the sensitivity of the first date on which the South Korean government could declare the end of the outbreak to a variety of threshold values (Figure, panel B). Examination of the probability of observing additional cases in the range of 0.5% to 10% indicated the end of the outbreak could have been declared from July 21 to July 24 (i.e., 9–12 days earlier than August 2).

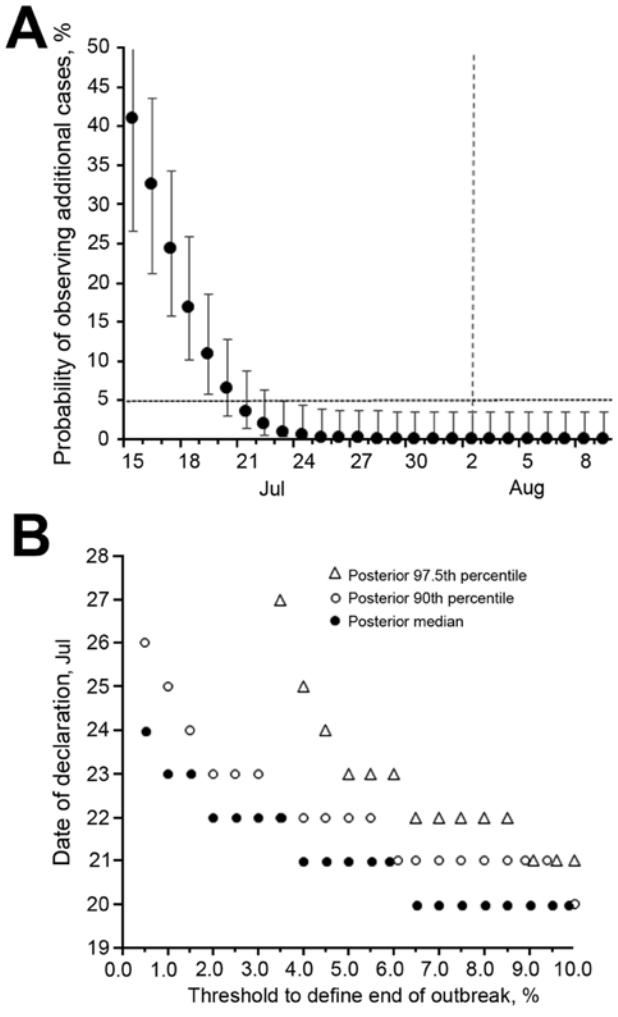

Figure. Estimated probability of observing additional cases of Middle East respiratory syndrome coronavirus infection, South Korea, 2015. A) Estimated probability of observing additional cases on each calendar date, given that no illness onset has been observed by the specified date. Circles represent posterior median values; whiskers extend to upper and lower 95% credible intervals. Horizontal dashed line represents 5%, a threshold level. Vertical line indicates August 2, 2015, on which the end of the outbreak might be declared if World Health Organization criteria were followed. B) Calendar date to declare the end of outbreak for different threshold probabilities and percentile points of posterior distribution. Horizontal axis corresponds to the probability of observing additional cases. Vertical axis shows the date of declaration which is calculated as 1 day plus the date at which the probability of observing additional cases lowered the specified threshold probability.

Our proposed method does not account for missing undiagnosed or mild cases, and underdiagnosis would considerably extend the time to declare the end of an outbreak (and thus the proposed method is not directly applicable to, for example, Ebola virus disease in West Africa, for which we are currently developing an alternative method). All possible contact with diagnosed case-patients in the late phase of the MERS outbreak in South Korea were traced (*6,7*); thus, we believe it was appropriate to ignore ascertainment bias in this specific setting. Although our proposed approach is simplistic, adopting the WHO criteria could have added >1 week to the elevated state of tension, and the use of the incubation period distribution would be fully supported only when the exact times of infection were known for exposed potential contacts. Although it is a posteriori reasoning, the original decision made by the South Korean government at an earlier date was ironically supported by our proposed method. Rather than adopting the use of "twice" and the "incubation period," which has not been theoretically justified, an objective decision of the end of an outbreak should explicitly rest on the risk of observing at least 1 more case on or after a specified date.

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Surveillance for Coronaviruses in Bats, Lebanon and Egypt, 2013–2015

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To the Editor: Coronaviruses (CoVs) in bats are genetically diverse, and evidence suggests they are ancestors of Middle East respiratory virus CoV (MERS-CoV), severe acute respiratory syndrome CoV, and human CoVs 229E and NL63 (*1–4*). We tested several bat species in Lebanon and Egypt to understand the diversity of bat CoVs there.

Samples were collected during February 2013–April 2015. A total of 821 bats were captured live in their caves; sampled (oral swab, rectal swab, serum); and released, except for 72 bats that died or were euthanized upon capture. Lungs and livers of euthanized bats were harvested and homogenized. Caves were in proximity to human-inhabited area but not in proximity to camels.

In Egypt, we sampled 3 bat species (online Technical Appendix 1, http://wwwnc.cdc.gov/EID/article/22/1/15-1397-Techapp1.pdf). Eighty-two Egyptian tomb bats (*Taphozous perforatus*) tested negative for CoV. We also sampled 31 desert pipistrelle bats (*Pipistrellus deserti*) and detected an HKU9-like betacoronavirus (b-CoV) in the liver of 1 bat (prevalence 3.2%). From 257 specimens from Egyptian fruit bats (*Rousettus aegyptiacus*), we detected b-CoV in 18 samples from 18 different bats (prevalence 7%). A murine hepatitis virus–like CoV was detected in the lung of 1 bat. HKU9-like viruses were detected in 5 oral, 2 lung, 5 liver, and 5 rectal samples. Overall, 5.1% of the bats tested positive.

In Lebanon, we sampled 4 bat species. Four *Rhinolophus hipposideros* bats and 6 *Miniopterus schribersii* bats tested negative. One of 3 *Rhinolophus ferrumequinum* bats sampled was positive. We sampled 438 *Rousettus aegyptiacus* bats from 10 different locations and detected HKU9-like viruses in 24 rectal swab specimens (prevalence 5.5%). Overall, 5.5% of the bats tested positive.

A subset of the samples (696 samples: 516 from Egypt, 180 from Lebanon) were tested for MERS-CoV by using the specific upstream of E quantitative reverse transcription PCR; all tested negative. Serum samples from 814 bats tested negative for MERS-CoV antibodies.

Phylogenetic analysis revealed that the RNA-dependent RNA polymerase (*RdRp*) genes of viruses detected in *R. aegyptiacus* bats in Lebanon and Egypt were closely related to the *RdRp* gene of HKU9 CoV (Figure). Our viruses clustered in 3 groups: A, B, and C. Group A viruses were closely related to HKU9-10-2 virus and included viruses from Egypt. Group B included viruses from both countries and were closely related to HKU9-1 and HKU9-4 viruses. Group C also included viruses from both countries that were related to HKU9-3 and HKU9-5 viruses. The *RdRp* fragments sequenced had <90% nt similarity among groups A, B, and C. Within-group nucleotide similarity was >90%, and amino acid variability was 2%–4% (online Technical Appendix 2, http://wwwnc.cdc.gov/EID/article/22/1/15-1397-Techapp2.xlsx). The phylogenetic tree of the N gene also showed proximity of the viruses detected in our study to HKU9 viruses (online Technical Appendix 1). Viruses from Lebanon clustered together as did the viruses from Egypt.

Most of the positive samples were detected in Egyptian fruit bats. These are cave-dwelling species that inhabit regions of East Africa, Egypt, the Eastern Mediterranean, Cyprus, and Turkey (*5*). This species is a reservoir for several viruses, including Marburg, Kasokero, and Sosuga

[1]These authors contributed equally to this article.

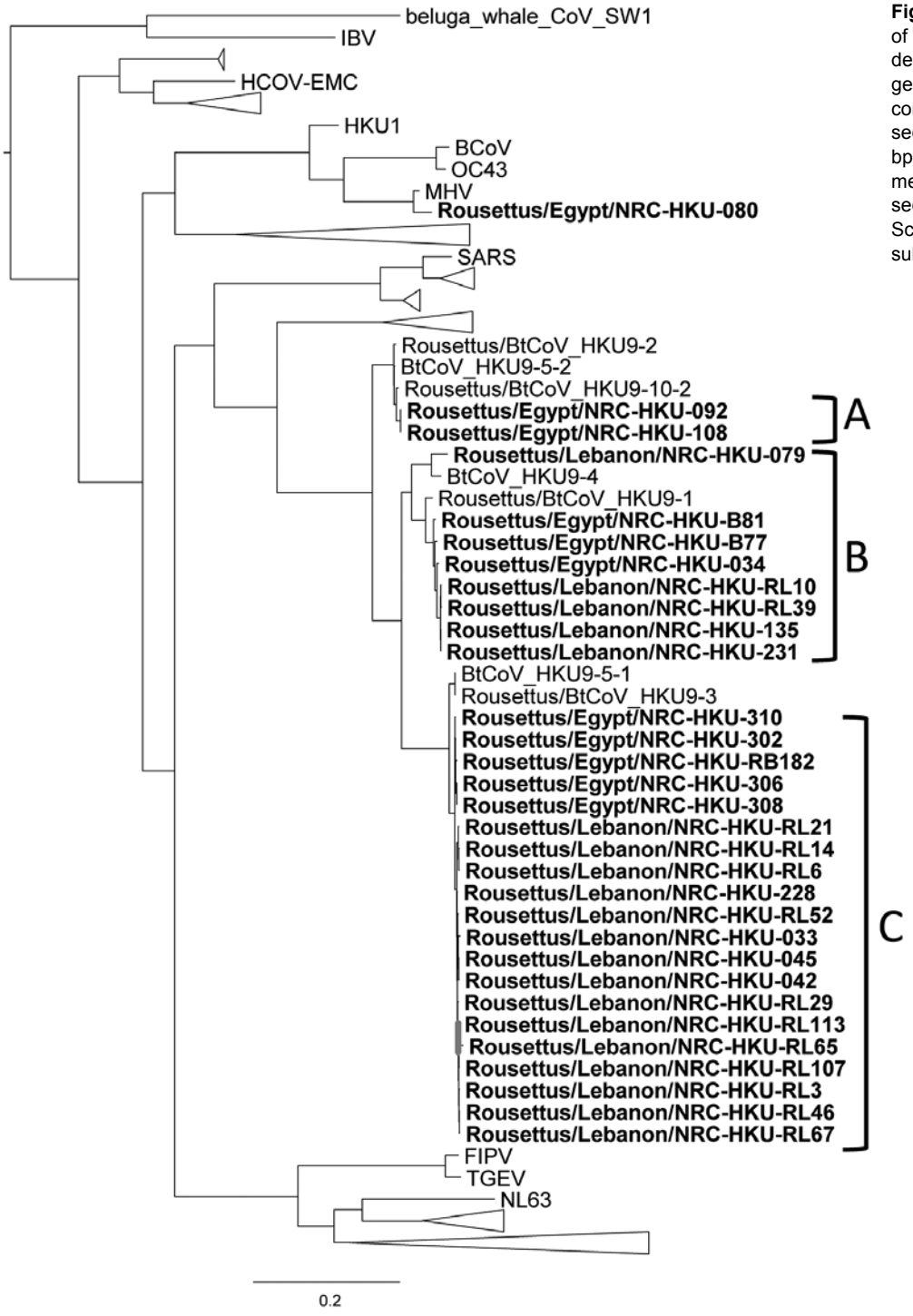

Figure. Phylogenetic tree of the coronavirus RNA-dependent RNA polymerase gene. This tree was constructed on the basis of a sequence alignment of 330 bp using the neighbor-joining method. Bold text indicates sequences found in this study. Scale bar indicates nucleotide substitutions per site.

viruses (*6*–*8*). The b-CoVs HKU9 and HKU10 were detected in Chinese fruit bats (*9*). All but 1 of the detected viruses were HKU9-like. However, there was enough genetic variability within the sequenced *RdRp* fragments to suggest the circulation of at least 3 diverse groups comprising 3 different CoV species.

Our detection of CoVs in oral, rectal, lung, and liver samples suggests that CoV infection in those bats was systemic, although the bats were apparently healthy. One bat had a murine hepatitis virus–like infection. This bat was captured from a brood that inhabited the windowsills of a historic building in urban Cairo. This infection might

have been a cross-species infection from mice to bats in the same habitat.

Although bats rarely come in direct contact with humans, humans can come into more frequent contact with bat urine and feces and, in the case of fruit bats, bat saliva through partially eaten fruits. Bats in the Middle East are not eaten for food but are occasionally hunted. In this study, HKU9-related viruses were detected in apparently healthy fruit bat species from Egypt and Lebanon and appear to cause systemic infection. HKU9-related viruses are not known to cause human disease. MERS-CoV was not detected in bats sampled in this study. More surveillance for bat CoVs in the Middle East is needed, and the zoonotic potential for bat-CoVs requires further study.

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Ebola Virus Disease Complicated by Late-Onset Encephalitis and Polyarthritis, Sierra Leone

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To the Editor: Ebola virus (EBOV) disease is usually an acute illness, but increasing evidence exists of persistent infections and post-Ebola syndromes. We report a case of EBOV encephalitis.

A 30-year-old woman with no known EBOV contact sought treatment at an Ebola isolation unit in Freetown, Sierra Leone, on January 1, 2015 (day 7 of illness). She was afebrile and weak, but ambulatory, with a history of fever, vomiting, diarrhea, headache, and muscle and joint pain. According to local protocol, she was given oral antimalarial, antimicrobial, and antiemetic drugs and oral rehydration therapy. On day 8 of illness, after testing EBOV PCR–positive (cycle threshold [C_t] value of 23.5) (*1*), she was given intravenous ceftriaxone (2 g) for 7 days, artesunate (180 mg) for 3 days, and Ringer's lactate (4–6 L) with supplemental KCl for 5 days.

During days 13–15, the patient improved, moving independently and talking. On day 16, she became confused; by day 20, she was unresponsive to voices. Intravenous ceftriaxone (2 g) and artesunate (180 mg) were administered for an additional 7 and 3 days, respectively. On days 28 and 29, she was still unconscious; serum PCR test results on both days were negative for EBOV. On day 29, she was transferred to Connaught Hospital in Freetown, where she had a Glasgow Coma Scale score of 9/15 (E3, V1, M5) but no localizing or focal signs. She was

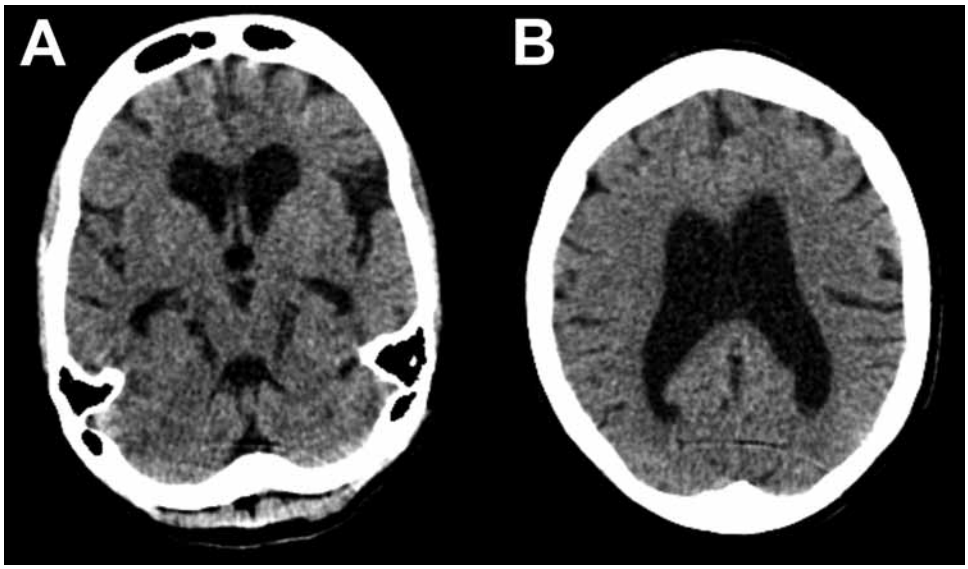

Figure. Representative axial cuts from noncontrast head computed tomography scan imaging of a 30-year-old woman with encephalitis resulting from Ebola virus infection, Sierra Leone. Images show global atrophy in keeping with nonobstructive ventriculomegaly and no periventricular low attenuation: A) subcortical atrophy; B) cortical atrophy. There was no evidence of hydrocephalus, previous stroke, or intracranial hemorrhage. A cavum septum pellucidum was noted in other images.

given intravenous fluconazole (800 mg 1×/d). Admission blood test results showed anemia, elevated alanine aminotransferase and C-reactive protein, and low creatinine (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-1212-Techapp1.pdf). HIV test results were negative.

On day 34, large-joint polyarthritis of the right shoulder, left elbow, and left knee developed. Affected joints appeared normal on radiographs, and synovial fluid (15 mL) from the left knee was EBOV PCR negative. She was given diclofenac (50 mg 2×/d) and 1 intramuscular dose of methylprednisolone (80 mg). Concurrent blood PCR on day 34 was negative.

By day 41 she was more alert, although her family reported she had slowed responses. Lumbar puncture was performed; opening pressure (30 cm H_2O) was elevated, and cerebrospinal fluid (CSF) was EBOV PCR–positive (C_t value 37.6), as determined by using the Public Health England in-house, optimized version of the Trombley assay (*2*) with a cutoff C_t value of 40. Concurrent catheter specimens of urine and blood samples tested EBOV-negative. FilmArray (BioFire Diagnostics, Salt Lake City, UT, USA) testing showed methicillin-resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* in CSF and mixed pathogens in urine. A computer tomographic scan image of the patient's head showed substantial cerebral atrophy without hydrocephalus (Figure).

On day 44, an underarm sweat swab sample was PCR-positive (C_t value 39.6) and a buccal swab sample PCR-negative for EBOV. Ongoing painful synovitis was treated with an additional 80-mg intramuscular dose of methylprednisolone. On day 51, a midstream urine sample was EBOV PCR–positive (C_t value 35.7), and an underarm sweat swab sample was EBOV PCR–negative. The patient was discharged; her family was advised to minimize contact with her body fluids.

At follow-up on day 64, the patient's family reported she had impaired short-term memory and ongoing slowness. She had a score of 18/23 on the Mini–Mental State Examination, but general neurologic exam results were normal. A midstream urine test was still EBOV PCR–positive (C_t value 39.6); PCR of her sweat swab sample was inhibited (online Technical Appendix). She was referred to the local survivors' clinic; no contact cases were reported.

The depressed mental status and presence of EBOV in this case-patient's CSF are consistent with encephalitis, a finding in autopsies of persons with Marburg virus infection (*3*,*4*) and in EBOV nonhuman primate models (*5*). The general atrophy seen in computer tomographic scan images is consistent with a rapidly developing complication of a diffuse inflammatory process. Given inadequate antimicrobial drug doses for meningitis and clinical improvement, we believe methicillin-resistant *S. aureus* and *K. pneumoniae* were CSF sample contaminants.

This case shows the brain's immune privilege is incomplete for EBOV and prompts a broader discussion regarding neurovirulence in Ebola virus disease. Our finding that EBOV can be present in CSF, even after serum clearance, adds to the knowledge of neurologic symptoms in acute infection and of postinfectious sequelae in observational clinical studies (*6*–*8*). This finding raises the possibility that EBOV persistence elsewhere in the body, or in multiple organs, could be an indicator of or risk for central nervous system invasion.

Our report has limitations. We could not perform many blood chemistry tests, in-country virus cultures, or deep sequencing on samples. Likewise, diagnosis of coma was challenging because of the lack of CSF cell counts, biochemistry values, and paired EBOV IgG and IgM titers in CSF and blood.

This case raises the practical issue that Ebola treatment requires understanding of multiorgan virologic and inflammatory complications; survivor care and research programs should screen for neurocognitive impairment and consider appropriate imaging. The case confirms previously reported intermittent EBOV PCR positivity in urine (*9*). The development of arthritis with synovitis, treated with corticosteroids, supports the diagnosis of reactive arthritis.

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Louseborne Relapsing Fever in Young Migrants, Sicily, Italy, July–September 2015

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To the Editor: During the early 20th century, at the end of World War I, and during World War II, louseborne relapsing fever (LBRF) caused by *Borrelia recurrentis* was a major public health problem, especially in eastern Europe and northern Africa (*1,2*). Currently, poor living conditions, famine, war, and refugee camps are major risk factors for epidemics of LBRF in resource-poor countries, such as those in the Horn of Africa (*3,4*).

Increased migration from resource-poor countries and war/violence create new routes for spread of vectorborne diseases. Recently, several cases of LBRF have been reported among asylum seekers from Eritrea in the Netherlands, Switzerland, and Germany (*5–8*). All of these asylum seekers had been in refugee camps in Libya or Italy. We report 3 cases of LBRF in migrants from Somalia to refugee camps in Sicily, Italy.

Patient 1 was a 13-old-boy from Somalia who arrived in Palermo, Italy, on July 11, 2015, after traveling though Libya. He was admitted to G. Di Cristina Hospital in Palermo 5 days after arrival because of high fever, headache, and general malaise, which developed 2 days after arrival. The patient had skin lesions on his fingers and legs and a conjunctival infection. He had thrombocytopenia (79,000 platelets/μL [reference range 150 platelets/μL–400 platelets/μL]), creatine phosphokinase level 967 mg/L [reference range 0.001 mg/L–0.10 mg/L], aspartate aminotransferase level 30 U/L (reference value 37 U/L), and alanine aminotransferase level 21 U/L (reference value 41 U/L). He was given ceftriaxone (2 g/d) and intravenous hydration. His conditions worsened ≈10 hours after treatment: high fever (temperature 40°C), chills, and profuse sweating (Jarish-Herxheimer reaction). The patient recovered

after 15 days of treatment with ceftriaxone. A Giemsa-stained blood smear was negative for *Plasmodium* spp. but showed large numbers of spirochetes. Serologic screening results for *B. burgdorferi* were negative.

Patient 2 was a 17-old-boy from Somalia who arrived in Lampedusa, Italy, on August 27, 2015, after traveling through Libya. Fever and artromyalgia developed 6 days after his arrival, and he was admitted to Hospital Paolo Giaccone in Palermo. Blood analyses showed increased levels of aminotransferases, thrombocytopenia (69,000 platelets/μL), and mild anemia (hemoglobin level 94 g/L [reference range 130 g/L–160 g/L]). A blood smear was negative for *Plasmodium* spp. but positive for spirochetes. Serologic screening results were negative for malaria, leptospirosis, infection with *Rickettsia conorii*, and dengue. An ELISA result was positive for *B. burgdorferi*, and a Western blot result was positive for *Borrelia* spp. proteins p10, p41, and OspC. The patient recovered after treatment with doxycycline (100 mg/d) and ceftriaxone (2 g/d) for 10 days.

Patient 3 was a 17-year-old boy from Somalia who arrived in Trapani, Italy, on September 4, 2015. He reported that he stayed for 5 months in Libya before arriving in Italy. Fever, artromyalgia, severe dehydration, renal failure, and mental confusion developed 3 days after his arrival, and he was admitted to Hospital Paolo Giaccone. He had severe thrombocytopenia (4,000 platelets/μL); mild anemia (hemoglobin level 88 g/L); increased levels of aminotransferases (aspartate aminotransferase 282 U/L, alanine aminotransferase 489 U/L), lactate dehydrogenase (1,041 U/L [reference range 105 U/L–333 U/L]), D-dimer (6,311 ng/mL [reference range 10 ng/mL–250 ng/mL]), C-reactive protein (237.8 mg/dL [reference range 0 mg/dL–10 mg/dL]), and creatinine (2.6 mg/dL [reference range 0.6 mg/dL–1.2 mg/dL]); and azotemia (blood urea nitrogen level 150 mg/dL [reference range 7 mg/dL–20 mg/dL]). A blood smear was negative for *Plasmodium* spp., but a Giemsa-stained thick blood smear was positive for spirochetes. Serologic screening results were negative for malaria, leptospirosis, infection with *B. burgdorferi*, and dengue. The patient recovered after treatment with doxycycline (100 mg/d) and ceftriaxone (2 g/d) for 10 days.

DNA was extracted from blood specimens from the 3 patients and used for molecular identification and characterization of the etiologic agent of LBRF. We used a species-specific real-time PCR for *B. recurrentis* and *B. duttonii*, which targeted an internal region of the *recN* gene. Multispacer sequence typing of the 16S rRNA gene was used for bacterial identification and genotyping (*9,10*). All blood samples were positive for *B. recurrentis* by real-time PCR. Multispacer sequences showed 100% identity with sequences of *B. recurrentis* reference strain A1 (GenBank accession no. CP000993) for isolates from all patients.

We report 3 patients in Italy with LBRF who migrated from Somalia. These patients arrived in Italy after traveling in several countries in Africa and crossing the Mediterranean Sea. The patients did not associate with each other during travel, and the place where they were infected is unknown. However, because they came from a disease-endemic country, they probably had been infested with body lice and were infected with *B. recurrentis* in Somalia or other neighboring countries.

Because the 3 cases we observed might indicate that more migrants and refugees are infected, LBRF should be considered an emerging disease among migrants and refugees. Diagnostic suspicion of LBRF should lead to early diagnosis among refugees from the Horn of Africa and in persons in migrant camps. Furthermore, improved public health measures and hygiene must be implemented for persons in refugee or migrant camps.

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Anticipated Negative Responses by Students to Possible Ebola Virus Outbreak, Guangzhou, China

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To the Editor: In 2014, a serious Ebola virus disease (EVD) outbreak occurred in West Africa (*1*). In a study on EVD-related perceptions, 85% of US respondents mistakenly believed that EVD could be transmitted through airborne droplets from patients' sneezes or coughs (*2*). EVD-related panic was reported in the United States (*3*) and the United Kingdom (*4*).

During November 15–December 20, 2014, we conducted a cross-sectional survey of 1,295 undergraduate students in Guangzhou, China, where the population of immigrants from Africa is high (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/1/15-0898-Techapp1.pdf). Our aim was to measure students' anticipated negative emotional responses and avoidance activities (dependent variables) to a possible outbreak of EVD (*5*). We constructed scales for the dependent and independent variables to assess EVD-related perceptions: 1) misconceptions/knowledge about transmission modes, 2) scenarios of an EVD outbreak in Guangzhou (chances, severity, control), 3) efficacy of preventive measures and self-protection, and 4) public stigma toward EVD survivors. MLwiN 2.30 (Centre for Multilevel Modeling, University of Bristol, Bristol, UK) was used for multilevel regression analyses (online Technical Appendix).

We analyzed data from 1,155 (89.2%) students who had heard of EVD. To the example of 2–3 EVD cases detected in Guangzhou, 31.0% showed ≥4 types of anticipated negative emotions (e.g., fear, panic, worry); 59.5% showed ≥3 types of anticipated unnecessary avoidance. Most (80.0%) indicated ≥1 misconception regarding transmission mode (e.g., believed it was droplet or waterborne) but knew that direct contact with the corpse of an infected person (69.0%) and body fluids (81.4%) could lead to infection and perceived EVD as fatal (85.6%,) and highly infectious (81.6%). About half of respondents believed that effective treatment and a vaccine were unavailable (51.9% and 59.1%, respectively); 22.2% anticipated EVD outbreaks among Africans in Guangzhou (during the next 12 months). Many students perceived severe consequences if a small EVD outbreak occurred in Guangzhou and believed an outbreak would have a high fatality rate (70.5%), EVD is highly infectious (65.4%), an outbreak would be of long duration (47.5%), and the number of infected persons would be high (39.9%); 52.5%–79.2% of respondents lacked confidence in the government's ability to control an outbreak (e.g., ability to provide adequate vaccines, medication, protective gear). Half or more of respondents believed that restricting travel by Africans to and from Africa and avoiding visiting African-inhabited areas were effective means of prevention. About 40% were confident that they could protect themselves or family members from EVD (online Technical Appendix Tables 1, 2).

Older age, female sex, longer school years, and rural origin were associated with negative emotional responses, avoidance, or both (online Technical Appendix Table 3). In multivariate analyses that adjusted for significant background variables, we found positive associations between both dependent variables and the following independent variables: perceived fatality of EVD, perceived nonavailability of treatment, misconceptions regarding modes of transmission, perceived severity of a Guangzhou outbreak, perceived efficacy of restricting Africans' travel, perceived efficacy of avoiding African-inhabited areas, and public stigma toward EVD survivors. Confidence in governmental control was negatively associated with both dependent variables. Some variables were positively associated with emotional response but not avoidance (perceived irreversible harm, perceived chance of outbreak in Guangzhou and in other parts in China, perceived self-efficacy for protection); 2 variables (perceived nonavailability of vaccine and knowledge of transmission mode) were positively associated with avoidance measures but not with emotional responses (Table).

Because EVD causes serious physical harm, negative emotional responses and unnecessary avoidance practices were anticipated. Such negative community responses might cause individual and societal harm, as witnessed during the epidemic of severe acute respiratory syndrome (*6*). Misconceptions concerning transmission modes were prevalent and significantly correlated with both dependent variables. More than 80% of respondents perceived that the virus was highly infectious, another significant factor.

About 20% of participants believed that an EVD outbreak would occur in Guangzhou in the next year. Among all participants, many anticipated severe outcomes but

Table. Factors associated with anticipated responses to EVD, adjusted for sociodemographic variables, Guangzhou, China, 2014*

Factor	Emotional Response to Ebola Scale†		Unnecessary Avoidance Scale‡	
	β (SE)	p value	β (SE)	p value
Perceived severity of EVD				
EVD is fatal	1.270 (0.928)	0.171	0.855 (0.388)	**0.027**
EVD causes irreversible harm to physical health	2.647 (0.637)	**<0.001**	0.504 (0.064)	0.064
Perceived fatality of EVD	2.545 (0.635)	**<0.001**	1.177 (0.269)	**<0.001**
Perceived high infectivity of EVD	1.568 (0.842)	0.063	1.273 (0.350)	**<0.001**
Treatment and vaccine availability				
Treatment not available	2.143 (0.639)	**<0.001**	1.108 (0.271)	**<0.001**
Vaccine not available	1.236 (0.654)	0.059	0.786 (0.276)	**0.005**
Misconceptions and knowledge about modes of transmission of EVD				
Misconceptions about Mode of Transmission Scale	0.406 (0.113)	**<0.001**	0.214 (0.048)	**<0.001**
Knowledge about Mode of Transmission Scale	0.285 (0.171)	0.095	0.369 (0.071)	**<0.001**
Perceptions related to EVD outbreak				
Chances of Outbreak Scale—Guangzhou	0.688 (0.091)	**<0.001**	0.064 (0.039)	0.100
Perceived Chances of Outbreak Scale—Other Parts of China	0.986 (0.189)	**<0.001**	0.151 (0.081)	0.062
Perceived Severity of Outbreak in Guangzhou Scale	0.825 (0.072)	**<0.001**	0.222 (0.031)	**<0.001**
Confidence in Governmental Control Scale	–1.024 (0.086)	**<0.001**	–0.192 (0.038)	**<0.001**
Perceived efficacy and self-efficacy				
Perceived Efficacy of Restricting Africans Travel Scale	1.003 (0.176)	**<0.001**	0.543 (0.073)	**<0.001**
Perceived Efficacy of Avoidance Scale	0.544 (0.138)	**<0.001**	0.595 (0.056)	**<0.001**
Perceived Self-Efficacy for Protection against EVD Scale	–0.571 (0.145)	**<0.001**	–0.112 (0.062)	0.070
Public stigma toward EVD survivors				
Public Stigma Scale	0.231 (0.032)	**<0.001**	0.125 (0.013)	**<0.001**

*Among participants who had heard of EVD (n = 1,155). Bold indicates significance. β, multilevel linear regression coefficient adjusted by significant background variables; EVD, Ebola virus disease.
†Anticipated Emotional Response to Ebola Scale items included the following: "If there are 2–3 EVD cases in Guangzhou, how likely would you 1) worry about getting infected with EVD, 2) worry about family members getting infected with EVD, 3) be scared, 4) be uneasy, 5) be in panic, 6) feel helpless, 7) be depressed, 8) have insomnia, 9) be distressed, 10) have fluctuating emotions, and 11) be emotionally disturbed." Response categories: 1 = very unlikely, 5 = very likely.
‡Unnecessary Avoidance Scale items included the following: "If there are 2–3 EVD cases in Guangzhou, how likely would you be to 1) avoid going to other cities, 2) avoid going to work, 3) avoid going out if unnecessary, 4) avoid going to crowded places, 5) avoid going to hospitals, and 6) avoid taking airplanes." Response categories: 1 = very unlikely, 5 = very likely.

were not confident that the government was prepared for and could control such an outbreak.

The concentration of immigrants from Africa in this region might have increased perceived chances of an EVD outbreak and thus lead to avoidance of this population. The high percentages of those who believed that restricting Africans' travel was effective also might result in discrimination.

Public stigmatization toward EVD survivors, another significant factor, was a prominent attitude (*7*,*8*). Fear, misconceptions, and perceived likelihood of EVD to cause death may lead to patient stigmatizing, which could hinder case detection and patients' service seeking. The relationship between stigmatization and EVD-related perceptions should be investigated.

The study's limitations included the inability to assess real responses, inability to generalize findings to all university students and the general public, and the use of scales that had not been validated. Also, some students might have given exaggerated responses.

In summary, misconceptions and perceptions regarding EVD may result in negative community responses in Guangzhou. Health education is needed to clarify that EVD is not airborne or waterborne or highly infectious and that avoidance is not an effective preventive measure. In addition, the government should start developing and publicizing its preparedness plans.

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Multiple Fungicide-Driven Alterations in Azole-Resistant *Aspergillus fumigatus*, Colombia, 2015

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To the Editor: We read with interest the report by van der Linden et al. about the prevalence of azole-resistant *Aspergillus fumigatus* isolates from 19 countries, including 2 from the Americas (Brazil and the United States) (*1*). Recent reports have suggested a link between use of fungicides in agricultural practices and the presence of triazole-resistant *A. fumigatus* among azole-naive persons (*2*). These resistant strains harbored the TR34/L98H and TR46/Y121F/T289A mutations in the *CYP51A* gene and its promoter region. These novel mechanisms of resistance have been reported both in environmental and clinical samples in Europe, Asia, and Africa, suggesting a broad geographic spread. However, clinical isolates from 22 states in the United States (*3*) and a few isolates from Latin America (*1*,*4*) failed to show any fungicide-driven resistance in *A. fumigatus* in these continents, even though use of pesticides is a widespread practice in the Americas. Colombia was ranked fourth in the world in 2010 for the use of pesticides, reportedly using 14.5 tons/1,000 ha, 30% of which were fungicides (*5*). Among the fungicides approved by Colombia's regulatory agency, the Colombian Agricultural Institute (*6*), tebuconazole and difenoconazole are largely used in the flower industry, more specifically in Cundinamarca, where 60% of Colombia's flowers are produced.

In 2015, we conducted a study for which 60 soil samples from flower fields and greenhouses were collected in the outskirts of Bogota, Cundinamarca. Samples were inoculated on Sabouraud agar at 43°C, and positive samples were screened for azole-resistance on agar supplemented with either itraconazole (4 mg/L) or voriconazole (4 mg/L). Of the 38 resistant *Aspergillus* strains, 20 were selected (up to 5 colonies for each positive culture), identified as *A. fumigatus* by β-tubulin gene sequencing, and analyzed for *CYP51A* gene alterations (*7*). Results showed great diversity in molecular resistance with the presence of TR46/Y121F/T289A (n = 17), TR34/L98H (n = 1), and TR53 (n = 1) mutations; 1 isolate had a wild-type *CYP51* sequence (*8*).

Our study highlights the presence of *A. fumigatus* harboring fungicide-driven alterations in Colombia, South America. The results indicate the importance of initiating active agricultural surveillance along with close monitoring of drug resistance in clinical isolates from naive and azole-exposed patients in these countries. Clinical management of *Aspergillus* disease can be challenging because of unfavorable clinical outcomes after patients have acquired multi-azole–resistant strains from the environment (*9*). Additional studies are needed to evaluate the extent to which pesticide use in floriculture and agriculture (e.g., coffee and banana) contributes to azole resistance in Colombia.

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Azole Resistance of *Aspergillus fumigatus* in Immunocompromised Patients with Invasive Aspergillosis

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To the Editor: First-line antifungal therapy for invasive aspergillosis (IA) is voriconazole, which is challenged by the emergence of azole resistance (*1*). A recent article reported a 3.2% prevalence of *Aspergillus fumigatus* isolates that are resistant to azole from 3,788 isolates screened in Europe (*2*). Of the 1,911 patients from whom the isolates were collected, IA developed in 10 (3 proven, 1 probable, 6 possible). Prevalence of azole-resistant *A. fumigatus* disease among patient populations at risk of IA was unavailable.

As described (*3*), we screened every *A. fumigatus* isolate recovered from respiratory specimens from patients with probable or proven IA in our hospital in Paris, France, during January 2012–December 2014. Every isolate recovered from 2% malt extract agar plates or Sabouraud dextrose agar slants (Bio-Rad, Marnes-la-Coquette, France) was incubated at 30°C and tested as individual isolates or multiple ones from a single sample by using itraconazole, voriconazole, and posaconazole Etest strips (bioMérieux, Marcy l'Etoile, France). Resistance was assessed for MICs >2.0 μg/μL for voriconazole and itraconazole and >0.25 μg/μL for posaconazole by using European Committee on Antimicrobial Susceptibility Testing clinical breakpoints for fungi (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Antifungal_breakpoints_v_7.0.pdf).

Every 4 months, a local multidisciplinary medical team classified each IA case by using the 2008 criteria established by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (*4*). For 148 patients (127 with hematologic malignancies and 21 with other conditions), the team recorded 152 episodes: 9 proven and 143 probable IA episodes. Possible IA was not analyzed because of a lack of microbiologic criteria. For 51 probable IA episodes, galactomannan positivity in blood or bronchoalveolar lavage fluid samples was the only microbiologic criterion used for classification. Cultures of respiratory samples (i.e., bronchoalveolar lavage fluid, tracheal aspirate, and sputum) or biopsies were positive for 99 episodes: 68 with *A. fumigatus* isolates and 31 with other *Aspergillus* spp. isolates. Among the 68 *A. fumigatus* isolates, 1 (1.5%) associated with probable IA was resistant to azoles (*5*). The isolate harbored the TR_{34}/L98H mutation (*5*), leading to a rate of IA caused by azole-resistant *A. fumigatus* of 0.7% (1/152) for total episodes recorded and 1% (1/99) for culture-positive episodes only. Nineteen (36%) of 53 culture-negative patients and 35 (37%) of 95 culture-positive patients died.

Azole resistance of *A. fumigatus* warrants specific surveillance in hospitals treating immunocompromised patients. Prevalence of resistant isolates can differ by hospital location and underlying disease (e.g., immunodeficiency vs. chronic lung diseases). When focusing on patients with probable or proven IA, we did not observe an emergence of azole-resistant *A. fumigatus* isolates during 2006–2009 (*3*) and 2012–2014 in France. Consequently, our center does not question the use of voriconazole as first-line treatment or of posaconazole as prophylaxis.

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In Response:

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In Response: Alanio et al. comment that the prevalence of azole-resistant *Aspergillus* disease may differ, depending on location of the hospital where patients are admitted and the patients' underlying disease (*1*). Determining local or regional epidemiology, especially in areas where azole-resistant isolates are found in the environment, is indeed important. These isolates commonly harbor the TR_{34}/L98H or TR_{46}/Y121F/T289A resistance mechanism. Patients may inhale azole-resistant spores in the air and subsequently develop azole-resistant disease, even when they have never been treated with azoles (*2*). Although risk for inhalation of azole-resistant *Aspergillus* spores arguably might be similar for all patients, surveillance of *Aspergillus* isolates in the Netherlands indicates that resistance rates vary among hospitals. When all *A. fumigatus* isolates cultured from patients were investigated for azole resistance, resistance rates in the Netherlands ranged from 4.3% to 19.2% in 2013 and 3.8% to 13.3% in 2014 (*3*). The highest and lowest resistance rates were found in hospitals only 39 km from each other, supporting the observation made by Alanio et al. about variations in prevalence of azole-resistant *Aspergillus* disease (*1*).

More detailed surveillance is required to determine if local treatment guidelines should be reassessed. Two recent studies in the Netherlands investigated the risk of azole-resistant invasive aspergillosis in high-risk populations. One study conducted in a 33-bed tertiary-care university hospital intensive-care unit (ICU) showed that 26% of culture-positive patients with presumed invasive aspergillosis harbored azole-resistant isolates, a proportion 14% higher than that found in other departments in the hospital ($p = 0.06$) (*4*). The second study, which investigated azole resistance in the primary routine culture (including respiratory cultures) of 105 ICU and hematology patients, showed that the resistance rate (24.6%) for hematology patients was higher than the rate (4.5%) for ICU patients (*5*). Other countries have also reported higher prevalence of resistance in high-risk populations than in other populations.

One problem with assessing prevalence of azole resistance is that the recovery of *A. fumigatus* in culture may vary considerably among different patient groups. A recent audit in our hematology department over the past 5 years indicated that *A. fumigatus* was cultured in only 35% of patients who underwent bronchoalveolar lavage as part of a diagnostic work-up for pulmonary infection (P.E. Verweij, unpub. data). This outcome indicates that in culture-negative patients, presence of azole resistance will be missed.

In agreement with Alanio et al. (*1*), recent studies show a need to determine frequency of azole resistance at the hospital level and within different patient groups or departments. Although surveillance of unselected clinical cultures provides resistance rates at a national level and offers information about the epidemiology of resistance mechanisms, regular audits in specific patient populations are warranted to determine the frequency of azole resistance among different risk groups. These audits will enable clinicians to determine whether reassessment of azole monotherapy as a primary treatment option is necessary. Given the low and variable rates of positive cultures, culture-negative patients should also be included in azole-resistance surveillance programs.

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Schistosomiasis Screening of Travelers to Corsica, France

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To the Editor: As members of the French Ministry of Health Working Group on autochthonous urinary schistosomiasis, we read with interest the 2 recently published articles regarding schistosomiasis screening of travelers to Corsica, France (*1*,*2*). Surprisingly, the authors of both articles lacked evidence to support the diagnosis of schistosomiasis in most of what they referred to as confirmed cases. The diagnostic standard for confirmation of urinary schistosomiasis is identification of eggs by microscopic examination of urine samples (*3*–*5*). If this criterion were applied in both reports, only 1 patient of the 7 allegedly confirmed cases would actually be confirmed.

The low sensitivity of microscopy is well known. Therefore, different serologic tests have been developed, including Western blot (WB). In the study based on travelers from Italy (*1*), the SCHISTO II WB IgG test (LDBIO Diagnostics, Lyon, France) was used. This test, available since 2015, is based on both *Schistosoma haematobium* and *S. mansoni* antigens and has not been evaluated by anyone other than the manufacturer. Moreover, the authors did not report any details regarding the molecular weight and number of specific bands observed on the strip.

In the study by authors from the GeoSentinel Surveillance Network (*2*), both cases that could have been infected after 2013, since exposure occurred only in 2014, and 4 cases which reported bathing in rivers in Corsica other than the Cavu River had just 1 weakly positive serologic screening test. Hence, irrespective of the criteria for a confirmed case of schistosomiasis described above, it appears difficult to conclude that confirmation could rely on only 1 positive serologic test, even a WB.

Altogether, these 2 studies identified only 1 patient with parasitological evidence of infection that was attributable to the already known 2013 focus in Cavu River. Therefore, these articles do not provide evidence of transmission of schistosomiasis in Corsica after 2013 or outside the Cavu River.

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In Response: Regarding the comments by Berry et al. (*1*) on our previously published letter, we acknowledge that, in strict parasitological terms, confirmation of the diagnosis of urogenital schistosomiasis requires the identification of eggs by microscopic examination of urine. Nevertheless, we aimed at an operational case definition, providing criteria for identifying cases most likely to be true infections. We should not forget that microscopy has an unacceptably low sensitivity (*2*). We should also consider that currently available serologic tools are hampered by both a poor sensitivity and a poor specificity for *Schistosoma haematobium* (*3*). Regarding immunoblot, Berry et al. are correct in saying that there is not yet any formally published evidence of its accuracy for *S. haematobium* and that the high specificity declared, close to 100%, is based on data provided by the manufacturer. A formal study on the accuracy of this test is underway at the Centre for Tropical Diseases of Sacro Cuore Hospital. This assay has been less extensively assessed than that in which purified *S. mansoni* antigen is used, as described previously, which has shown very high accuracy (*4*). However, Western blot is already accepted as a diagnostic standard for the identification of other infectious diseases, including parasitic infections such as cysticercosis (for which, indeed, the direct parasitological confirmation is often impossible), and has become the test of choice for the latter (*5*).

Moreover, the population in our study was composed of persons not exposed to other parasites. Therefore, cross-reactions with other helminths would be extremely unlikely.

In conclusion, although we recognize that, by a strictly semantic definition, the term "confirmed" should be reserved for cases for which there is a parasitological proof, in operational terms, we could not rely on a direct test that has such a poor sensitivity in this particular patient population. Had we done so, we would have found a subestimated, and therefore totally incorrect, picture of the true prevalence, leading to inappropriate conclusions and actions (or lack thereof).

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In Response: We agree with Berry et al. (*1*) that the diagnostic standard for confirmation of urinary schistosomiasis is the identification of eggs by microscopic examination of urine, especially in patients living in endemic areas with high schistosome loads. However, this approach may not apply to travelers who have low parasite loads and in whom the diagnosis relies mainly on serologic testing (*2*,*3*). Given the very poor sensitivity of egg detection in non–schistosomiasis-endemic settings, most tropical and travel medicine clinics in Europe use conventional microscopy systematically combined with 2 different (commercial or in-house) serologic tests (*2*). The sensitivity of this approach (i.e., diagnosis of infection if combined ELISA and hemagglutination inhibition assay or an indirect fluorescent antibody test are positive) is >78% for chronic urinary schistosomiasis; specificity is 75%–98% when using various in-house

and commercial kits (*3*). Future availability of promising ultra-sensitive tests (e.g., PCR and antigenic tests) may overcome the limitations associated with conventional microscopy and serologic testing for low-parasite load schistosomiasis.

As stated in our manuscript, we cannot exclude the possibility that our case definition generated false-positives; the potential limitations of our findings have already been discussed (*4*). Furthermore, we were cautious with our interpretation of the serologic test results and, therefore, claimed only 2 confirmed cases (*4*), 1 on the basis of egg detection and the other on positive serologic test results by using 2 different methods. We believe, on the basis of our findings (*4*) and in accordance with the European Centre for Disease Control experts (*5*), that the possibility of transmission in the Cavu River during the summer of 2014 cannot be excluded. We also want to reiterate the possibility of transmission in other rivers in Corsica, including the Solenzara, Osu, and Tarcu rivers, where *Bulinus* snails, which can serve as intermediate hosts for *Schistosoma haematobium*, were found during a malacological survey in 2014 (*5*).

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Correction: Vol. 21, No. 11

Details regarding vaccine serotypes and surveillance programs were described incorrectly in Invasive Pneumococcal Disease 3 Years after Introduction of 10-Valent Pneumococcal Conjugate Vaccine, the Netherlands (M.J. Knol et al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/21/11/14-0780_article).

Correction: Vol. 21, No. 11

The affiliation for Laura Nic Lochlainn was listed incorrectly in Economic Costs of Measles Outbreak in the Netherlands, 2013–2014 (A.W.M. Suijkerbuijk et al.). She is with the European Programme for Intervention Epidemiology Training (EPIET) at the European Centre for Disease Prevention and Control, Stockholm, Sweden. The article has been corrected online (http://wwwnc.cdc.gov/eid/article/21/11/15-0410_article).

Correction: Vol. 21, No. 12

Several author names were listed incorrectly in Spillover of Peste des Petits Ruminants Virus from Domestic to Wild Ruminants in the Serengeti Ecosystem, Tanzania (M. Mahapatra et al.). The article has been corrected online (http://wwwnc.cdc.gov/eid/article/21/12/15-0223_article).

George Caleb Bingham (1811–1879), *The Jolly Flatboatmen,* 1846. Oil on canvas, 38 1/8 in x 48 1/2 in/96.8 cm x 123.2 cm. Open access digital image courtesy of the National Gallery of Art, Washington, DC, USA.

Flatboats, Travelers, Infectious Diseases, and Other River Thoughts

Byron Breedlove

> "I choose to listen to the river for a while, thinking river thoughts, before joining the night and the stars."
>
> —Edward Abbey, *Down the River*

A constellation of synonyms exist for the word "river": what distinguishes these naturally flowing watercourses from a beck, bourn, brook, burn, creek, rill, rivulet, runnel, or tributary may come down to the size of the stream or may simply be a regional preference. Rivers flow across every continent and on all but the smallest islands, carving, eroding, and reshaping the Earth's topography; connecting populations; enabling access to natural resources, commerce, and trade; defining boundaries; and offering sustenance and energy. Rivers have provided routes for explorers and adventurers and have roused the imaginations of writers and artists across cultures and history.

An iconic depiction of life on North American rivers in the 1840s, *The Jolly Flatboatmen*, this month's cover image, is one of the most celebrated works by American painter George Caleb Bingham. This well-preserved painting is considered an early example of luminism, an American painting style that depicts the effects of light played out across tranquil settings, calm waters, and hazy skies. Drifting downriver, Bingham's happy-go-lucky crew pays little mind to their surroundings: nothing in this painting hints at the potential dangers of river life—shifting sandbars and shoals, flash flooding, submerged trees, injuries and diseases, or scheming pirates.

The National Gallery of Art's overview states that "The composition is at once dynamic—the dancing man

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and the musicians—and elegantly stable in the way Bingham arranged the figures to form an isosceles triangle." The Gallery, which has borrowed and displayed this painting several times since the 1960s, finally purchased it from an undisclosed seller in May 2015.

The painting's symmetry is striking, a point noted in a review of Bingham's river paintings from the *New York Times*: the rectangular flatboat floats downstream, framed by a peaceful river, misty tree-lined riverbanks, and a pale blue, cloudless sky. A pair of long oars, locked in place, jut off to either side. Front and center, a capering young man, dressed in a red shirt, dances a jig, frozen in mid-step with hands held high. One crew member plays a fiddle, and another keeps time on a pan. Other crewmen watch and listen, several locking eyes with the viewer; another, seen from behind, stretches out in repose, his head resting on his interlocked hands.

A raccoon pelt hangs by the ladder; a serpentine coil of rope dangles from the top deck; a turkey thrusts its head between the slats of the wooden crate doubling as a stage; a rock anchors the blue shirt drying; bed rolls are tidily stashed below the top deck. Draped over the back of the boat, a sheet of newspaper and its reflection in the river form additional, smaller triangles. According the National Gallery of Art, Bingham's meticulous attention to details helped establish the artist's enduring reputation.

Bingham, who was born in Virginia and raised in Missouri, began his career as a self-taught portrait painter. In the mid-1840s, he started painting his genre works that idealized labor and leisure on the river. Even though flatboats had become essentially obsolete by that time, Bingham nonetheless saw his reputation soar when *The Jolly Flatboatmen* became the first piece of American art that, to borrow from today's social media lingo, "went viral." In 1847, the American Art-Union purchased the painting from the artist and distributed engravings to some 10,000 members across the United States, which made it one of the best known and most widely distributed works of art of its era.[1]

For a time, vast numbers of flatboats, forerunners of today's barges, conveyed agricultural commodities, raw materials, whiskey, livestock, and people downriver. Stops along the way served to increase contact between local populations and the flatboat crews and travelers. Those contacts and human behaviors created opportunities for rapid and efficient transmission of many types of pathogens—including those that can cause sexually transmitted infections. Infectious diseases could be transmitted from geographically isolated populations to more densely populated communities and, conversely, from urban populations to susceptible, isolated populations

Today the number of people who travel for work, leisure, and adventure has exponentially increased, and global mobility contributes to the spread of sexually transmitted infections. The *CDC Health Information for International Travel* (the Yellow Book) notes that an estimated 499 million cases of chlamydia, gonorrhea, syphilis, and trichomoniasis occur worldwide each year. Emerging and reemerging sexual infections, including antimicrobial resistant gonorrhea, chancroid, sexually transmitted hepatitis C, lymphogranuloma venereum, HIV infection, and human papillomavirus infection, further underscore the value of heightened public health surveillance and research.

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[1]From 1839 through 1851, the American Art-Union promoted American art by distributing engravings of the best American paintings of the day—including works by George Caleb Bingham, Thomas Cole, Jasper Francis Cropsey, Asher B. Durand, and William Sidney Mount—to its nationwide membership.

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Upcoming Issue

- Ebola and Its Control in Liberia, 2014–2015
- Epidemiology of Epidemic Ebola Virus Disease in Conakry and Surrounding Prefectures, Guinea, 2014–2015
- Sustained Transmission of Pertussis in Vaccinated, 1–5-Year-Old Children in a Preschool, Florida, USA
- Invasive Group A Streptococcus Infection among Children, Rural Kenya
- Feasibility of Xpert Ebola Assay in Médecins Sans Frontières Ebola Program, Guinea
- Prognostic Indicators for Ebola Virus Disease Survival
- Randomized Controlled Trial of Hospital-Based Hygiene and Water Treatment Intervention (CHoBI7) to Reduce Cholera
- Epidemiology of Serotype 1 Invasive Pneumococcal Disease, South Africa, 2003–2013
- Profile of the 2009 Influenza A (H1N1) Pandemic among Health Maintenance Organization Members, San Diego, California, USA
- Dogs and Opossums Positive for Vaccinia Virus during Outbreak Affecting Cattle and Humans, São Paulo State, Brazil
- *Candidatus* Coxiella massiliensis Infection and Eschar-Associated Illness
- Louseborne Relapsing Fever among East African Refugees, Italy, 2015
- Uveitis and Systemic Inflammatory Markers in Convalescent Phase of Ebola Virus Disease
- Microevolution of Outbreak-Associated Middle East Respiratory Syndrome Coronavirus, South Korea, 2015
- Ebola Virus RNA Stability in Human Blood and Urine in West Africa's Environmental Conditions
- Ebola Virus Persistence in Semen Ex Vivo
- Nanopore Sequencing as a Rapidly Deployable Ebola Outbreak Tool
- The Merits of Malaria Diagnostics during an Ebola Virus Disease Outbreak
- Hemorrhagic Fever with Renal Syndrome, Zibo City, China, 2006–2014
- Anaplasmataceae-Specific PCR for Diagnosis and Therapeutic Guidance of Symptomatic Neoehrlichiosis in an Immunocompetent Host

Complete list of articles in the February issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

February 8–10, 2016
ASM Biodefense and Emerging Diseases Research Meeting
Arlington, VA, USA
biodefense@asmusa.org

March 2–5, 2016
ISID
17th International Congress on Infectious Diseases
Hyderabad, India
http://www.isid.org

April 18–20, 2016
19th Annual Conference on Vaccine Research
Baltimore, MD, USA
http://www.cvent.com/events/19th-annual-conference-on-vaccine-research/event-summary-9c2a6b5301a64921afbd9c07a4cffa14.aspx?refid=spcoc

May 18–21, 2016
The Society for Heathcare Epidemiology of America
Atlanta, GA, USA
http://www.shea-online.org/Education/SHEASpring2016Conference.aspx

June 16–20, 2016
American Society for Microbiology
Boston, MA, USA
http://www.asmmicrobe.org/

July 18–22, 2016
21st International AIDS Conference
Durban, South Africa
http://www.aids2016.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

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Article Title

Epidemiology of *Haemophilus ducreyi* Infections

CME Questions

1. You are seeing a 21-year-old woman who recently emigrated from rural South Africa. She complains of genital ulcers. You consider whether *Haemophilus ducreyi* may be responsible for her symptoms. Which of the following statements regarding genital ulcer disease and *H. ducreyi* is most accurate?

A. *H. ducreyi* is a gram-positive organism
B. *H. ducreyi* promotes painless genital ulcers
C. Many patients with genital ulcer disease stemming from *H. ducreyi* have inguinal lymphadenitis
D. Most carriers of *H. ducreyi* are asymptomatic and are unaware of their infection

2. You perform an evaluation for genital ulcer disease in this patient. Which of the following statements regarding the diagnosis of *H. ducreyi* infection in the current study is most accurate?

A. Most cases of *H. ducreyi* were confirmed by PCR
B. All culture studies used low temperatures to grow *H. ducreyi*
C. All PCR studies used the hemolysin gene as a target
D. Multiplex studies that can detect *H. ducreyi*, *Treponema pallidum*, and herpes viruses were not used

3. Which of the following continents featured nations with the highest proportions of cases of *H. ducreyi* from 1980–1999?

A. South America
B. Australia and the South Pacific
C. Africa
D. Asia

4. Which of the following statements regarding trends in the diagnosis of chancroid in the current study is most accurate?

A. No study found a proportion of *H. ducreyi* genital ulcers above 10%
B. No African country reported rates of *H. ducreyi* below 10% between 2000 and 2014
C. More recent studies found that *H. ducreyi* was an emerging cause of chronic skin ulceration
D. The proportion of *H. ducreyi* found in genital ulcer disease remained relatively stable between 1980 and 2014

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

Falling *Plasmodium knowlesi* Malaria Death Rate among Adults despite Rising Incidence, Sabah, Malaysia, 2010–2014

CME Questions

1. According to the surveillance study by Rajahram and colleagues, which of the following statements about notification-fatality rates of *Plasmodium knowlesi* cases in Sabah, Malaysia, during 2010–2014 is correct?

A. Overall adult notification-fatality rate for *P. knowlesi, P. falciparum*, and *P. vivax* malaria was 3.4, 4.2, and 1.0 per 1,000 notifications, respectively
B. Of 16 malaria deaths reported during 2012–2014, there were 12 deaths from *P. knowlesi*
C. Notification-fatality rate for *P. knowlesi* increased from 2010 to 2014
D. From 2010 to 2014, there were 3 reported deaths from *P. knowlesi* in children

2. Your patient is a 60-year-old woman in Sabah with suspected *P. knowlesi*. According to the surveillance study by Rajahram and colleagues, which of the following statements about the clinical characteristics of fatal cases of *P. knowlesi* in Sabah during 2012–2014 is correct?

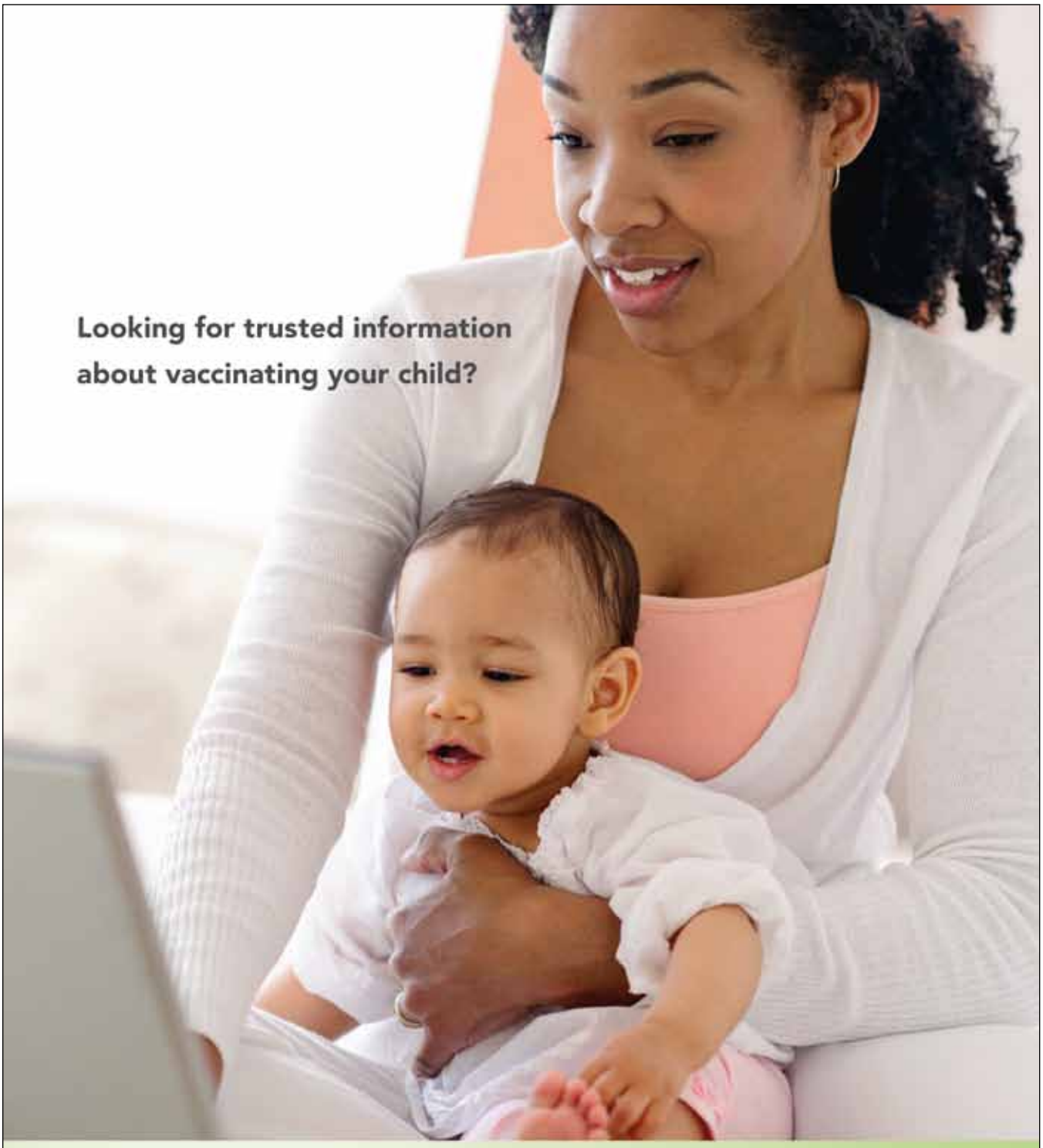
A. Most patients were men 30 to 49 years old
B. Hyperparasitemia, respiratory distress, shock, jaundice, and acute kidney injury were common in this series and in previous series
C. Respiratory acidosis occurred in 5 of 7 patients
D. Most patients had prolonged coma

3. According to the surveillance study by Rajahram and colleagues, which of the following statements about the management details of fatal cases of *P. knowlesi* in Sabah, Malaysia, during 2012–2014 is correct?

A. Microscopic diagnosis of *P. knowlesi* was correct in 6 of 7 cases
B. All 5 patients with *P. knowlesi* recognized to have severe malaria on admission received intravenous chloroquine
C. Most patients received oral therapy and converted to parenteral administration when oral therapy was ineffective
D. World Health Organization guidelines recommend intravenous artesunate for all patients with *P. knowlesi* malaria and parasite count of more than 100,000/μL, or more than 20,000/μL if testing for laboratory criteria for severe malaria is unavailable

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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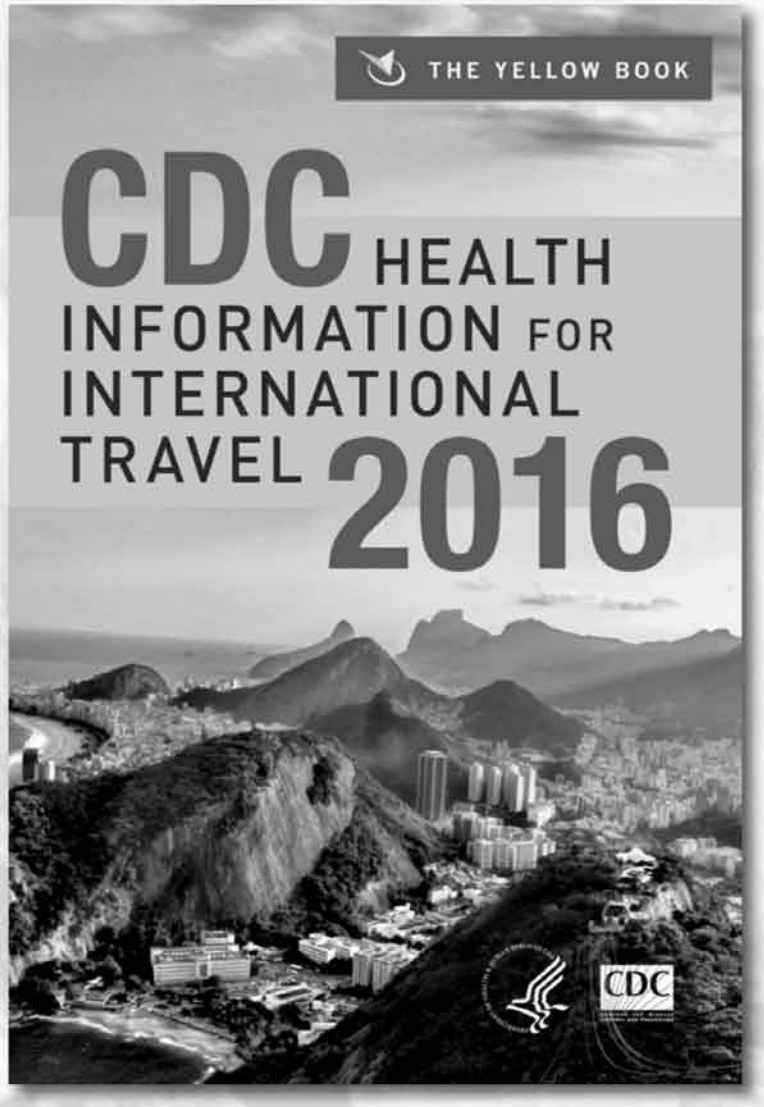

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Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

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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.

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Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

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