EMERGING INFECTIOUS DISEASES®

April 2012

Vector-borne Infections

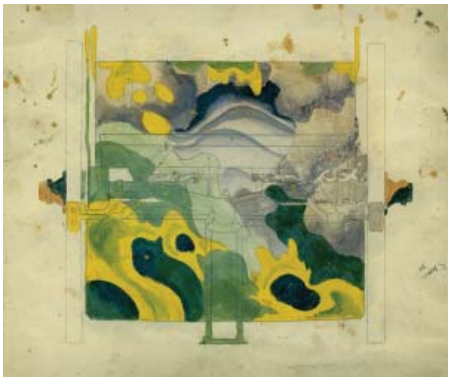
EMERGING INFECTIOUS DISEASES®

Emerging Infectious Diseases is published monthly by the Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop D61, Atlanta, GA 30333, USA. Telephone 404-639-1960, fax 404-639-1954, email eideditor@cdc.gov.

∞ Emerging Infectious Diseases is printed on acid-free paper that meets the requirements of ANSI/NISO 239.48-1992 (Permanence of Paper)

EMERGING INFECTIOUS DISEASES

April 2012



On the Cover

Charles E. Burchfield (1893–1967)
Camouflage design (1918)
Watercolor and graphite on paper mounted on black paper (27.3 cm x 32.1 cm)

The Charles E. Burchfield Foundation Archives at the Burchfield Penney Art Center at Buffalo State College, Buffalo, New York
Gift of the Charles E. Burchfield Foundation, 2006

About the Cover p. 706

p. 612

Research

Medscape EDUCATION **ACTIVITY**

Medscape EDUCATION **ACTIVITY**

p. 617

EMERGING INFECTIOUS DISEASES
April 2012

Policy Review

Historical Reviews

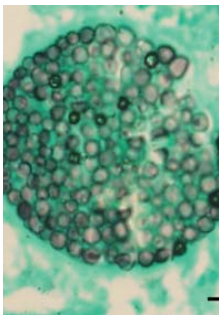
p. 626

Dispatches

p. 664

EMERGING INFECTIOUS DISEASES
April 2012

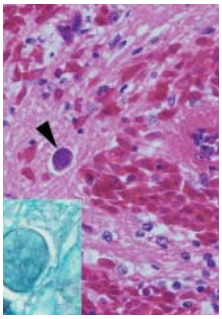
Another Dimension



p. 669

Letters

p. 694



About the Cover

Online Report

Multidisciplinary and Evidence-based Method for Prioritizing Diseases of Food-producing Animals and Zoonoses
http://wwwnc.cdc.gov/eid/article/18/4/11-1151_article.htm

The EID journal is now using **Quick Response codes** (QR codes) to make its content more interactive, engaging, and accessible. QR codes can be interpreted by numerous smartphone apps. Free downloads of readers are available online. These codes will allow readers to access CME, articles, podcasts, and other relevant content, easily from smartphones.

CDC FOUNDATION

Helping CDC Do More, Faster

Helping CDC Do More, Faster

Established by Congress as an independent, nonprofit organization, the CDC Foundation connects the Centers for Disease Control and Prevention (CDC) with private-sector organizations and individuals to build public health programs that support CDCís work 24/7 to save lives and protect people from health, safety and security threats.

Since 1995, the CDC Foundation has provided more than $300 million to support CDCís work, launched more than 500 programs around the world and built a network of individuals and organizations committed to supporting CDC and public health.

Each CDC Foundation program involves a talented team of experts at CDC and at least one outside funding partner. Sometimes, a program begins with a CDC scientist who has a great idea and wants to collaborate with an outside partner to make it happen. At other times, organizations in the private sector recognize that they can better accomplish their own public health goals by working with CDC through the CDC Foundation.

JOIN US www.cdcfoundation.org

Photos: David Snyder / CDC Foundation

Determinants for Autopsy after Unexplained Deaths Possibly Resulting from Infectious Causes, United States

Lindy Liu, Laura S. Callinan, Robert C. Holman, and Dianna M. Blau

Medscape EDUCATION ACTIVITY

Medscape, LLC is pleased to provide online continuing medical education (CME) for this journal article, allowing clinicians the opportunity to earn CME credit.

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of Medscape, LLC and Emerging Infectious Diseases. Medscape, LLC is accredited by the ACCME to provide continuing medical education for physicians.

Medscape, LLC designates this Journal-based CME activity for a maximum of 1 *AMA PRA Category 1 Credit(s)*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

All other clinicians completing this activity will be issued a certificate of participation. To participate in this journal CME activity: (1) review the learning objectives and author disclosures; (2) study the education content; (3) take the post-test with a 70% minimum passing score and complete the evaluation at **www.medscape.org/journal/eid**; (4) view/print certificate.

Release date: March 16, 2012; Expiration date: March 16, 2013

Learning Objectives

Upon completion of this activity, participants will be able to

- Assess characteristics of cases of unexplained deaths possibly resulting from infectious causes
- Distinguish the age group most likely to receive an autopsy after unexplained death
- Evaluate other variables associated with a higher likelihood of receiving an autopsy after unexplained death

Editor

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

CME Author

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

Authors

Disclosures: ***Lindy Liu, MPH; Laura S. Callinan; Robert C. Holman, MS;*** *and* ***Dianna M. Blau, DVM, PhD,*** *have disclosed no relevant financial relationships.*

We analyzed US multiple cause-of-death data for 2003–2006 for demographic and clinical determinants for autopsy in unexplained deaths possibly resulting from infectious causes. For 96,242 deaths, the definition for unexplained death was met and autopsy status was recorded. Most decedents were male, 40–49 years of age, and white. To identify factors associated with unexplained death, we used data from Arizona records. Multivariate analysis of Arizona records suggested that decedents of races other than white and black and decedents who had clinicopathologic syndromes in the cardiovascular, sepsis/shock, and multisyndrome categories recorded on the death certificate were least likely to have undergone autopsy; children with unexplained death were the most likely to have undergone autopsy. Improved understanding of unexplained deaths can provide opportunities for further studies, strengthen collaboration between investigators of unexplained deaths, and improve knowledge and awareness of infectious diseases of public health concern.

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

DOI: http://dx.doi.org/10.3201/eid1804.111311

Many factors can influence the dynamics of pathogen ecology, increase the mobility of microbial agents, and elevate the risk for infectious disease posed to humans. Outbreaks and novel pathogens identified in recent decades are reminders that historical and newly recognized

infectious diseases remain threats to the health of the global community (*1*–*3*). Unexplained deaths possibly resulting from infectious causes (unexplained deaths) also present public health challenges. Many fatal infectious etiologies are never identified because of inadequate testing or inherent difficulties of detecting certain pathogens (*4*).

An autopsy can verify an existing diagnosis or provide a diagnosis if one is not determined before death, which might facilitate provision of prophylaxis or treatment of contacts of decedents with communicable diseases. Autopsies also contribute to epidemiologic data, provide insights into disease pathogenesis, and create educational opportunities for physicians and medical students (*5*). Recent disease descriptions facilitated by autopsy findings include hantavirus pulmonary syndrome, West Nile virus, and severe acute respiratory syndrome (SARS) (*6*–*8*); pathogens for these diseases were recognized only after substantial numbers of illnesses and deaths. Although autopsies of persons who died of unexplained causes can help build public health capacity to respond to emerging infectious diseases, the declining rate of autopsies performed in US hospitals reduces the possibility of early detection of such diseases (*9*). Because most autopsies in the United States are now performed by medical examiners and coroners (*10*), medicolegal death investigation system–based surveillance for unexplained death can serve as a sentinel system to identify new agents, recognize unique characteristics of known pathogens, or detect acts of bioterrorism (*11*). Medical examiner and coroner systems contribute to national mortality data and autopsy-based information (*12*), and specimens collected at autopsy of persons whose deaths are unexplained could lead to diagnoses from advancements in diagnostic methods that have enabled identification and characterization of new infectious agents.

Although an earlier study measured unexplained deaths and critical illnesses (*4*), the demographic characteristics and clinicopathologic syndromes of persons whose deaths are unexplained who undergo autopsies have not been described. Understanding the types of persons who died of unexplained causes and who undergo autopsies might help identify specimens for diagnostic testing and improve epidemiologic and mortality data. We analyzed demographic characteristics and infectious disease–related syndromes associated with unexplained death in decedents for whom an autopsy was performed in the United States.

Methods

Data Source and Study Population

We obtained multiple cause-of-death data with autopsy status for 2003–2006 in the 50 states and the District of Columbia from the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (*13*). Multiple cause-of-death data contain information from all death certificates for US residents, including demographic information and causes of death that have been translated to International Classification of Diseases, 10th Revision (ICD-10), codes (*14*).

On the basis of the previous definition of unexplained death (*15*) that was refined to use ICD-10 codes, we used 99 codes likely to represent deaths from unexplained infectious causes to select decedents for this study (online Appendix Table, wwwnc.cdc.gov/EID/article/18/4/11-1311-TA1.htm). These codes aimed to capture deaths from infectious causes that lacked an identifiable etiologic agent or deaths with unknown causes. Unexplained deaths were defined as deaths of previously healthy US residents 1–49 years of age for whom the death certificate had ≥1 codes for unexplained infections. Decedents with unexplained infections for whom any of the ICD-10 codes listed in the Table 1 as an underlying cause of death were not considered previously healthy and were excluded from analysis. Decedents outside the age range also were excluded. We excluded infants (<1 year of age) to eliminate deaths attributed to congenital problems and persons ≥50 years of age because of the expected increased proportion of unexplained deaths from noninfectious causes (*15*). Analyses were restricted to unexplained deaths for which we could ascertain from the selected death certificate data whether an autopsy had been performed.

Study Measures and Statistical Analysis

Decedents were described by age, sex, and race and by the syndromic category recorded on the death certificate. Age was categorized as 1–17 years (children), 18–39 years, and 40–49 years. Race categories were white, black, and other, as recorded on death certificates and obtained from NCHS (*13*). Death certificates enabled reporting of ≥1 race, including any combination of white, black or African

Table 1. Excluded ICD-10 codes and cause-of-death categories for unexplained deaths possibly resulting from infectious causes, United States, 2003–2006*

ICD-10 code	Cause-of-death category
B20-B24	HIV disease
C00-D48	Neoplasms
D73 (except D73.3)	Diseases of spleen
D80-D89	Certain disorders involving immune mechanism
E10-E14	Diabetes mellitus
F02.4	Dementia in HIV disease
R75	Inconclusive laboratory evidence of HIV
S00-T98	Injury, poisoning and certain consequences of external causes
V01-V99	Transport accidents
W00-X59	Other external causes of accidental injury
X60-X84	Intentional self-harm
X85-Y09	Assault
Y10-Y34	Event of undetermined intent
Y40-Y84	Complications of medical and surgical care

*ICD-10, International Classification of Diseases, 10th Revision.

American, American Indian or Alaska Native, Asian, and Native Hawaiian or Other Pacific Islander, and decedents were imputed to a single race according to their combination of races, Hispanic origin, sex, and age indicated on the death certificate (*16*). On the basis of the selected ICD-10 codes, unexplained deaths were also grouped into 6 clinicopathologic syndromes: gastrointestinal, neurologic, respiratory, cardiovascular, sepsis/shock, and unknown/other (Table 2). Unexplained deaths for which ICD-10 codes were recorded as belonging to ≥2 syndromic categories were classified as multisyndrome.

We calculated odds ratios (ORs) with 95% CIs for selected characteristics by using logistic regression analysis. Characteristics considered univariately associated ($p<0.1$) with autopsy were further assessed through multivariate logistic regression models to determine which variables were independently associated with autopsy. We considered $p<0.05$ as significant. Because of the large sample size, statistical but not meaningful significance was found for most variables in the logistic regression model, including all unexplained deaths during 2003–2006 (data not shown). To further evaluate the variables, we created a multivariate logistic regression model using unexplained death data from Arizona for 2003–2006 (*17*). This subset of data was selected because of the minimal amount of missing autopsy data (0.2%) and the Unexplained Deaths Investigation Protocol, which identifies deaths that might be of public health concern, established by the Arizona Department of Health Services (*18*).

Table 2. Syndromic classification of selected ICD-10 codes and cause of death for unexplained deaths possibly resulting from infectious causes, United States, 2003–2006*

Syndrome	ICD-10 codes
Gastrointestinal	A04.9, A05.9, A07.9, A08.4, A09, B82.0, B82.9, K29.7, K29.9, K51.9, K65.9, K85.9, R11, R85.5, R85.6, R85.7
Neurologic	A81.9, A83.9, A84.9, A85.2, A86, A87.9, A89, A92.9, A94, G00.9, G03.9, G04.9, G06.2, R29.8, R40.2, R83.5
Respiratory	J01.9, J02.9, J03.9, J06.9, J12.9, J15.9, J18.0, J18.1 J18.2, J18.8, J18.9, J20.9, J21.9, J22, R04.9, R84.5, R84.6, R84.7
Cardiovascular	D59.4, D59.9, D61.9, D64.9, D69.6, I01.9, I30.9, I33.9, I40.9, I42.8, I42.9, I51.4, I77.6, L95.9
Unknown/other	A28.9, A49.8, A49.9, A64, A68.9, A99, B09, B34.9, B49, B64, B83.9, B88.9, B89, B94.9, B99, D73.3, M60.0, N10.9, O98.9, P36.9, P37.9, P39.9, R50.9, R56.8, R59.9, R69, R89.5, R89.6, R89.7, R96.0, R96.1, R98, R99
Sepsis/shock	A41.9, R57.9

*ICD-10, International Classification of Diseases, 10th Revision.

Results

United States

A total of 153,476 deaths were reported for persons 1–49 years of age for whom the selected ICD-10 codes (online Appendix Table) were recorded in the multiple cause-of-death data for 2003–2006. Of these, 111,160 (72.4%) met the definition for unexplained death, and information on autopsy status was available for 96,242 (86.6%). Of decedents for whom autopsy status was known, 38,332 (39.8%) had undergone autopsy.

Of decedents for whom autopsy status was known, 59.5% were male (Table 3). Most decedents whose deaths were unexplained (55.1%) were 40–49 years of age; children accounted for 9.2%. Whites composed 71.7% of unexplained deaths, followed by blacks (24.6%) and others (3.7%). For most unexplained deaths, cause was coded as unknown/other syndrome (33.1%). Sepsis/shock accounted for 21.6%, and gastrointestinal and neurologic causes accounted for only 1.8% each.

More male than female decedents underwent autopsies (41.5% vs. 37.4%) (Table 3). The highest percentage of autopsies was performed for white decedents (40.7%); autopsies were performed for 38.1% of black decedents and 34.0% of other decedents. Children whose deaths were unexplained underwent the highest percentage of autopsies (50.5%), followed by persons 18–39 years (48.4%) and 40–49 years of age (32.5%). The highest percentage of autopsies were performed on decedents whose cause of death was coded as unknown/other syndrome (65.3%); the lowest percentage of autopsies were performed on decedents whose deaths were coded as sepsis/shock syndrome (15.9%).

Table 3. Characteristics of decedents 1–49 years of age and autopsies conducted for unexplained deaths possibly resulting from infectious causes, United States*

Characteristic	No. (%) decedents	No. (%) autopsies
Overall	96,242 (100)	38,332 (39.8)
Sex		
M	57,238 (59.5)	23,753 (41.5)
F	39,004 (40.5)	14,579 (37.4)
Race		
White	69,053 (71.7)	28,125 (40.7)
Black	23,657 (24.6)	9,006 (38.1)
Other†	3,532 (3.7)	1,201 (34.0)
Age group, y		
1–17	8,844 (9.2)	4,468 (50.5)
18–39	34,382 (35.7)	16,640 (48.4)
40–49	53,016 (55.1)	17,224 (32.5)
Syndrome		
Gastrointestinal	1,736 (1.8)	837 (48.2)
Neurologic	1,765 (1.8)	676 (38.3)
Respiratory	15,229 (15.8)	5,607 (36.8)
Cardiovascular	12,487 (13.0)	4,404 (35.3)
Sepsis/shock	20,762 (21.6)	3,298 (15.9)
Multisyndrome	12,371 (12.9)	2,688 (21.7)
Unknown/other	31,892 (33.1)	20,822 (65.3)

*Numbers reflect decedents for whom autopsy information was available; autopsy information was not available for 13.4% of the 111,160 persons who died of unexplained causes.
†American Indian or Alaska Native, Asian Indian, Chinese, Filipino, Japanese, Korean, Vietnamese, Other Asian, Native Hawaiian, Guamanian or Chamorro, Samoan, other Pacific Islander, and other.

Arizona

Of the 2,097 persons in Arizona who died from unexplained possibly infectious causes and for whom autopsy status was known, most (55.2%) were 40–49 years of age (Table 4). Whites composed 78.6% of such decedents, followed by others (14.4%) and blacks (7.1%). Most (33.9%) unexplained deaths resulted from unknown/other causes; unexplained deaths from gastrointestinal causes accounted for 1.2%.

Percentages of decedents for whom an autopsy was performed were similar for whites (35.2%) and blacks (35.8%) (Table 4). The highest percentages of autopsies were performed on children whose deaths were unexplained (44.5%), followed by persons 18–39 years (38.0%) and 40–49 (28.1%) years of age. Of the 7 syndromic classifications, gastrointestinal cause of death accounted for the highest percentage of autopsies (60.0%) and sepsis/shock for the lowest percentage (14.5%).

Univariate analysis of data on persons who died from unexplained infectious causes in Arizona indicated that race, age group, and syndromic category, but not sex, were significantly associated with autopsy. Multivariate logistic regression analysis indicated that race, age group, and syndromic category remained independent predictors of autopsy. Persons of other races were less likely than white persons to undergo autopsy (OR 0.5, 95% CI 0.4–0.7) (Table 4). Children whose deaths were unexplained (OR 1.9, 95% CI 1.4–2.6) and persons 18–39 years of age (OR 1.6, 95% CI 1.3–2.0) were more likely to have undergone autopsy than were persons 40–49 years of age (Table 4). Persons with cardiovascular conditions, sepsis/shock, and multisyndrome conditions were less likely to have undergone autopsy than were persons with unknown/other unexplained deaths (Table 4).

Discussion

Unlike other studies that have described and analyzed characteristics that influence autopsies overall (*19*,*20*), ours describes demographic characteristics and clinicopathologic syndromes associated with autopsy of persons who died of unexplained infectious causes in the United States. The overall percentage of autopsies performed on such decedents during 2003–2006 (39.8%) was higher than estimates of the proportion of overall autopsies in the United States (≈8.5%) (*21*). The higher percentage of autopsies for persons whose deaths were unexplained might reflect the frequent inclusion of complete autopsies in investigations of natural disease deaths by medical examiners and coroners (*22*).

Our finding that most characteristics in the multivariate regression analysis were highly significant when complete data for 2003–2006 were included in the analysis probably resulted from the large number of persons in the study whose deaths were unexplained. Unexplained deaths among persons with a history of fever have been reportable in Arizona since 2004, and medical examiners and health care providers are required to report these unexplained deaths to their local health departments (*18*). The Arizona Unexplained Deaths Investigation Protocol identifies appropriate specimens and clinical data needed for investigation, and the Arizona data might elucidate true demographic characteristics and syndromic trends of unexplained deaths in the United States. The analysis of data for Arizona decedents suggests that race, age, and clinicopathologic syndrome are potentially major factors for whether persons who died of unexplained infectious causes undergo autopsy.

Data on religious preferences are not collected on death certificates, but race might have been a proxy for

Table 4. Association between having undergone autopsy and demographic characteristics of decedents 1–49 years of age and clinicopathologic syndrome for unexplained deaths possibly resulting from infectious causes, Arizona, USA*

Characteristic	No. (%) decedents	No. (%) autopsies	Adjusted odds ratio (95% CI)
Total	2,097 (100.0)	696 (33.2)	
Race			
White	1,648 (78.6)	580 (35.2)	Reference
Black	148 (7.1)	53 (35.8)	1.0 (0.7–1.5)
Other†	301 (14.4)	63 (20.9)	0.5 (0.4–0.7)
Age group, y			
1–17	211 (10.1)	94 (44.5)	1.9 (1.4–2.6)
18–39	728 (34.7)	277 (38.0)	1.6 (1.3–2.0)
40–49	1,158 (55.2)	325 (28.1)	Reference
Syndrome			
Gastrointestinal	25 (1.2)	15 (60.0)	1.8 (0.8–4.1)
Neurologic	54 (2.6)	28 (51.9)	1.2 (0.7–2.1)
Respiratory	319 (15.2)	142 (44.5)	0.9 (0.7–1.2)
Cardiovascular	213 (10.2)	59 (27.7)	0.4 (0.3–0.6)
Sepsis/shock	428 (20.4)	62 (14.5)	0.2 (0.2–0.3)
Multisyndrome	348 (16.6)	56 (16.1)	0.2 (0.2–0.3)
Unknown/other	710 (33.9)	334 (47.0)	Reference

*By multivariate logistic regression analysis. Numbers reflect decedents for whom autopsy information was available; autopsy status was not available for 0.2% of the 2,102 persons who died of unexplained causes. Variables are independently associated with autopsy.
†American Indian or Alaska Native, Asian Indian, Chinese, Filipino, Japanese, Korean, Vietnamese, Other Asian, Native Hawaiian, Guamanian or Chamorro, Samoan, Other Pacific Islander, and Other.

cultural and religious preferences. Religious objections and lack of understanding about cultural or religious influences have been reported as reasons a family might not consent to an autopsy (*23*,*24*). For example, many American Indian tribes have traditions contrary to autopsy in which organ specimens are retained by medical examiners and pathologists (*25*). The observed lower odds for autopsy of decedents of other races possibly resulted from the larger American Indian population in Arizona (5%) than in the United States (1%) (*26*).

Results from the analysis of the Arizona subset suggest that children and young adults whose deaths resulted from unexplained possibly infectious causes are more likely than older adults to have undergone autopsies. Although some studies have suggested that children are more likely to undergo autopsies (*19*,*20*), the literature regarding the association between age and autopsy is limited, and findings have been inconclusive (*27*,*28*). Particularly when children die suddenly or unexpectedly, which is often from infectious causes (*29*), autopsies can contribute to families' understanding of the circumstances of death or expand medical knowledge (*19*,*30*).

Persons whose unexplained deaths were coded as from cardiovascular, sepsis/shock, or multisyndrome causes were less likely than those whose deaths were coded as unknown/other to undergo autopsies. These results could reflect differences in the availability and resources of investigators of unexplained deaths from possibly infectious causes. Sepsis, in particular, remains perplexing and costly, and despite efforts to understand the systemic inflammation and multisystem organ failure characteristics of severe sepsis, the reason many of these patients die remains unknown (*31*,*32*). Furthermore, investigators of unexplained deaths or family members of decedents might have believed that additional studies, including autopsy, would not yield substantial findings. According to an opinion survey of pathology and medicine resident physicians, reasons families refuse autopsies included beliefs that the patient has suffered enough and that the autopsy would not be useful (*33*). Routine microscopic examination has been argued to not provide additional information in forensic pathology cases for which the cause and manner of death are apparent at the time of autopsy (*34*). However, the reduced likelihood of autopsy or further evaluation of these challenging unexplained deaths could also result in the failure to recognize infectious diseases. For example, Chong et al. illustrated the difficulty of differentiating an emerging disease (SARS) from other causes of sudden cardiovascular death at autopsy (*35*). Of the14 autopsies performed on persons with suspected or probable SARS, 8 confirmed SARS only on the basis of clinical history, histopathologic evaluation, and testing of autopsy specimens. Therefore, an autopsy should be pursued especially for those whose unexplained deaths were possibly of infectious causes.

Reasons for differences in likelihood of autopsy with respect to race, age, and clinicopathologic syndrome could be multifactorial, and results from our study are subject to limitations. The availability, training, and resources of investigators of unexplained natural deaths differ among institutions and jurisdictions and might account for differences in autopsy performance, testing capabilities, and reporting of autopsy data (*12*,*36*). Unfortunately, multiple cause-of-death data do not capture whether autopsies are performed by medical examiners or by hospital-based pathologists, and differences in autopsy rates between medicolegal death investigation systems and hospital-based pathologists in unexplained death remains unknown. Inaccuracy in death certification and reliance solely on ICD-10 classification for unexplained death also has limitations. Codes might be assigned by persons not directly familiar with decedents and who therefore might not be aware of known diagnoses. Death certificates might not have been amended when organism-specific etiologies (i.e., *Streptococcus pneumoniae*) were determined after broad ICD-10 codes (i.e., bacterial meningitis) were assigned. Results from our study also are limited by the restriction of analyses to unexplained deaths for which autopsy status is known and the large variation of autopsy data reported by states to NCHS. Of deaths that met the unexplained death definition, the percentage of missing autopsy status data by state ranged from 0 to 99% during 2003–2006. Additional data on autopsy status reported to NCHS could have more accurately described unexplained death.

Additional studies are needed to assess the similarities in demographic characteristics and clinicopathologic syndromes of persons who died of unexplained possibly infectious causes and characteristics found in autopsies overall. The statistically significant findings of such characteristics as age and race in this study could reflect general trends of autopsies performed and might not be unique to persons whose infectious deaths are unexplained. Furthermore, results from the analysis of Arizona data might not necessarily reflect unexplained deaths in other states or nationally.

Additional insight into persons who died of unexplained infectious causes and underwent autopsies might help pinpoint areas in which diagnostic capabilities or resources are needed (*15*) and provide opportunities for additional studies. Retrospective studies using postmortem specimens and improved diagnostic tools could benefit the broader community. Improved understanding by health departments and medical examiners of a specific type of unexplained death for which an autopsy is conducted could increase overall awareness of unexplained deaths from

infectious causes; improve approaches in the collection of medical history and laboratory results in the forensic setting (*37*); and strengthen collaboration between health departments, clinicians, and medical examiners. Awareness of the types of unexplained death for which autopsies are less likely to be conducted is also imperative. Clinicians and pathologists challenged by cultural or religious restrictions can consider alternative methods for diagnosis such as taking biopsy samples (*38*), collecting appropriate antemortem specimens, or performing virtual autopsies (*39*,*40*). Retrospective studies evaluating perceptions by families, physicians, and medical examiners on autopsies of persons who died of unexplained infectious causes also might be helpful. Improving education about unexplained death and autopsy, identifying areas where diagnostic resources are needed, and maintaining cooperation between investigators should be considered. Autopsy findings, in conjunction with clinical history and diagnostic tools, can assist surveillance and investigations of infectious diseases of public health concern.

Acknowledgments

We thank Amy Denison, Christopher Paddock, and Sherif Zaki for their helpful discussion and critical review of this article.

Ms Liu is an epidemiologist at the Infectious Diseases Pathology Branch, US Centers for Disease Control and Prevention. Her research interests include infectious disease epidemiology and the role of pathology in investigating unexplained deaths resulting from infectious causes.

References

1. Campbell GL, Hughes JM. Plague in India: a new warning from an old nemesis. Ann Intern Med. 1995;122:151–3.
2. Fischer SA, Graham MB, Kuehnert MJ, Kotton CN, Srinivasan A, Marty FM, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. N Engl J Med. 2006;354:2235–49. http://dx.doi.org/10.1056/NEJMoa053240
3. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team; Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009;360:2605–15. http://dx.doi.org/10.1056/NEJMoa0903810
4. Hajjeh RA, Relman D, Cieslak PR, Sofair AN, Passaro D, Flood J, et al. Surveillance for unexplained deaths and critical illnesses due to possibly infectious causes, United States, 1995–1998. Emerg Infect Dis. 2002;8:145–53. http://dx.doi.org/10.3201/eid0802.010165
5. McPhee SJ. Maximizing the benefits of autopsy for clinicians and families. What needs to be done. Arch Pathol Lab Med. 1996;120:743–8.
6. Zaki SR, Greer PW, Coffield LM, Goldsmith CS, Nolte KB, Foucar K, et al. Hantavirus pulmonary syndrome. Pathogenesis of an emerging infectious disease. Am J Pathol. 1995;146:552–79.
7. Shieh WJ, Guarner J, Layton M, Fine A, Miller J, Nash D, et al. The role of pathology in an investigation of an outbreak of West Nile encephalitis in New York, 1999. Emerg Infect Dis. 2000;6:370–2. http://dx.doi.org/10.3201/eid0604.000407
8. Nicholls JM, Poon LL, Lee KC, Ng WF, Lai ST, Leung CY, et al. Lung pathology of fatal severe acute respiratory syndrome. Lancet. 2003;361:1773–8. http://dx.doi.org/10.1016/S0140-6736(03)13413-7
9. Dalen JE. The moribund autopsy. DNR or CPR? Arch Intern Med. 1997;157:1633. http://dx.doi.org/10.1001/archinte.1997.00440360019001
10. Hanzlick R. Medical examiners, coroners, and public health: a review and update. Arch Pathol Lab Med. 2006;130:1274–82.
11. Nolte KB, Fischer M, Reagan S, Lynfield R. Guidelines to implement medical examiner/coroner-based surveillance for fatal infectious diseases and bioterrorism ("Med-X"). Am J Forensic Med Pathol. 2010;31:308–12. http://dx.doi.org/10.1097/PAF.0b013e3181c187b5
12. Hanzlick R. The conversion of coroner systems to medical examiner systems in the United States: a lull in the action. Am J Forensic Med Pathol. 2007;28:279–83. http://dx.doi.org/10.1097/PAF.0b013e31815b4d5a
13. National Center for Health Statistics. Vital statistics mortality data, multiple cause detail, 2003–2006. Public use data tape contents and documentation package. Hyattsville (MD): The Center; 2008.
14. Centers for Disease Control and Prevention. Multiple cause of death 1999–2006 [cited 2011 Nov 15]. http://wonder.cdc.gov/wonder/help/mcd.html
15. Perkins BA, Flood JM, Danila R, Holman RC, Reingold AL, Klug LA, et al. Unexplained deaths due to possibly infectious causes in the United States: defining the problem and designing surveillance and laboratory approaches. The Unexplained Deaths Working Group. Emerg Infect Dis. 1996;2:47–53. http://dx.doi.org/10.3201/eid0201.960106
16. Ingram DD, Parker JD, Schenker N, Weed JA, Hamilton B, Arias E, et al. United States Census 2000 population with bridged race categories. Vital Health Stat 2. 2003;(135):1–55.
17. Kleinbaum D, Klein M. Logisitc regression: a self-learning text. 2nd ed. New York: Springer-Verlag; 2002.
18. Arizona Department of Health Services, Infectious Disease Epidemiology Program. Unexplained deaths with history of fever (UNEX) [cited 2011 Nov 15]. http://www.azdhs.gov/phs/oids/epi/unex/index.htm
19. Sinard JH. Factors affecting autopsy rates, autopsy request rates, and autopsy findings at a large academic medical center. Exp Mol Pathol. 2001;70:333–43. http://dx.doi.org/10.1006/exmp.2001.2371
20. Andrews-Joseph A, Bourgeois SS, Ratard RC. Louisiana autopsy patterns 1999–2006. J La State Med Soc. 2009;161(2):97, 99–102.
21. Hoyert DL. The changing profile of autopsied deaths in the United States, 1972–2007. NCHS data brief. No. 67, August 2011. Hyattsville (MD): US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Health Statistics; 2011 [cited 2011 Nov 15]. http://www.cdc.gov/nchs/data/databriefs/db67.htm
22. Wolfe MI, Nolte KB, Yoon SS. Fatal infectious disease surveillance in a medical examiner database. Emerg Infect Dis. 2004;10:48–53.
23. Geller SA. Religious attitudes and the autopsy. Arch Pathol Lab Med. 1984;108:494–6.
24. Perkins HS, Supik JD, Hazuda HP. Autopsy decisions: the possibility of conflicting cultural attitudes. J Clin Ethics. 1993;4:145–54.
25. Krinsky CS, Lathrop SL, Reichard RR. A policy for the retention and extended examination of organs at autopsy. J Forensic Sci. 2010;55:418–22. http://dx.doi.org/10.1111/j.1556-4029.2009.01271.x
26. US Census Bureau. State and county quick facts: Arizona [cited 2011 Nov 15]. http://quickfacts.census.gov/qfd/states/04000.html
27. Whitehouse SR, Kissoon N, Singh N, Warren D. The utility of autopsies in a pediatric emergency department. Pediatr Emerg Care. 1994;10:72–5. http://dx.doi.org/10.1097/00006565-199404000-00002

28. Ahronheim JC, Bernholc AS, Clark WD. Age trends in autopsy rates. Striking decline in late life. JAMA. 1983;250:1182–6. http://dx.doi.org/10.1001/jama.1983.03340090038026
29. Taggart MW, Craver R. Causes of death, determined by autopsy, in previously healthy (or near-healthy) children presenting to a children's hospital. Arch Pathol Lab Med. 2006;130:1780–5.
30. Beckwith JB. The value of the pediatric postmortem examination. Pediatr Clin North Am. 1989;36:29–36.
31. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med. 2001;29:1303–10. http://dx.doi.org/10.1097/00003246-200107000-00002
32. Torgersen C, Moser P, Luckner G, Mayr V, Jochberger S, Hasibeder WR, et al. Macroscopic postmortem findings in 235 surgical intensive care patients with sepsis. Anesth Analg. 2009;108:1841–7. http://dx.doi.org/10.1213/ane.0b013e318195e11d
33. Hull MJ, Nazarian RM, Wheeler AE, Black-Schaffer WS, Mark EJ. Resident physician opinions on autopsy importance and procurement. Hum Pathol. 2007;38:342–50. http://dx.doi.org/10.1016/j.humpath.2006.08.011
34. Molina DK, Wood LE, Frost RE. Is routine histopathologic examination beneficial in all medicolegal autopsies? Am J Forensic Med Pathol. 2007;28:1–3. http://dx.doi.org/10.1097/01.paf.0000257388.83605.0a
35. Chong PY, Chui P, Ling AE, Franks TJ, Tai DY, Leo YS, et al. Analysis of deaths during the severe acute respiratory syndrome (SARS) epidemic in Singapore: challenges in determining a SARS diagnosis. Arch Pathol Lab Med. 2004;128:195–204.
36. Wilson ML, Gradus S, Zimmerman SJ. The role of local public health laboratories. Public Health Rep. 2010;125(Suppl 2):118–22.
37. Christiansen LR, Collins KA. Natural death in the forensic setting: a study and approach to the autopsy. Am J Forensic Med Pathol. 2007;28:20–3. http://dx.doi.org/10.1097/01.paf.0000233553.19938.a0
38. Huston BM, Malouf NN, Azar HA. Percutaneous needle autopsy sampling. Mod Pathol. 1996;9:1101–7.
39. Thali MJ, Yen K, Schweitzer W, Vock P, Boesch C, Ozdoba C, et al. Virtopsy, a new imaging horizon in forensic pathology: virtual autopsy by postmortem multislice computed tomography (MSCT) and magnetic resonance imaging (MRI)—a feasibility study. J Forensic Sci. 2003;48:386–403.
40. Thali MJ, Dirnhofer R, Becker R, Oliver W, Potter K. Is 'virtual histology' the next step after the 'virtual autopsy'? Magnetic resonance microscopy in forensic medicine. Magn Reson Imaging. 2004;22:1131–8. http://dx.doi.org/10.1016/j.mri.2004.08.019

Address for correspondence: Lindy Liu, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop G32, Atlanta, GA 30333, USA; email: fuz3@cdc.gov

Influenza-associated Hospitalizations by Industry, 2009–10 Influenza Season, United States

Sara E. Luckhaupt, Marie Haring Sweeney, Renee Funk, Geoffrey M. Calvert, Mackenzie Nowell,[1] Tiffany D'Mello, Arthur Reingold, James Meek, Kimberly Yousey-Hindes, Kathryn E. Arnold, Patricia Ryan, Ruth Lynfield, Craig Morin, Joan Baumbach, Shelley Zansky, Nancy M. Bennett, Ann Thomas, William Schaffner, and Timothy Jones

Medscape EDUCATION ACTIVITY

Medscape, LLC is pleased to provide online continuing medical education (CME) for this journal article, allowing clinicians the opportunity to earn CME credit.

This activity has been planned and implemented in accordance with the Essential Areas and policies of the Accreditation Council for Continuing Medical Education through the joint sponsorship of Medscape, LLC and Emerging Infectious Diseases. Medscape, LLC is accredited by the ACCME to provide continuing medical education for physicians.

Medscape, LLC designates this Journal-based CME activity for a maximum of 1 *AMA PRA Category 1 Credit(s)*™. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

All other clinicians completing this activity will be issued a certificate of participation. To participate in this journal CME activity: (1) review the learning objectives and author disclosures; (2) study the education content; (3) take the post-test with a 70% minimum passing score and complete the evaluation at **www.medscape.org/journal/eid**; (4) view/print certificate.

Release date: March 15, 2012; Expiration date: March 15, 2013

Learning Objectives

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

- Distinguish the effect of employment on the risk for hospitalization for influenza
- Assess the risk for hospitalization for influenza across different sectors of industry
- Evaluate the interaction between underlying medical illness and the risk for hospitalization for influenza among workers
- Analyze other factors potentially associated with the risk for hospitalization for influenza among workers.

Editor

Thomas J. Gryczan, MS, Technical Writer/Editor, *Emerging Infectious Diseases. Disclosure: Thomas J. Gryczan, MS, has disclosed no relevant financial relationships.*

CME Author

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

Authors

*Disclosures**: Sara E. Luckhaupt, MD, MPH; Mackenzie Nowell, MPH; Tiffany D'Mello, MPH; Arthur Reingold, MD; James Meek, MPH; Kimberly Yousey-Hindes; Renee Funk, DVM; Patricia Ryan, MS; Ruth Lynfield, MD; Craig Morin, MPH; Joan Baumbach, MD, MPH; Shelley Zansky, PhD; Nancy M. Bennett, MD, MS; Ann Thomas, MD, MPH;** and **Timothy Jones, MD,** have disclosed no relevant financial relationships. **Marie Haring Sweeney, PhD, MPH,** has disclosed the following relevant financial relationships: owns stock, stock options, or bonds from P&G, Apple, Lucent, J&J. **Geoffrey M. Calvert, MD, MPH,** has disclosed the following relevant financial relationships: owns stock, stock options, or bonds from Bristol-Myers Squibb, Abbott, Amgen, Baxter, Gen-Probe, Johnson & Johnson, Pfizer, Gilead, St. Jude Medical, Stryker, Teva, Novartis. **Kathryn E. Arnold, MD,** has disclosed the following relevant financial relationships: owns stock, stock options, or bonds from Steris Corp, Kimberley Clark Corp. **William Schaffner, MD,** has disclosed the following relevant financial relationships: served as an advisor or consultant for Merck, sanofi-Pasteur, Pfizer, GSK, Dynavax.*

Author affiliations: Centers for Disease Control and Prevention, Cincinnati, Ohio, USA (S.E. Luckhaupt, M. H. Sweeney, G.M. Calvert); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (R. Funk, M. Nowell, T. D'Mello); University of California, Berkeley, California, USA (A. Reingold); Yale University, New Haven, Connecticut, USA (J. Meek, K. Yousey-Hindes); Georgia Division of Public Health, Atlanta (K. E. Arnold); Maryland Department of Health and Mental Hygiene, Baltimore, Maryland, USA (P. Ryan), Minnesota Department of Health, Minneapolis, Minnesota, USA (R. Lynfield, C. Morin); New Mexico Department of Health, Santa Fe, New Mexico, USA (J. Baumbach); New York State Department of Health, Albany, New York, USA (S. Zansky); University of Rochester, Rochester, New York, USA (N. M. Bennett); Oregon Public Health Division, Portland, Oregon, USA (A. Thomas); Vanderbilt University, Nashville, Tennessee, USA (W. Schaffner); and Tennessee Department of Health, Nashville (T. Jones)

DOI: http://dx.doi.org/10.3201/eid1804.110337

[1]Current affiliation: Florida Department of Health, Orlando, Florida, USA.

In response to pandemic (H1N1) 2009, data were collected on work status and industry of employment of 3,365 adults hospitalized with laboratory-confirmed influenza during the 2009–10 influenza season in the United States. The proportion of workers hospitalized for influenza was lower than their proportion in the general population, reflecting underlying protective characteristics of workers compared with nonworkers. The most commonly represented sectors were transportation and warehousing; administrative and support and waste management and remediation services; health care; and accommodation and food service.

Although the Occupational Safety and Health Administration, Department of Labor, and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention (CDC), have issued guidance to assist workplaces in responding to influenza pandemics (*1*,*2*), more information is needed about which specific groups of workers are at highest risk for acquiring or having complications from pandemic and seasonal influenza. Specifically, this information is needed for recognizing and responding to increased risks for infection among key occupational groups (e.g., health care workers, school teachers, retail and food service workers, and others with substantial exposure to the general public); informing persons who develop guidance for key policy questions, including the prioritization of groups to receive vaccine, school closing policies, and appropriate personal protective equipment use; and providing data that might trigger more in-depth case studies of clusters of disease occurring among specific workers.

During the influenza (H1N1) 2009 pandemic, NIOSH explored multiple sources of data on the occupations of affected persons. The occupational distribution of all confirmed (H1N1) 2009 pandemic influenza case-patients from 4 states during the early phase of the pandemic (April–July 2009) has been reported by Suarthana et al. (*3*) but as the pandemic progressed and case counts rapidly increased, it became impossible to collect occupational information on all case-patients.

Thus, during the fall wave of the pandemic, NIOSH worked with the CDC Emerging Infections Program (EIP) to collect data on the industry and occupation of the subset of adults hospitalized with laboratory-confirmed influenza. Hospitalized case-patients, many of whom have underlying medical risk factors for severe disease, are not representative of all persons who acquire influenza. Thus, studying them provides little insight into the risk of acquiring influenza. However, examining the distribution of industry of employment of these persons provides some clues about specific groups of workers that might be most commonly affected by severe influenza.

Methods

EIP Data for Hospitalized Influenza Case-Patients

The EIP is coordinated and funded by CDC. It consists of a network of 10 state health departments (California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee) and their collaborators in local health departments, academic institutions, other federal agencies, and public health and clinical laboratories. The network comprises a catchment area of ≈16.8 million persons ≥18 years of age. The population studied by the EIP is roughly representative of the US population on the basis of demographic characteristics such as age, sex, race, urban residence, and population density and percentage at or below the poverty level. During the 2009–10 influenza season (September 1, 2009–April 30, 2010), the 10 EIP sites performed active population-based surveillance for laboratory-confirmed influenza-related hospitalizations (see www.cdc.gov/ncpdcid/deiss/eip/index.html for information about EIP). This period coincided with circulation and dominance of the pandemic (H1N1) 2009 virus strain. Institutional Review Board approval for EIP adult influenza-associated hospitalization surveillance activities during the 2009–10 influenza season was obtained from all sites, including CDC.

As described elsewhere (*4*), cases were defined as persons ≥18 years of age hospitalized for community-onset, laboratory-positive influenza infection. Case-patients were residents of the defined EIP catchment areas and were admitted to a surveillance-area hospital during the influenza season and within 14 days of receiving a positive influenza test result. Laboratory confirmation of influenza was obtained by virus culture, immunofluorescence antibody staining, reverse transcription PCR, or a commercially available rapid diagnostic test. Written documentation of a positive influenza test result in the medical chart was acceptable as evidence of laboratory confirmation. Persons who had positive results for influenza >3 days after hospital admission were considered to have nosocomial influenza and were excluded from this study. Staff at each EIP site identified cases by contacting hospital laboratories, medical records departments, and infection control practitioners, and by reviewing databases of state-reportable conditions.

Once a person who met the surveillance case definition was identified, his or her hospital medical chart was abstracted and a standardized data collection instrument was completed. The instrument included a question about employment in selected health care occupations and an open text field to record other occupational information.

NIOSH received a deidentified dataset of 4,511 hospitalized cases of influenza from the 2009–10 influenza season from the CDC Influenza EIP program, as of May

18, 2010. One hundred forty-eight (3.3.%) case-patients were excluded from analyses because of incomplete data collection. An additional 11 (0.2%) case-patients were excluded because they were hospitalized outside the EIP catchment area, although they resided in a catchment area. We classified case-patients by work status (worker, nonworker, or unknown). Nonworkers included students, homemakers, retired persons, disabled persons, and nonworkers not elsewhere classified. Nonworkers not elsewhere classified included occupation text entries of none, not employed, unemployed, incarcerated, and homeless. All nursing home residents, regardless of occupational information recorded, were grouped with disabled persons because of their similar high prevalence of underlying medical conditions. If the text field for occupation was blank or indicated that the work status of the case-patient was unknown, we classified the work status as unknown. Trained NIOSH coders used responses to the health care worker question and occupational text entries to assign 2-digit codes for industry sector from the North American Industrial Classification System (*5*) and 2-digit codes for occupational group from the Standard Occupation Classification system (*6*) to the workers.

The EIP data collection instrument also included information about several underlying conditions associated with increased risk for influenza (asthma, cystic fibrosis, other chronic lung disease, chronic cardiovascular disease, renal disease, chronic metabolic disease [including diabetes], hemoglobinopathy, neuromuscular disorder, diagnosis of cancer [excluding nonmelanoma skin cancer] in the past 12 months, immunosuppressive condition, seizure disorder, Guillain-Barré syndrome, lymphoma or leukemia, cognitive dysfunction, pregnancy, and obesity). The EIP program does not collect any data on tobacco use, socioeconomic factors (e.g., income), or access to primary care among hospitalized influenza case-patients.

National Health Interview Survey Reference Data for Employed US Population

Reference data for the employed US population was obtained from the 2010 National Health Interview Survey (NHIS) public use dataset (www.cdc.gov/nchs/nhis/quest_data_related_1997_forward.htm). The NHIS is a cross-sectional in-person household survey conducted annually by the National Center for Health Statistics, CDC. Data are collected on the civilian noninstitutionalized population of the United States, and thus exclude persons in long-term care facilities (e.g., nursing homes), correctional facilities, active-duty Armed Forces personnel (although civilian family members are included), and US nationals living in foreign countries. The survey uses a multistage clustered sample design with oversampling of black, Hispanic, and Asian persons and produces nationally representative data on health insurance coverage, health care access and use, health status, health behavior, and other health-related topics.

Data Analyses

The proportions of adults hospitalized for influenza (EIP cases) by employment status were compared with expected proportions in the US population according to the 2010 NHIS, by age group. Among employed adults hospitalized for influenza, the proportions employed in each industry sector were compared with proportions of the US population employed in each industry sector. Ratios >1.0 indicated overrepresentation of an industry sector in the EIP dataset compared with what would be expected if workers from all industry sectors had the same risk for hospitalization because of influenza, which would lead to equal distributions of industry sectors between the 2 datasets. Confidence intervals were calculated by using the χ^2 statistic to approximate the Poisson distribution.

We also used data from the 2010 NHIS to estimate the proportion of US adults employed in each sector who reported ≥1 underlying medical conditions, were current smokers, had relatively low annual earnings (<$35,000), and had a usual place to go for health care. We included health conditions reported in the NHIS that most closely matched the underlying health conditions for which data were collected in the EIP influenza study (i.e., asthma, emphysema, chronic bronchitis, cardiovascular disease [hypertension, coronary heart disease, angina, history of myocardial infarction, other heart condition, and/or history of stroke], renal failure, diabetes, diagnosis of cancer [excluding nonmelanoma skin cancer] in the past 12 months, epilepsy, pregnancy, and obesity [body mass index ≥30]). These estimates provide some group-level background information about underlying characteristics of workers employed in each industry sector that might affect their risks of hospitalization because of influenza (*4*,*7*–*12*).

Data analyses were performed by using SAS version 9.2 for Windows (SAS Institute, Cary, NC, USA). To account for the complex sampling design of the NHIS, all analyses of NHIS data were completed by using SAS survey procedures appropriate for complex samples and sampling information included in the public use dataset. Estimates based on NHIS data with relative SE >30% are not presented because of low reliability/precision.

Results

Data were available for 4,352 case-patients who had laboratory-confirmed influenza and who were hospitalized during September 1, 2009–April 24, 2010. Of these case-patients, 3,365 (77.3%) had adequate information recorded to classify them according to work status: 1,283 workers, 96 students, 86 homemakers, 472 retired persons, 535 nursing

home residents and disabled persons, and 893 nonworkers not elsewhere classified.

Overall, workers represent a much lower proportion of EIP hospitalized influenza case-patients compared with their proportions in the general US population according to NHIS data for every age group (EIP:NHIS ratio range 0.61–0.66) (Table 1). A total of 1,070 (83.4%) current workers were assigned 2-digit North American Industrial Classification System industry codes. Industry sectors with overrepresentation among hospitalized influenza case-patients (EIP data) compared with the 2010 NHIS reference population data were transportation and warehousing (ratio 1.53, 95% CI 1.18–1.94), administrative and support and waste management and remediation services (ratio 1.51, 95% CI 1.18–1.91), health care (ratio 1.47, 95% CI 1.26–1.70), and accommodation and food services (ratio 1.35, 95% CI 1.10–1.65) (Table 2).

In general, industry sectors with the highest prevalence of underlying chronic medical conditions (per NHIS, e.g., social assistance, public administration) were not overrepresented in the EIP database (Table 2). For every industry sector, the proportion of workers with underlying medical conditions among hospitalized influenza (EIP) case-patients was higher than the proportion of workers in the general population (NHIS) with underlying medical conditions (Table 2). These ratios varied by industry sector, but neither the industry sector with the highest proportion of workers with underlying medical conditions among hospitalized influenza case-patients (education services) nor the industry sector with the highest ratio of case-patients with underlying conditions compared with workers in the general population with underlying conditions (arts, entertainment, and recreation) were overrepresented in the EIP database (Table 2).

According to the 2010 NHIS reference population data, some industry sectors overrepresented in the EIP database had a higher prevalence of demographic characteristics that might place them at increased risk for influenza-associated hospitalization. For example, the accommodation and food services industry sector had the highest prevalence of current smokers (30.9%) and workers with relatively low earnings (<$35,000 per year) (92.3%) (Table 2). With regards to health care access, the industry sectors with the lowest proportion of workers who report having a usual place to go for health care (other than an emergency department) include accommodation and food services (63.9%) and administrative and support and waste management and remediation services (67.7%) (Table 2).

Discussion

Pandemic (H1N1) 2009 virus, which emerged in April 2009, resulted in substantial illness and deaths among working-aged adults. As reported, cumulative rates of laboratory-confirmed, influenza-associated hospitalizations in EIP sites were 2.4/10,000 population for all persons 18–49 years of age during August 30, 2009–March 27, 2010, which was ≈6× higher than the influenza-associated hospitalization rate for this age group in 2008–09, when seasonal influenza (H1N1) virus was the predominant strain (*13*).

We found that workers made up a much lower proportion of EIP hospitalized influenza case-patients than the general US population. This finding was not unexpected because of favorable underlying characteristics of workers compared with nonworkers, such as younger age and lower prevalence of diagnosed underlying medical conditions, which make workers less likely to be hospitalized for influenza. Furthermore, among workers, certain industry sectors are overrepresented in the EIP dataset than what would be expected if workers from all industry sectors had the same risk for hospitalization for influenza, suggesting that the risk for severe influenza varied among different groups of workers during the 2009–10 influenza season.

To date, most evaluations of work-related risk for influenza have focused on health care workers (*14–17*).

Table 1. Employment status among US adults hospitalized with influenza September 1, 2009–April 24, 2010 by age group, compared with data from the 2010 NHIS*

Status	No. (%) cases†	Estimated US populations in thousands (weighted %) from NHIS†	Ratio (95% CI)
Age 18–49 y			
Employed	825 (45.2)	94,862 (71.3)	0.63 (0.59–0.68)
Not employed	999 (54.8)	38,170 (28.7)	1.91 (1.79–2.03)
Employment status unknown	586 (24.3)	36 (0)	NA
Age 50–64 y			
Employed	409 (41.2)	36,045 (62.4)	0.66 (0.60–0.73)
Not employed	583 (58.8)	21,675 (37.6)	1.56 (1.44–1.70)
Employment status unknown	300 (23.2)	24 (0)	NA
Age ≥65 y			
Employed	49 (8.9)	5,697 (14.7)	0.61 (0.45–0.80)
Not employed	500 (91.1)	32,980 (85.3)	1.07 (0.98–1.17)
Employment status unknown	101 (15.5)	15 (0)	NA

*NHIS, National Health Interview Survey; NA, not applicable.
†Percentage among case-patients/respondents with known work status and among all case-patients/respondents with unknown work status.

There is some evidence that household exposures are more predictive of influenza infection among these workers than occupational exposures (*16*), but there is also evidence that occupational acquisition occurs (*14,17*). We found that persons working in the health care industry were overrepresented among hospitalized persons with influenza compared with what would be expected if workers from all industry sectors had the same risk for hospitalization because of influenza. However, health care workers were not the only worker group overrepresented.

Although the ratios were only modestly increased, these results suggest that groups of workers other than those employed in health care may also be at increased risk for influenza severe enough to result in hospitalization. Overrepresentation of an industry sector in the EIP dataset may be related to demographic and underlying health

Table 2. Hospitalized influenza case-patients by industry sector per EIP (September 1, 2009–April 24, 2010), ratios compared with distribution of employed US adults per NHIS, and characteristics of employed US adults by industry sector per NHIS*

Industry sector of employment (NAICS code)	% Employed hospitalized influenza case-patients, EIP	Weighted % employed adults, NHIS†	Ratio (95% CI)‡	% Case-patients with underlying condition, EIP§	% Adults, NHIS†			
					With underlying condition¶	Who smoke	Annual earnings <$35,00	With usual place for health care
Transportation and warehousing (48, 49)	6.26	4.10	1.53 (1.18–1.94)	76.12	54.07	20.79	48.23	78.36
Administrative and support and waste management and remediation (56)	6.64	4.38	1.51 (1.18–1.91)	73.24	49.82	25.34	80.74	67.69
Health care (62, except for 624)	16.17	11.01	1.47 (1.26–1.70)	81.40	52.46	16.24	59.12	87.72
Accommodation and food (72)	9.07	6.70	1.35 (1.10–1.65)	73.20	39.32	30.88	92.27	63.92
Other (81)	6.26	5.15	1.22 (0.94–1.54)	75.00	44.88	17.37	78.74	77.23
Social assistance (624)	3.18	2.70	1.18 (0.82–1.64)	82.35	60.66	14.32	81.83	89.66
Information (51)	2.90	2.54	1.14 (0.78–1.62)	87.10	51.19	15.54	44.20	84.80
Retail trade (44, 45)	12.43	11.03	1.13 (0.94–1.34)	85.61	46.35	22.64	80.93	75.84
Finance and insurance (52)	4.95	4.41	1.12 (0.84–1.47)	69.81	48.59	14.60	41.36	87.91
Education (61)	9.63	10.25	0.94 (0.77–1.14)	89.32	50.00	8.40	53.79	91.72
Professional, scientific, and technical (54)	6.26	6.84	0.92 (0.71–1.16)	71.64	41.06	13.35	28.65	85.08
Arts, entertainment, and recreation (71)	1.78	2.04	0.87 (0.52–1.36)	78.95	39.32	21.40	75.67	80.40
Construction (23)	5.05	6.61	0.76 (0.57–1.00)	57.41	46.50	29.09	63.51	70.01
Real estate and rental and leasing (53)	1.21	1.92	0.63 (0.34–1.08)	76.92	45.90	23.33	63.24	78.29
Public administration (92)	3.18	5.44	0.58 (0.40–0.82)	82.35	57.93	14.64	34.99	92.16
Manufacturing (31–33)	4.30	9.56	0.45 (0.33–0.60)	71.74	52.02	21.75	49.79	81.93
Utilities (22)	–	1.00	–	–	53.67	18.20	24.09	88.59
Agriculture, forestry, fishing, and hunting (11)	–	1.18	–	–	44.75	19.36	82.97	71.40
Wholesale trade (42)	–	2.58	–	–	49.76	23.71	48.68	82.70
Mining (21)	–	0.50	–	–	44.93	27.03	35.78	78.99
All employed	38.10	59.50	0.64 (0.59–0.70)	77.05	48.53	19.17	60.39	81.09
All nonemployed	61.90	40.50	1.53 (1.42–1.65)	87.56	65.20	19.62	96.77	84.52

*EIP, Emerging Infections Program; NHIS, National Health Interview Survey; NAICS, North American Industry Classification System; –, <5 cases.
†Weighted estimates based on 2010 NHIS public use dataset (www.cdc.gov/nchs/nhis/quest_data_related_1997_forward.htm).
‡Proportion among EIP cases:proportion of employed adults, per NHIS.
§Include asthma, cystic fibrosis, other chronic lung disease, chronic cardiovascular disease, renal disease, chronic metabolic disease (including diabetes), hemoglobinopathy, neuromuscular disorder, cancer (excluding nonmelanoma skin cancer) in past 12 mo, immunosuppressive condition, seizure disorder, Guillain-Barré syndrome, lymphoma or leukemia, cognitive dysfunction, pregnancy, and obesity.
¶Include asthma, emphysema, chronic bronchitis, cardiovascular disease (hypertension, coronary heart disease, angina, myocardial infarction, other heart condition, or stroke), renal failure, diabetes, cancer (excluding nonmelanoma skin cancer) in past 12 mo, epilepsy, pregnancy, and obesity (body mass index $\geq$30).

characteristics of the sector's work force that put them at increased risk for acquiring influenza and for being hospitalized with influenza, but it may also partially reflect occupational risk factors for influenza (e.g., exposure to ill members of the public).

Because the EIP data only include cases, it is difficult to assess the potential reasons for overrepresentation of certain industry sectors, but general population estimates of potential contributing factors based on 2010 NHIS data provide some clues. We examined 4 factors for which data or expert consensus suggests associations with hospitalization (or severe outcomes in general) caused by influenza: underlying medical conditions (*4*,*7*,*8*), current smoking behavior (*8*,*9*), low income (*10*–*12*), and timely access to primary care (*8*,*10*), as measured by reporting a usual place to go for health care.

For example, the most highly overrepresented groups of workers among EIP cases, transportation and warehousing and administrative and support and waste management and remediation services, also had the highest prevalence of some unfavorable demographic characteristics, which might place them at increased risk for influenza-associated hospitalization. Workers in the accommodation and food services sector were also overrepresented among EIP cases. It seems logical that these workers may be at increased occupational risk for acquiring influenza because of their high level of interaction with the general public, but NHIS data also suggest some demographic factors that might increase their risk for hospitalization because of severe influenza (e.g., low earnings, smoking, lack of access to medical care).

On the other hand, variation in underlying health status, socioeconomic status, and access to health care by industry group does not appear to explain all of our findings regarding overrepresentation of groups among hospitalized influenza case-patients compared with the general working population. Health care workers are overrepresented among EIP cases despite being relatively healthy and having relatively high earnings and access to health care. Construction workers are underrepresented among EIP cases despite having relatively low earnings and access to health care. These workers in construction might have a relatively low risk for acquiring influenza because of low interaction with the public.

We also found some industry sectors in which we would expect a relatively high level of interaction with the public (e.g., public administration, education) that were not overrepresented among EIP cases. Even if these workers have an increased risk for acquiring influenza from the public, they might have a low risk for progressing to severe influenza requiring hospitalization because of their relatively high earnings and access to health care.

This study has several limitations in addition to the major limitation of relying on a secondary data source (NHIS) for information on the characteristics of workers by industry sector. No useful information on work status was available for 22.7% of EIP hospitalized influenza case-patients. This fact likely reflects the inconsistency of occupational data available in typical hospital records. There is the potential for misclassification of work status and, among workers, misclassification of industry sector because of inconsistency in narrative data recorded for occupation. Although we called the variable we collected occupation, there were more entries that reflected codable industry sectors than reflected codable occupational groups. Thus, we only reported results by industry. Furthermore, in most cases, the available information only enabled industry to be coded at a broad, nonspecific level. For example, it was impossible to distinguish whether many of the health care workers worked in inpatient or outpatient settings.

Our study examined systematically collected influenza surveillance data according to occupational variables. Benefits of using data from the EIP program include laboratory confirmation of influenza and representation of a large population from geographically diverse areas in the United States. Although we were able to identify specific groups of workers that were most heavily affected by severe influenza during the 2009–10 influenza season, more research is needed to understand the reasons for the increased incidence of severe influenza among specific groups of workers. Concurrently, any interventions that focus on these groups of workers should be evaluated for effectiveness and efficiency.

Acknowledgments

We thank state health departments in California, Colorado, Connecticut, Georgia, Maryland, Minnesota, New Mexico, New York, Oregon, and Tennessee; collaborators in local health departments; academic institutions; other federal agencies; public health and clinical laboratories; infection preventionists; and healthcare providers for contributing data to the EIP network; and Laurie Kamimoto, Liping Pan, Pam Schumacher, Matthew Hirst, Elizabeth Smith, Jia Li, SangWoo Tak, and David Weissman for contributing to the study and the manuscript.

Dr Luckhaupt is a medical officer at CDC in Cincinnati, Ohio. Her research interests include surveillance and epidemiology of various chronic and acute health outcomes among various groups of workers.

References

1. Occupational Safety and Health Administration, US Department of Labor. Guidance on preparing workplaces for an influenza pandemic. OSHA 3327–05R, 2009 [cited 2010 Jul 8]. http://www.osha.gov/Publications/OSHA3327pandemic.pdf

2. Centers for Disease Control and Prevention. Guidance for businesses and employers to plan and respond to the 2009–2010 influenza season. Updated February 22, 2010 [cited 2010 Jul 8]. http://www.cdc.gov/h1n1flu/business/guidance/#top
3. Suarthana E, McFadden JD, Laney AS, Kreiss K, Anderson HA, Hunt DC, et al. Occupational distribution of persons with confirmed 2009 H1N1 influenza. J Occup Environ Med. 2010;52:1212–6. http://dx.doi.org/10.1097/JOM.0b013e3181fd32e4
4. Dao CN, Kamimoto L, Nowell M, Reingold A, Gershman K, Meek J, et al. Adult hospitalizations for laboratory-positive influenza during the 2005–2006 through 2007–2008 seasons in the United States. J Infect Dis. 2010;202:881–8. http://dx.doi.org/10.1086/655904
5. Office of Management and Budget. North American industry classification system (NAICS). United States 2007 [cited 2012 Jan 6]. http://www.census.gov/naics
6. Office of Management and Budget. Standard occupational classification manual (SOC). United States 2000 [cited 2012 Jan 6]. http://stats.bls.gov/soc/soc_home.htm
7. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, et al. Hospitalized patients with 2009 H1N1 influenza in the United States, April—June 2009. N Engl J Med. 2009;361:1935–44. http://dx.doi.org/10.1056/NEJMoa0906695
8. Gilca R, De Serres G, Boulianne N, Ouhoummane N, Papenburg J, Douville-Fradet M, et al. Risk factors for hospitalization and severe outcomes of 2009 pandemic H1N1 influenza in Quebec, Canada. Influenza Other Respi Viruses. 2011;5:247–55. http://dx.doi.org/10.1111/j.1750-2659.2011.00204.x
9. Huttunen R, Heikkinen T, Syrjänen J. Smoking and the outcome of infection. J Intern Med. 2011;269:258–69. http://dx.doi.org/10.1111/j.1365-2796.2010.02332.x
10. Bouye K, Truman BI, Hutchins S, Richard R, Brown C, Guillory JA, et al. Pandemic influenza preparedness and response among public-housing residents, single-parent families, and low-income populations. Am J Public Health. 2009;99:S287–93. http://dx.doi.org/10.2105/AJPH.2009.165134
11. Thompson DL, Jungk J, Hancock E, Smelser C, Landen M, Nichols M, et al. Risk factors for 2009 pandemic influenza A (H1N1)–related hospitalization and death among racial/ethnic groups in New Mexico. Am J Public Health. 2011;101:1776–84.
12. Yousey-Hindes KM, Hadler JL. Neighborhood socioeconomic status and influenza hospitalizations among children: New Haven County, Connecticut, 2003–2010. Am J Public Health. 2011;101:1785–9.
13. Centers for Disease Control and Prevention. Update: influenza—United States, August 30, 2009–March 27, 2010, and composition of the 2010–11 influenza vaccine. MMWR Morb Mortal Wkly Rep. 2010;59:423–30.
14. Centers for Disease Control and Prevention. Novel influenza A (H1N1) virus infections among healthcare personnel—United States, April–May 2009. MMWR Morb Mortal Wkly Rep. 2009;58:641–5.
15. Santos CD, Bristow RB, Vorenkamp JV. Which healthcare workers were most affected during the spring 2009 H1N1 pandemic? Disaster Med Public Health Prep. 2010;4:47–54.
16. Williams CJ, Schweiger B, Diner G, Haaman F, Krause G, Nienhaus A, et al. Seasonal influenza risk in hospital healthcare workers is more strongly associated with household than occupational exposures: results from a prospective cohort study in Berlin, Germany, 2006/07. BMC Infect Dis. 2010;10:8. http://dx.doi.org/10.1186/1471-2334-10-8
17. Wise ME, De Perio M, Halpin J, Jhung M, Magill S, Black SR, et al. Transmission of pandemic (H1N1) 2009 influenza to healthcare personnel in the United States. Clin Infect Dis. 2011;52:S198–204. http://dx.doi.org/10.1093/cid/ciq038

Address for correspondence: Sara E. Luckhaupt, Centers for Disease Control and Prevention, 4676 Columbia Pkwy, Mailstop R17, Cincinnati, OH 45226-1998, USA; email: pks8@cdc.gov

Identification of Risk Factors for Chronic Q Fever, the Netherlands

Linda M. Kampschreur, Sandra Dekker, Julia C.J.P. Hagenaars, Peter J. Lestrade, Nicole H.M. Renders, Monique G.L. de Jager-Leclercq, Mirjam H.A. Hermans, Cornelis A.R. Groot, Rolf H.H. Groenwold, Andy I.M. Hoepelman, Peter C. Wever, and Jan Jelrik Oosterheert

Since 2007, the Netherlands has experienced a large Q fever outbreak. To identify and quantify risk factors for development of chronic Q fever after *Coxiella burnetii* infection, we performed a case–control study. Comorbidity, cardiovascular risk factors, medications, and demographic characteristics from 105 patients with proven (n = 44), probable (n = 28), or possible (n = 33) chronic Q fever were compared with 201 patients who had acute Q fever in 2009 but in whom chronic Q fever did not develop (controls). Independent risk factors for development of proven chronic Q fever were valvular surgery, vascular prosthesis, aneurysm, renal insufficiency, and older age.

Q fever, a zoonosis caused by the intracellular gram-negative bacterium *Coxiella burnetii*, is prevalent worldwide (*1*,*2*) and has various acute and chronic clinical manifestations. Acute Q fever is mostly a self-limiting, mild, influenza-like disease, sometimes complicated by severe pneumonia or hepatitis. Asymptomatic acute infection occurs in 50%–60% of patients (*3*–*5*). Among patients infected by *C. burnetii*, infection progresses to chronic Q fever in 1%–5%, months to years after primary infection (*2*,*4*,*6*). Previous data, mainly from France, show that endocarditis is the most common clinical manifestation (±75%), followed by infections of aortic aneurysms and vascular prostheses (±10%) (*5*,*7*–*9*). In the Netherlands, however, an equal distribution of endocarditis and vascular infections has been seen (*10*).

Chronic Q fever leads to high illness and death rates if untreated, which makes early case finding and preventive measures critical for patients at high risk. Treatment for Q fever consists of long-term antimicrobial drug therapy, preferably a combination of doxycycline and hydroxychloroquine for 18–24 months. Previously identified risk factors for chronic Q fever are preexisting cardiac valvulopathy, vascular grafts and aneurysms, immunosuppression, and pregnancy; however, most published studies have been descriptive, lacked statistical quantification, or included specific high-risk groups only (*6*–*9*,*11*,*12*).

Since 2007, a large Q fever outbreak has been ongoing in the Netherlands, with >4,000 acute Q fever cases reported (*13*). Because of asymptomatic disease and overlap with other febrile diseases, however, the actual number of Q fever infections is probably much higher. Although the acute Q fever epidemic in the Netherlands has subsided, the number of patients with chronic Q fever is rising (*10*,*14*). In this unique population, we conducted a case–control study to identify and quantify risk factors for development of chronic Q fever after *C. burnetii* infection.

Methods

Study Design and Setting

Case-patients and controls were recruited from Jeroen Bosch Hospital in 's-Hertogenbosch and Bernhoven Hospital in Oss and Veghel; both are regional hospitals located in the center of the Q fever epidemic area in the

Author affilations: University Medical Centre Utrecht, Utrecht, the Netherlands (L.M. Kampschreur, R.H.H. Groenwold, A.I.M Hoepelman, J.J. Oosterheert); VU University, Amsterdam, the Netherlands (S. Dekker); Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands (J.C.J.P. Hagenaars, P.J. Lestrade, N.H.M. Renders, M.H.A. Hermans, P.C. Wever); and Bernhoven Hospital, Oss/Veghel, the Netherlands (M.G.L. de Jager-Leclercq, C.A.R. Groot)

DOI: http://dx.doi.org/10.3201/eid1804.111478

Netherlands. The study design was approved by the Medical Research Ethics Committee of the University Medical Centre Utrecht.

Patient Selection

We used existing datasets and spontaneous notifications from the 2 hospitals to identify all chronic Q fever diagnoses among patients >18 years of age during January 1, 2007–May 1, 2011. In the past, diagnosis of chronic Q fever has relied on results of serologic testing and PCR. Chronic Q fever is considered proven if *C. burnetii* is detected by PCR in blood or tissue in the absence of acute infection, but sensitivity of this technique is only ≈50% (*15*,*16*). Persisting high levels of IgG to phase I antigens (phase I IgG) and, to a lesser extent, phase II antigens (phase II IgG) are also indicative of chronic Q fever (*1*). The optimal immunofluorescence assay (IFA) cutoff value for phase I IgG titer is still matter of debate and is dependent on the test used but is probably within the range of 800–1,600 (*7*,*17*–*19*).

Recently, the Dutch Q Fever Consensus Group proposed a new diagnostic approach that combines PCR, serologic testing, and clinical data and categorizes cases into proven, probable, or possible chronic Q fever (*20*). Proven cases are those among patients with positive PCR results for *C. burnetii* in blood or tissue or a phase I IgG titer of ≥1,024 in combination with a vascular infection proven by positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI), or endocardial involvement according to the major criteria of the modified Duke criteria on echocardiogram (*21*). Probable cases are those among patients with phase I IgG titers of ≥1,024 and known risk factors: nonmajor valvulopathy according to the modified Duke criteria (*21*), suspected nonvascular or noncardial localization of chronic Q fever infection, or aspecific signs of chronic infection. Possible cases are those among patients with phase I IgG titers of ≥1,024 without other risk factors as listed for probable or proven chronic Q fever. In contrast to the other 2 subgroups, in general, possible chronic Q fever patients do not receive long-term antimicrobial drug treatment but instead enter a follow-up program; many demonstrate spontaneous decline in phase I IgG titers. We defined cases according to these definitions (*20*) (Table 1).

Controls were selected from an existing cohort of patients with acute Q fever, seen by general practitioners in 2009, who had positive PCR results for *C. burnetii* in serum samples. Controls were included if they were >18 years of age at the time of acute Q fever and if the serologic profile was not suggestive of chronic Q fever during >1 year of follow-up (i.e., decreasing antibody titers and phase I IgG titer <1,024). Patients with serologic follow-up of <1 year after the episode of acute Q fever were excluded from analysis. All case-patients except 1 and all controls lived in the same postal code area (5000–5400) in the Netherlands.

Table 1. Classification of chronic Q fever according to Dutch Q Fever Consensus Group guidelines*

Classification	Definition
Proven	Any of the following: • Positive PCR for *Coxiella burnetii* in serum, plasma, or tissue in the absence of acute Q fever • IFA phase I titer ≥1,024 with definite endocarditis according to the revised Duke criteria (*21*) • Indication of vascular infection on PET/CT, CT, MRI, or ultrasound testing
Probable	IFA phase I IgG titer ≥1,024 and any of the following clinical manifestations: • Valvulopathy not meeting the criteria of endocardial involvement of the major modified Duke criteria (*22*) • Aneurysm, vascular prosthesis or prosthetic valve without signs of infection on PET/CT, CT, MRI, or ultrasound testing • Signs of possible chronic Q fever infection of noncardiac or vascular origin on PET/CT, CT, or ultrasound testing • Pregnancy • Clinical symptoms of chronic infection (i.e., fever, night sweats, weight loss, hepatosplenomegaly) • Histopathologic proven granulomatous inflammation • Immune disorder
Possible	IFA phase I IgG titer ≥1,024 without clinical manifestations as described above

*Described in (*20*). IFA, immunofluorescence assay; PET, positron emission tomography; CT, computed tomography; MRI, magnetic resonance imaging.

Microbiological Analyses

Microbiological diagnostics for chronic Q fever case-patients consisted of IFA (Focus Diagnostics, Inc., Cypress, CA, USA) of serum samples and PCR for *C. burnetii* DNA in serum, plasma, and tissue samples. The diagnostic workup to evaluate *C. burnetii* infection in control patients with documented acute Q fever had been performed according to a diagnostic algorithm for acute Q fever introduced in May 2009. In brief, serum samples were screened with ELISA for IgM against *C. burnetii* phase II antigens (MII-screen; Institut Virion Serion GmbH, Würzburg, Germany). Depending on date of onset of disease and inpatient or outpatient setting, PCR for *C. burnetii* DNA was performed if the MII-screen result was negative (*23*–*25*). In patients with confirmed acute Q fever, serologic follow-up was performed at 3, 6, and 12 months, consisting of IFA for IgM and IgG against *C. burnetii* phase I and phase II antigens.

Data Collection and Storage

We collected patient characteristics including demographic variables, medical history, medication, pathology and microbiology results, imaging records, therapy, and outcome for case-patients and controls. Case-patient information was already available in the hospital registration systems and was interpreted by 2 researchers (L.K. and S.D.). All controls were sent a questionnaire and an informed consent form that asked for permission to request patient's data from the general practitioner and from the hospital registration system.

Although debatable, routine echocardiographic screening after diagnosis of acute Q fever is not the standard of care in the Netherlands because no benefit was found in an earlier evaluation (*26*,*27*). Therefore, for chronic Q fever case-patients and acute Q fever controls, details about cardiac valvulopathy were retrieved by review of medical records. The obtained information was processed and stored anonymously with the use of coded data. SPSS version 18.0 was used for storage and analysis of the collected data (SPSS Inc., Chicago, IL, USA).

Statistical Analysis

Within this study, we conducted 3 analyses: 1) an overall analysis of all chronic Q fever cases (i.e., proven, probable, and possible); 2) an analysis of proven and probable chronic Q fever cases; and 3) an analysis of proven chronic Q fever cases only. We performed these analyses to determine whether exclusion of possible chronic Q fever and, to a lesser extent, probable chronic Q fever (the groups in which disease status is doubtful) influenced the overall results. Univariate and subsequent multivariate logistic regression analyses were performed to calculate odds ratios (ORs), corresponding 95% CIs, and p values for the development of chronic Q fever. In univariate analysis, missing values were excluded. Variables with no observations among case-patients and <2 observations in the control group (or vice versa) were excluded (i.e., hematologic malignancies, bone marrow transplantation, dialysis, renal transplant, nonrenal organ transplant, congenital cardiac deviation, pulmonary diseases, and autoimmune disorder). For potential dichotomous risk factors, i.e., those that had 0 observations among either the case-patients or controls but >2 observations in the other, we applied a Fisher exact test to calculate p values. Variables with ≥1 observations and ≤25% missing values in case-patients and controls, a p value of <0.10 in univariate analysis, or known association in previous reports with the development of chronic Q fever were subsequently analyzed in a multivariate model.

The variables vascular history and valvulopathy were not included in multivariate analysis because they were included in variables that were listed separately (i.e., vascular prosthesis, aneurysm, other vascular surgery, peripheral arterial disease, cerebrovascular disease, valvular surgery, and nonsurgical valvular disease). Eighteen case-patients and 0 controls had a history of valvular surgery. Because of the expected importance of this risk factor and the high incidence among case-patients, we considered its inclusion in the multivariate analysis critical; moreover, the logistic regression model could not be fitted with this variable excluded. Therefore, we randomly changed one of the observations of the control group from 0 to 1, which artificially reduced the association but enabled us to fit the regression model.

The variables age, vascular history, vascular prosthesis, aneurysm, other vascular surgery, cerebrovascular disease, peripheral vascular disease, valvulopathy, valvular surgery, valvular deviation, ischemic heart disease, other cardiovascular diseases, hypertension, dyslipidemia, diabetes, nonhematologic malignancy, and renal insufficiency could be included in multivariate analysis of all groups. The variable immune disorder was also included in multivariate analysis for the probable and proven and the proven subgroups. The variable pacemaker was also included in multivariate analysis for the proven group.

Differences between case-patients and controls were shown in use of statins, clopidogrel, acenocoumarol, and proton pump inhibitors and hospitalization and adequate treatment during acute Q fever (p<0.10). However, these variables could not be included in the multivariate analysis because >25% of values were missing, most among case-patients in whom an acute Q fever episode had gone unrecognized.

After selecting predictors for our final multivariate model, we evaluated their possible interactions by including 2-way interactions in consecutive models. Interactions were not significant and therefore not included in the model. To assess the goodness-of-fit of the final model, we plotted sensitivity and specificity by using a receiver operating characteristic curve and estimated the area under the curve (c-statistic). p≤0.05 was considered significant.

Results

We identified 105 case-patients with proven, probable, or possible chronic Q fever; 44 (42%) had proven, 28 (27%) probable, and 33 (31%) possible disease. Of the case-patients with proven chronic Q fever, 27 (61%) had positive PCR results for *C. burnetii* in blood only, 5 (11%) in tissue only, 8 (18%) in tissue and blood, and 4 (9%) in neither blood nor tissue. The focus of infection in cases of proven chronic Q fever was endocarditis for 12 case-patients (27%) and endovascular infection for 26 (59%); 6 (14%) had no clear infection focus. Of the case-patients with probable chronic Q fever, suspected foci were cardiac valves in 12 (43%), endovascular lesions in 1 (4%), and another focus (e.g., pregnancy or clinical symptoms of

infection such as weight loss, night sweats, and fever) in 15 (54%).

Long-term antimicrobial drug treatment was started for 40/44 case-patients (91%) with proven chronic Q fever, 18/28 case-patients (64%) with probable chronic Q fever, and 5/32 case-patients (15%) with possible chronic Q fever. Three patients with proven chronic Q fever patients died before diagnosis of chronic Q fever; 1 refused therapy.

In all, 289 controls who had PCR-proven acute Q fever in 2009 were sent a questionnaire. Of these, 201 (69.6%) responded, signed the informed consent form, and fulfilled the inclusion criteria (Figure 1).

Results of the univariate analysis are listed in Table 2. Comparisons for age, vascular history, vascular prosthesis, aneurysm, other vascular surgery, cerebrovascular disease, peripheral vascular disease, valvulopathy, valvular surgery, valvular deviation, ischemic heart disease, other cardiovascular diseases, hypertension, dyslipidemia, diabetes, nonhematologic malignancy (defined as several kinds of solid tumors), renal insufficiency, and pregnancy showed significant differences between case-patients and controls.

Results of the multivariate analyses are shown in Table 3. Valvular surgery (OR 31.5, 95% CI 3.99–249), vascular prosthesis (OR 10.4, 95% CI 2.17–50.0), aneurysm (OR 8.65, 95% CI 1.74–42.9), nonhematologic malignancy (OR 3.90, 95% CI 1.33–11.5), and age (OR 1.03, 95% CI 1.01–1.06) were independently associated with the development of chronic Q fever. The final discriminative performance was good, with a c-statistic of 0.71 (95% CI 0.71–0.83) (Figure 2).

Patient risk factors identified in the analysis of the proven cases, representing the most definite chronic Q fever cases, were valvular surgery (OR 43.6, 95% CI 4.70–405), vascular prosthesis (OR 26.8, 95% CI 4.88–147), aneurysm (OR 25.9, 95% CI 4.55–147), renal insufficiency (OR 16.0, 95% CI 2.06–123), and age (OR 1.06, 95% CI 1.02–1.11). The final discriminative performance was good, with a c-statistic of 0.91 (95% CI 0.85–0.97) (Figure 2).

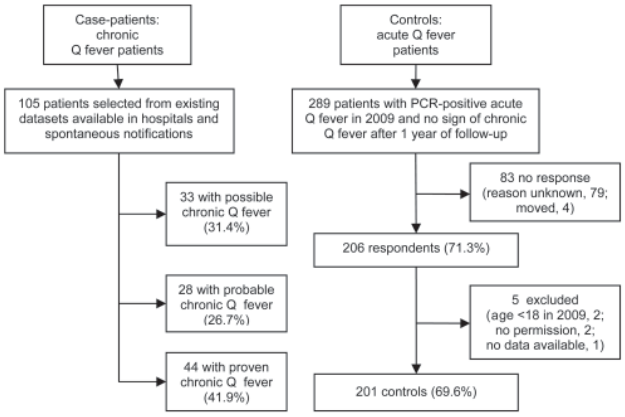


Figure 1. Enrollment, selection, and inclusion criteria forcase-patients and controls for case–control study to identify risk factors for chronic Q fever, the Netherlands.

Discussion

To our knowledge, this is the first study that analyzed a large number of potential risk factors for chronic Q fever in a large number of patients. Most former studies have been limited by a low number of cases and evaluation of few risk factors. Moreover, quantification of these risk factors was lacking (*6*–*9*,*11*,*12*).

In our study, we focused mainly on case-patients with proven chronic Q fever becausethis group included patients with the most definite form of chronic Q fever. Proven chronic Q fever also showed the strongest correlation with the identified risk factors. In multivariate analysis, valvular surgery, vascular prosthesis, aneurysms, renal insufficiency, and age were significant risk factors for the development of chronic Q fever in patients with proven cases. In the analysis of all patients with chronic Q fever cases, nonhematologic malignancy also seemed to be a risk factor; however, this could not be reproduced in the subanalyses of the more definite cases (e.g., proven and probable cases). Hence, nonhematologic malignancy as a risk factor remains uncertain. Valvular surgery, vascular prostheses, and aneurysms were the strongest predictors in this study, which confirms observational findings from earlier studies. Explanation lies in the association with the preferred localization of chronic Q fever infection.

A novel finding is the association between mild renal insufficiency and chronic Q fever. The majority of patients with chronic Q fever and renal disease in our study had stage 3 renal insufficiency according to Kidney Disease Outcome Quality Initiative guidelines (*28*). Although terminal renal insufficiency can decrease the immune response, this association was not found for mild renal disease (*29*). Renal insufficiency is associated with vascular disease, which may explain the elevated incidence of chronic Q fever in these patients (*30*).

Increasing age also predisposes for the development of chronic Q fever; this predisposition was also illustrated in a recent report of van der Hoek et al. (*24*). The explanation probably lies in the increased prevalence of cardiovascular diseases and the decreased cellular immunity during aging (*31*,*32*). Age >60 years appeared the best cutoff above which the risk for chronic Q fever increases significantly.

Preexisting cardiac valvulopathy has been found to give an estimated risk of 39% for the development of chronic Q fever after infection with *C. burnetii* (*6*,*33*,*34*). In contrast, recent reports showed no elevated risk for patients with mild valvulopathy in the ongoing outbreak in

the Netherlands (*26*,*27*). Although our univariate analyses showed that nonsurgical cardiac valvulopathy increased the risk for the development of chronic Q fever, this finding was not confirmed in the multivariate analysis. This finding can be explained by the fact that 9/17 (53%) case-patients with nonsurgical valvulopathy also had a history of valvular surgery of one of the other valves. The location and type of valvular defects did not differ significantly between case-patients and controls (Table 2). A possible explanation for the discrepancy with previous observations lies in the fact that our study was conducted 4 years after start of the Q fever epidemic, but chronic Q fever endocarditis

Table 2. Results of univariate analysis of risk factors for chronic versus acute Q fever, the Netherlands*

Risk factor	Acute Q fever, no. (%), n = 201	All chronic Q fever, n = 105			Proven chronic Q fever, n = 44		
		No. (%)	OR (95% CI)	p value	No. (%)	OR (95% CI)	p value
Male	129 (64.2)	70 (66.7)	1.12 (0.68–1.84)	0.665	32 (72.7)	1.49 (0.72–3.07)	0.281
Mean age, y (±SD)	52.5 (±13.7)	63.9 (±13.5)	1.06 (1.04–1.09)†	0.000	68.4 (±10.8)	1.11 (1.07–1.15)†	0.000
Smoker	85 (42.5)	43 (44.3)	1.08 (0.66–1.76)	0.765	22 (55.0)	1.65 (0.84–3.27)	0.149
Medical history							
Vascular abnormality	9 (4.5)	33 (31.4)	9.78 (4.46–21.4)	0.000	23 (52.3)	23.4 (9.57–57.1)	0.000
Vascular prosthesis	2 (1.0)	15 (14.3)	16.5 (3.71–74.0)	0.000	14 (31.8)	46.4 (10.0–215)	0.000
Aneurysm	2 (1.0)	12 (11.4)	12.8 (2.82–58.5)	0.001	9 (20.5)	25.6 (5.30–123)	0.000
Other vascular surgery	3 (1.5)	7 (6.7)	4.71 (1.19–18.6)	0.027	4 (9.1)	6.60 (1.42–30.6)	0.016
Peripheral arterial disease	6 (3.0)	11 (10.5)	3.80 (1.37–10.6)	0.011	6 (13.6)	5.13 (1.57–16.8)	0.007
Cerebrovascular disease‡	8 (4.0)	11 (10.5)	2.82 (1.10–7.25)	0.031	5 (11.4)	3.09 (0.96–9.96)	0.058
Valvulopathy	10 (5.0)	25 (23.8)	5.97 (2.47–13.0)	0.000	13 (29.5)	8.01 (3.23–19.8)	0.000
Valvular disease, NS§	10 (5.0)	17 (16.2)	3.69 (1.62–8.39)	0.002	9 (20.5)	4.91 (1.86–13.0)	0.001
Valvular surgery	1 (0.5)¶	18 (17.1)	41.4 (5.44–315)	0.000	10 (22.7)	58.8 (7.29–474)	0.000
Congenital cardiac disease	1 (0.5)	1 (1.0)	1.92 (0.12–31.1)	0.645	NA	NA	NA
Ischemic cardiac disease#	17 (8.5)	28 (26.7)	3.94 (2.04–7.61)	0.000	17 (38.6)	6.82 (3.11–14.9)	0.000
Pacemaker	2 (1.0)	3 (2.9)	2.93 (0.48–17.8)	0.244	3 (6.8)	7.28 (1.18–45.0)	0.033
Other cardiac history**	12 (6.0)	26 (24.8)	5.18 (2.49–10.8)	0.000	15 (34.1)	8.15 (3.47–19.1)	0.000
Hypertension	56 (27.9)	44 (41.9)	1.87 (1.14–3.07)	0.013	24 (54.5)	3.11 (1.59–6.06)	0.001
Dyslipidemia	39 (19.4)	32 (30.5)	1.82 (1.06–3.13)	0.031	16 (36.4)	2.37 (1.17–4.81)	0.017
Diabetes mellitus, type 1 or 2	13 (6.5)	15 (14.3)	2.41 (1.10–5.28)	0.028	7 (15.9)	2.74 (1.02–7.32)	0.045
Nonhematologic malignancy	6 (3.0)	16 (15.2)	5.84 (2.21–15.4)	0.000	6 (13.6)	5.13 (1.57–16.8)	0.007
Immune disorder††	2 (1.0)	4 (3.8)	3.94 (0.71–21.9)	0.117	3 (6.8)	7.28 (1.18–45.0)	0.033
COPD	14 (7.0)	13 (12.4)	1.89 (0.85–4.18)	0.117	6 (13.6)	2.11 (0.76–5.84)	0.151
Other pulmonary disease‡‡	6 (3.0)	3 (2.9)	0.96 (0.23–3.90)	0.950	NA	NA	NA
Liver disease	1 (0.5)	3 (2.9)	5.88 (0.60–57.3)	0.127	1 (2.3)	4.65 (0.29–75.8)	0.280
Renal insufficiency	2 (1.0)	12 (11.4)	12.8 (2.82–58.5)	0.001	9 (20.5)	25.6 (5.30–123)	0.000
Autoimmune disease§§	2 (1.0)	1 (1.0)	0.96 (0.09–10.7)	0.971	NA	NA	NA
Pregnancy¶¶	0 (0)	3 (2.9)	NA	0.040	1 (2.3)	NA	0.180
Medication at time of acute Q fever							
Proton pump inhibitors¶¶	15 (7.5)	7 (11.7)	1.63 (0.63–4.20)	0.313	5 (23.8)	3.85 (1.24–12.0)	0.020
Statin¶¶	29 (14.5)	19 (31.7)	2.73 (1.40–5.35)	0.003	13 (61.9)	9.58 (3.65–25.1)	0.000
Carbasalate calcium¶¶	6 (3.0)	2 (3.3)	1.12 (0.22–5.67)	0.896	2 (9.5)	3.40 (0.64–18.0)	0.150
Acenocoumarol¶¶	6 (3.0)	7 (11.7)	4.27 (1.38–13.3)	0.012	2 (9.5)	3.40 (0.64–18.0)	0.150
Clopidogrel¶¶	2 (1.0)	3 (5.0)	5.21 (0.85–31.9)	0.074	2 (9.5)	10.4 (1.39–78.2)	0.023
Acute Q fever							
Adequate treatment##	157 (89.7)	37 (84.1)	0.61 (0.24–1.56)	0.298	12 (70.6)	0.28 (0.09–0.87)	0.028
Hospitalization	36 (18.0)	26 (35.1)	2.47 (1.36–4.49)	0.003	9 (34.6)	2.41 (0.99–5.84)	0.051

*No. (%) case patients. n indicates no. patients with information available for that category. OR, odds ratio; NS, nonsurgical; NA, not applicable; COPD, chronic obstructive pulmonary disease. An expanded version of this table that includes proven and probable chronic Q fever cases is available online (wwwnc.cdc.gov/EID/article/18/4/11-1478-T2.htm).
†OR per year of increasing age.
‡Cerebrovascular disease and transient ischemic attack.
§Case-patients: aortic valve defects, 10 (no bicuspid valves); mitral valve defects, 9 (no prolapse); tricuspid valve defects, 4. Controls: aortic valve defects, 6 (no bicuspid valves); mitral valve defects, 3 (1 prolapse).
¶n = 0 in reality.
#Angina pectoris and myocardial infarction.
**Atrial fibrillation, congestive heart failure, pericarditis, bradycardia, ischemic cardiomyopathy, and left ventricular hypertrophy.
††Prednisone cumulative dose >750 mg; use of tumor necrosis factor α–blocker, methotrexate, mycofenolate mofetil; splenectomy.
§§Asthma, recurrent pneumonia, rheumatoid arthritis.
¶¶>25% missing in case groups.
##Defined as 10–14 d of doxycycline treatment.

Table 3. Results of multivariate analyses of risk factors for development of chronic Q fever, the Netherlands*

	All chronic Q fever		Proven and probable chronic Q fever		Proven chronic Q fever	
Risk factor*†	OR (95% CI)	p value	OR (95% CI)	p value	OR (95% CI)	p value
Valvular surgery†‡	31.5 (3.99–249)	0.001	47.7 (5.87–387)	0.000	43.6 (4.70–405)	0.001
Vascular prosthesis‡§	10.4 (2.17–50.0)	0.003	14.9 (2.96–75.2)	0.001	26.8 (4.88–147)	0.000
Aneurysm§¶	8.65 (1.74–42.9)	0.008	13.5 (2.60–70.4)	0.002	25.9 (4.55–147)	0.000
Renal insufficiency¶#	–	–	9.08 (1.44–57.2)	0.019	16.0 (2.06–123)	0.008
Nonhematologic malignancy	3.90 (1.33–11.5)	0.013	–	–	–	–
Age, continuous	1.03 (1.01–1.06)#	0.005	1.06 (1.03–1.09)#	0.000	1.06 (1.02–1.11)#	0.005

*OR, odds ratio.
†Possible risk factors entered in all analyses: age, vascular prosthesis, aortic aneurysm, other vascular surgeries, peripheral arterial disease, cerebrovascular disease, valvular surgery, valvular disease (nonsurgical), ischemic cardiac disease, other cardiac history, hypertension, dyslipidemia, diabetes, nonhematologic malignancy, renal insufficiency. Immune disorder was also entered in the analyses of proven and probable chronic Q fever and of proven chronic Q fever. Pacemaker was also entered in the analysis of proven chronic Q fever.
‡Valvular surgeries in the proven group are subdivided into biological valve (n = 6), prosthetic valve (n = 3), and valve repair (n = 1) all located in the aortic valve (n = 10). Within the controls there were no patients with history of valvular surgery.
§Locations of vascular prostheses in proven group were infrarenal and iliac (n = 6), infrarenal (n = 4), thoracic (n = 2), and unknown (n = 2). Types of vascular prosthesis were Y-prosthesis (n = 7), endovascular aneurysm repair (n = 2), stent graft (n = 2), Bentall (n = 1), and unknown (n = 2). For the 2 control patients, specifications of the prostheses were unknown.
¶Locations of aneurysms in proven group were infrarenal (n = 6), infrarenal and iliac (n = 2), and suprarenal, infrarenal, and iliac (n = 1). Within the control group, aneurysms were infrarenal and iliac (n = 2).
#Observed stages of chronic kidney disease according to the Kidney Disease Outcome Quality Initiative guidelines (*28*) in the proven group were stage 3 (n = 6), stage 4 (n = 2), and stage 5 (n = 1) and in the controls solely stage 3 (n = 2).
#OR per year of increasing age.

in patients with nonsurgical cardiac valvulopathy might become evident later (*6*,*8*). Furthermore, strain-specific differences in clinical signs and symptoms might also be of importance (*26*). Presence of valvulopathy in case-patients and controls could have been missed becausethis was assessed only through review of medical records. However, echocardiography, which was standard care for all patients with suspected cases of chronic Q fever, revealed no additional congenital or bicuspid valve defects, in comparison to assessment of valvulopathy through review of medical records. From other than the above-mentioned defects, it could not be determined by these echocardiograms if these were preexisting or caused by chronic Q fever.

Immunosuppression, although not well defined, has been indicated as a risk factor in former reports, but clear definition and statistical empowerment is lacking (*8*). Although our univariate analysis did show an elevated risk for immunosuppression, especially for patients with proven chronic Q fever cases, this elevated risk was not confirmed in multivariate analysis. Immunocompromised patients may be underrepresented in our study becauseit was conducted in a peripheral hospital setting. Further evaluation of this risk factor should be performed in future studies.

Pregnancy, another formerly reported risk factor, showed an association with the development of chronic Q fever in univariate analysis. However, because there were

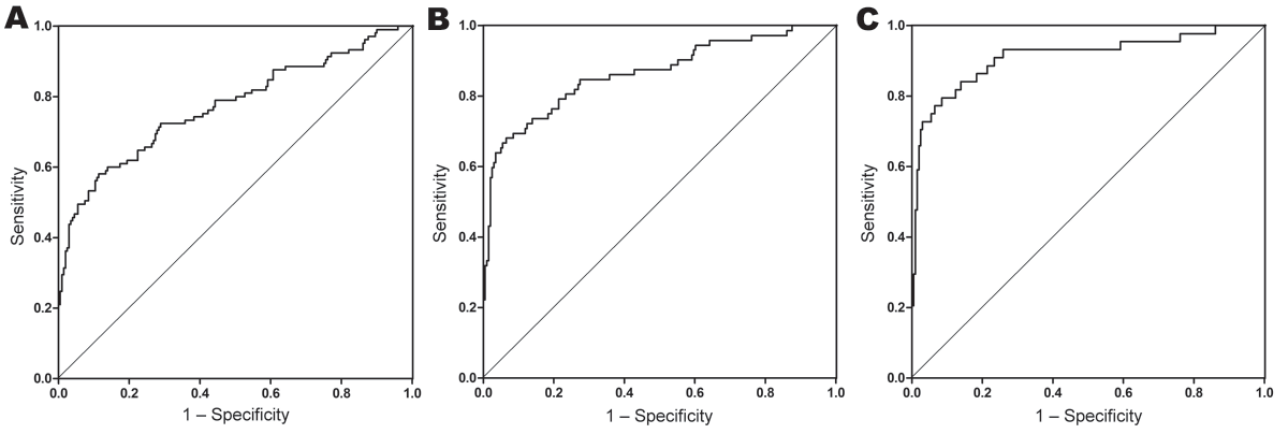


Figure 2. Goodness-of-fit models for case–control study to identify risk factors for chronic Q fever, the Netherlands. A) All chronic Q fever cases (n = 105); area under the curve (c-statistic) 0.77 (95% CI 0.71–0.83); p<0.001. B) Proven and probable chronic Q fever cases (n = 72); c-statistic 0.86 (95% CI 0.81–0.92); p<0.001. C) Proven chronic Q fever cases (n = 44); c-statistic 0.91 (95% CI 0.85–0.97); p<0.001. Patient risk factors included in the model (no. observations): A) valvular surgery (18); vascular prosthesis (15); aneurysm (12); nonhematologic malignancy (16); age, continuous, mean 63.9 y; B) valvular surgery (18); vascular prosthesis (15); aneurysm (12); renal insufficiency (12); age, continuous, mean 67.3 y; C) valvular surgery (10); vascular prosthesis (14); aneurysm (9); renal insufficiency (9); age, continuous, mean 68.4.

no pregnant women in the control group and only 3 pregnant women in all case groups, evaluation of pregnancy in multivariate analyses was not possible. A study specifically designed to evaluate associations between pregnancy and Q fever is ongoing in the Netherlands (*35*).

In our opinion, our data were representative for this large Q fever outbreak because they were well documented data and patients were willing to participate. The fact that case-patients and controls were living in the same area increases the comparability of these groups and strengthens the results. However, our study does have potential weaknesses.

First, all controls had an acute episode in 2009, but information about their signs and symptoms was obtained in 2011, introducing possible recall bias. We tried to reduce this bias by requesting additional information from the general practitioners and by reviewing clinical test results and physicians' reports in the hospital registration systems. Because information bias could also have been introduced by the subjective interpretation of physicians' reports for case-patients and controls, 2 of our researchers interpreted the results independently.

Serologic follow-up of the controls after the acute Q fever episode lasted only 1 year, which is the normal follow-up period in the Netherlands. However, because chronic Q fever can become manifest years after initial infection, development of chronic Q fever after this follow-up period is still possible (*6*,*22*). Still, 75% of chronic Q fever cases develop within 6 months after primary infection (*22*). Moreover, according to the observed decrease in antibody titers of these patients, progression to chronic Q fever is not likely. In addition, as a consequence of the inclusion of patients with symptomatic acute Q fever as a control group, the results can only be generalized to patients with symptomatic acute Q fever, although the results probably provide an adequate indication of risks factors for patients with mild or asymptomatic primary Q fever.

Notably, almost all controls received antimicrobial drug treatment at time of acute Q fever, in contrast to the case-patients, among which only a minority had symptomatic acute Q fever. Thus, antimicrobial drug treatment might influence the chance of chronic Q fever development, although there is no quantitative evidence that treatment for acute Q fever reduces the chance for chronic Q fever (*4*).

Chronic Q fever cases were selected and classified according to the definitions of the Dutch Q Fever Consensus Group (*20*), which still need confirmation. The definition of probable chronic Q fever contains several patient criteria that we also included as potential risk factors in our study (e.g., valvular disease, vascular prosthesis, aneurysm, and immunosuppressive state). Nevertheless, proven chronic Q fever, for which these criteria are not part of the definition, was also predicted with the identified risk factors in multivariate analysis, thereby confirming the independent risk association of these variables.

Some chronic Q fever cases were identified during screening programs for patients who had valvular surgery, aneurysms, or vascular prostheses. Patients with these risk factors may therefore be overrepresented within our study, although all proven case-patients had symptomatic disease.

Last, the results of this study have to be considered in view of a predominant *C. burnetii* strain that is responsible for the majority of Q fever cases in humans in the Netherlands (*36*). Worldwide, Q fever manifestations differ geographically, which might result from differences in *C. burnetii* strains (*4*).

In conclusion, previous valvular surgery, vascular prosthesis, aneurysms, renal insufficiency, and age were identified as major risk factors for the development of chronic Q fever among persons infected with *C. burnetii*. Because untreated chronic Q fever comes with serious risk for illness and death, awareness is required in people with acute Q fever possessing the identified risk factors. This may require close follow-up or even prophylactic treatment in high-risk groups. Moreover, in case of large Q fever outbreaks, screening is advisable for patients with these identified risk factors.

Dr Kampschreur is a medical doctor and PhD student at the Division of Medicine, Department of Internal Medicine and Infectious Diseases, University Medical Center Utrecht, the Netherlands. Her primary research interest is chronic Q fever in the Netherlands.

References

1. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. Lancet Infect Dis. 2005;5:219–26. http://dx.doi.org/10.1016/S1473-3099(05)70052-9
2. Parker NR, Barralet JH, Bell AM. Q fever. Lancet. 2006;367:679–88. http://dx.doi.org/10.1016/S0140-6736(06)68266-4
3. Angelakis E, Raoult D. Q fever. Vet Microbiol. 2010;140:297–309. http://dx.doi.org/10.1016/j.vetmic.2009.07.016
4. Maurin M, Raoult D. Q fever. Clin Microbiol Rev. 1999;12:518–53.
5. Tissot-Dupont H, Raoult D. Q fever. [ix.]. Infect Dis Clin North Am. 2008;22:505–14. http://dx.doi.org/10.1016/j.idc.2008.03.002
6. Fenollar F, Fournier PE, Carrieri MP, Habib G, Messana T, Raoult D. Risks factors and prevention of Q fever endocarditis. Clin Infect Dis. 2001;33:312–6. http://dx.doi.org/10.1086/321889
7. Frankel D, Richet H, Renvoise A, Raoult D. Q fever in France, 1985–2009. Emerg Infect Dis. 2011;17:350–6.
8. Raoult D, Tissot-Dupont H, Foucault C, Gouvernet J, Fournier PE, Bernit E, et al. Q fever 1985–1998. Clinical and epidemiologic features of 1,383 infections. Medicine (Baltimore). 2000;79:109–23. http://dx.doi.org/10.1097/00005792-200003000-00005
9. Botelho-Nevers E, Fournier PE, Richet H, Fenollar F, Lepidi H, Foucault C, et al. *Coxiella burnetii* infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. Eur J Clin Microbiol Infect Dis. 2007;26:635–40. http://dx.doi.org/10.1007/s10096-007-0357-6

10. Delsing CE, Kullberg BJ, Bleeker-Rovers CP. Q fever in the Netherlands from 2007 to 2010. Neth J Med. 2010;68:382–7.
11. Brouqui P, Dupont HT, Drancourt M, Berland Y, Etienne J, Leport C, et al. Chronic Q fever. Ninety-two cases from France, including 27 cases without endocarditis. Arch Intern Med. 1993;153:642–8. http://dx.doi.org/10.1001/archinte.1993.00410050074010
12. Tissot-Dupont H, Vaillant V, Rey S, Raoult D. Role of sex, age, previous valve lesion, and pregnancy in the clinical expression and outcome of Q fever after a large outbreak. Clin Infect Dis. 2007;44:232–7. http://dx.doi.org/10.1086/510389
13. van der Hoek W, Dijkstra F, Schimmer B, Schneeberger PM, Vellema P, Wijkmans C, et al. Q fever in the Netherlands: an update on the epidemiology and control measures. Euro Surveill 2010;15:pii=19520 [cited 2011 May 20]. http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19520
14. Hogema BM, Slot E, Molier M, Schneeberger PM, Hermans MH, van Hannen EJ, et al. *Coxiella burnetii* infection among blood donors during the 2009 Q-fever outbreak in the Netherlands. Transfusion. Epub 2011 Jul 14.
15. Fenollar F, Fournier PE, Raoult D. Molecular detection of *Coxiella burnetii* in the sera of patients with Q fever endocarditis or vascular infection. J Clin Microbiol. 2004;42:4919–24. http://dx.doi.org/10.1128/JCM.42.11.4919-4924.2004
16. Musso D, Raoult D. *Coxiella burnetii* blood cultures from acute and chronic Q-fever patients. J Clin Microbiol. 1995;33:3129–32.
17. Healy B, van Woerden H, Raoult D, Graves S, Pitman J, Lloyd G, et al. Chronic Q fever: different serological results in three countries—results of a follow-up study 6 years after a point source outbreak. Clin Infect Dis. 2011;52:1013–9. http://dx.doi.org/10.1093/cid/cir132
18. Dupont HT, Thirion X, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol. 1994;1:189–96.
19. Rolain JM, Lecam C, Raoult D. Simplified serological diagnosis of endocarditis due to *Coxiella burnetii* and *Bartonella.* Clin Diagn Lab Immunol. 2003;10:1147–8.
20. Wegdam-Blans MCA, Kampschreur LM, Nabuurs-Franssen MH, Renders NHM, Delsing CE, Bijlmer HA. Dutch consensus chronic Q fever [in Dutch]. Tijdschr Infect. 2011;6:71.
21. Li JS, Sexton DJ, Mick N, Nettles R, Fowler VG Jr, Ryan T, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. Clin Infect Dis. 2000;30:633–8. http://dx.doi.org/10.1086/313753
22. Landais C, Fenollar F, Thuny F, Raoult D. From acute Q fever to endocarditis: serological follow-up strategy. Clin Infect Dis. 2007;44:1337–40. http://dx.doi.org/10.1086/515401
23. Wegdam-Blans MC, Nabuurs-Franssen MN, Horrevorts AM, Peeters MF, Schneeberger PM, Bijlmer HA. Laboratory diagnosis of acute Q fever [in Dutch]. Ned Tijdschr Geneeskd. 2010;154:A2388.
24. van der Hoek W, Versteeg B, Meekelenkamp JC, Renders NH, Leenders AC, Weers-Pothoff I, et al. Follow-up of 686 patients with acute Q fever and detection of chronic infection. Clin Infect Dis. 2011;52:1431–6. http://dx.doi.org/10.1093/cid/cir234
25. Jager MM, Weers-Pothoff G, Hermans MH, Meekelenkamp JC, Schellekens JJ, Renders NH, et al. Evaluation of a diagnostic algorithm for acute Q fever in an outbreak setting. Clin Vaccine Immunol. 2011;18:963–8. http://dx.doi.org/10.1128/CVI.00009-11
26. Limonard GJ, Nabuurs-Franssen MH, Weers-Pothoff G, Wijkmans C, Besselink R, Horrevorts AM, et al. One-year follow-up of patients of the ongoing Dutch Q fever outbreak: clinical, serological and echocardiographic findings. Infection. 2010;38:471–7. http://dx.doi.org/10.1007/s15010-010-0052-x
27. Limonard GJ, Nabuurs-Franssen MH, Dekhuijzen PN, Groot CA. Prevention of Q fever endocarditis. Lancet Infect Dis. 2011;11:82–3. http://dx.doi.org/10.1016/S1473-3099(11)70016-0
28. Kidney Disease Outcome Quality Initiative clinical practice guidelines for chronic kidney disease: evaluation, classification and stratification. National Kidney Foundation. 2002 [cited 2011 June 13]. http://www.kidney.org/professionals/kdoqi/guidelines_ckd/p4_class_g1.htm
29. Betjes MG, Langerak AW, van der Spek A, de Wit EA, Litjens NH. Premature aging of circulating T cells in patients with end-stage renal disease. Kidney Int. Epub 2011 Apr 27.
30. El Nahas M. Cardio-Kidney-Damage: a unifying concept. Kidney Int. 2010;78:14–8. http://dx.doi.org/10.1038/ki.2010.123
31. Weiskopf D, Weinberger B, Grubeck-Loebenstein B. The aging of the immune system. Transpl Int. 2009;22:1041–50. http://dx.doi.org/10.1111/j.1432-2277.2009.00927.x
32. Lloyd-Jones DM, Leip EP, Larson MG, D'Agostino RB, Beiser A, Wilson PW, et al. Prediction of lifetime risk for cardiovascular disease by risk factor burden at 50 years of age. Circulation. 2006;113:791–8. http://dx.doi.org/10.1161/CIRCULATIONAHA.105.548206
33. Fenollar F, Thuny F, Xeridat B, Lepidi H, Raoult D. Endocarditis after acute Q fever in patients with previously undiagnosed valvulopathies. Clin Infect Dis. 2006;42:818–21. http://dx.doi.org/10.1086/500402
34. Million M, Thuny F, Richet H, Raoult D. Long-term outcome of Q fever endocarditis: a 26-year personal survey. Lancet Infect Dis. 2010;10:527–35. http://dx.doi.org/10.1016/S1473-3099(10)70135-3
35. Munster JM, Leenders AC, van der Hoek W, Schneeberger PM, Rietveld A, Riphagen-Dalhuisen J, et al. Cost-effectiveness of a screening strategy for Q fever among pregnant women in risk areas: a clustered randomized controlled trial. BMC Womens Health. 2010;10:32. http://dx.doi.org/10.1186/1472-6874-10-32
36. Huijsmans CJ, Schellekens JJ, Wever PC, Toman R, Savelkoul PH, Janse I, et al. Single-nucleotide-polymorphism genotyping of *Coxiella burnetii* during a Q fever outbreak in the Netherlands. Appl Environ Microbiol. 2011;77:2051–7. http://dx.doi.org/10.1128/AEM.02293-10

Address for correspondence: Linda M. Kampschreur, Division of Medicine, Department of Internal Medicine and Infectious Diseases, Room F02-107, University Medical Centre Utrecht, PO Box 85500, 3508 GA Utrecht, the Netherlands; email: l.m.kampschreur@umcutrecht.nl

Geographic Distribution of Hantaviruses Associated with Neotomine and Sigmodontine Rodents, Mexico

Mary L. Milazzo,[1] Maria N.B. Cajimat,[1] Hannah E. Romo, Jose G. Estrada-Franco, L. Ignacio Iñiguez-Dávalos, Robert D. Bradley, and Charles F. Fulhorst

To increase our knowledge of the geographic distribution of hantaviruses associated with neotomine or sigmodontine rodents in Mexico, we tested 876 cricetid rodents captured in 18 Mexican states (representing at least 44 species in the subfamily Neotominae and 10 species in the subfamily Sigmodontinae) for anti-hantavirus IgG. We found antibodies against hantavirus in 35 (4.0%) rodents. Nucleotide sequence data from 5 antibody-positive rodents indicated that Sin Nombre virus (the major cause of hantavirus pulmonary syndrome [HPS] in the United States) is enzootic in the Mexican states of Nuevo León, San Luis Potosí, Tamaulipas, and Veracruz. However, HPS has not been reported from these states, which suggests that in northeastern Mexico, HPS has been confused with other rapidly progressive, life-threatening respiratory diseases. Analyses of nucleotide sequence data from 19 other antibody-positive rodents indicated that El Moro Canyon virus and Limestone Canyon virus are geographically widely distributed in Mexico.

Hantavirus pulmonary syndrome (HPS) is a potentially fatal zoonosis caused by hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) that are principally associated with members of the rodent family Cricetidae, more specifically, members of the subfamily Neotominae or Sigmodontinae (*1*,*2*). The viruses known to cause HPS on the North American continent are Bayou virus, Black Creek Canal virus (BCCV), Choclo virus (CHOV), New York virus, and Sin Nombre virus (SNV) (*3*–*7*). Other hantaviruses that are principally associated with neotomine or North American sigmodontine rodents include Carrizal virus (CARV), Catacamas virus, El Moro Canyon virus (ELMCV), Huitzilac virus (HUIV), Limestone Canyon virus (LSCV), Montano virus (MTNV), Muleshoe virus (MULV), Playa de Oro virus, and Rio Segundo virus (RIOSV) (*8*–*14*).

Specific rodents (usually 1 or 2 closely related species) are the principal hosts of the hantaviruses, for which natural host relationships have been well characterized. The current principal host relationships of some hantaviruses seem to represent a long-term association between viruses in the genus *Hantavirus* and rodents in the family Cricetidae. Evidence for this ancient relationship includes the association of phylogenetically closely related hantavirus species with phylogenetically closely related allopatric rodent species. For example, Catacamas virus is associated with Coues's rice rat (*Oryzomys couesi*) in Honduras, and Bayou virus is associated with the marsh rice rat (*Oryzomys palustris*) in the southeastern United States (*9*,*15*).

The rodent fauna of Mexico comprises the brush mouse (*Peromyscus boylii*), the deer mouse (*P. maniculatus*), the western harvest mouse (*Reithrodontomys megalotis),* the hispid cotton rat (*Sigmodon hispidus*), the fulvous pygmy rice rat (*Oligoryzomys fulvescens*), and 122 other species in the Neotominae or Sigmodontinae (*16*).

Author affiliations: University of Texas Medical Branch, Galveston, Texas, USA (M.L. Milazzo, M.N.B. Cajimat, H.E. Romo, J.G. Estrada-Franco, C.F. Fulhorst); Universidad de Guadalajara, Autlán de Navarro, Mexico (L.I. Iñiguez-Dávalos); and Texas Tech University, Lubbock, Texas, USA (R.D. Bradley)

DOI: http://dx.doi.org/10.3201/eid1804.111028

[1]These authors contributed equally to this article.

In the southwestern United States, LSCV, SNV, ELMCV, and MULV are principally associated with rodents of the species *P. boylii*, *P. maniculatus*, *R. megalotis,* and *S. hispidus*, respectively (*10–12,17*), and that in Panama, CHOV is principally associated with *O. fulvescens* (*18*). Hypothetically, LSCV, SNV, ELMCV, and/or MULV—in association with deer mice (*Peromyscus* spp.), harvest mice (*Reithrodontomys* spp.), or cotton rats (*Sigmodon* spp.)—are widely distributed in northern Mexico, and the hantavirus assemblage of southern Mexico includes CHOV or hantaviruses that are phylogenetically closely related to CHOV.

Our knowledge of the rodent-associated hantaviruses in Mexico includes the following findings: HUIV RNA in a western harvest mouse (*R. megalotis*) captured in Morelos (*8*); CARV RNA in a Sumichrast's harvest mouse (*R. sumichrasti*) and MTNV RNA in an Orizaba deer mouse (*P. beatae*) from Guerrero (*8*); Playa de Oro virus RNA in a Mexican oryzomys (*Oryzomys mexicanus*) and Jaliscan cotton rat (*S. mascotensis*) from Colima (*13*); ELMCV RNA and SNV RNA in western harvest mice from Zacatecas (*14*); antibody against hantavirus in nimble-footed mice (*P. levipes*) captured in Tamaulipas (*19*); and antibody against hantavirus in a North American deer mouse (*P. maniculatus*), transvolcanic mice (*P. hylocetes*), black-eared mice (*P. melanotis*), and Sumichrast's harvest mouse captured in the state of Mexico (*20,21*). The purpose of this study was to extend our knowledge of the geographic distribution of hantaviruses associated with neotomine or sigmodontine rodents in Mexico.

Materials and Methods

Blood samples from 876 rodents, representing at least 44 species in the Neotominae and 10 species in the Sigmodontinae, were tested for anti-hantavirus IgG. The 876 rodents were captured during 1998–2008 at 43 localities in 18 states in Mexico (online Appendix Table 1, wwwnc.cdc.gov/EID/article/18/4/11-1028-TA1.htm). Blood samples from all of the rodents and lung samples from the antibody-positive rodents were acquired from the Natural Science Research Laboratory, Museum of Texas Tech University, Lubbock, Texas, USA.

The blood samples were tested for IgG to Caño Delgadito virus (CADV) strain VHV-574 by using an ELISA in which CADV can be highly cross-reactive with SNV, BCCV, and other North American hantaviruses (*22*). The antibody titers in the antibody-positive blood samples were recorded as 320, 1,280, or ≥5,120.

Samples of lung tissue from the antibody-positive rodents were tested for hantavirus nucleocapsid (N) protein gene RNA. Subsequently, we determined the nucleotide sequences of a 1,078-nt fragment of the glycoprotein precursor (GPC) genes of 11 of the hantaviruses associated with the antibody-positive rodents. We chose these 11 viruses to represent the geographic distribution and natural host associations of the hantaviruses in Mexico included in this study. Total RNA was isolated from 30 mg to 45 mg of lung tissue by using Tri Reagent (Sigma-Aldrich, St. Louis, MO, USA). First-strand cDNA was synthesized from small (S) segment and medium (M) segment RNA by using SuperScript II RNase H^- Reverse Transcriptase (Invitrogen Life Technologies, Inc., Carlsbad, CA, USA) in conjunction with oligonucleotide 5′-GGTGGTTGTGGTAGTAGTAGACTCC-3′ (*23*). The first-round and second-round (hemi-nested) PCR assays used the MasterTaq Kit (Eppendorf North America, Inc., Westbury, NY, USA). (The sequences of the oligonucleotides that were used to prime the PCR are available from the corresponding author.) The sizes of the N protein gene amplicons from the second-round assays ranged from 377 to 545 bp, the sizes of the GPC gene amplicons from the second-round assays ranged from 607 to 631 bp (M1 amplicon) and 571 to 618 bp (M2 amplicon), and the lengths of the overlaps between the M1 and M2 amplicons ranged from 125 to 134 bp. Together, the nucleotide sequences of the M1 and M2 amplicons encoded a 359-aa fragment of the G_C glycoprotein.

The sequences in each dataset were aligned by using the computer program ClustalW version 2.0.12 (*24*).

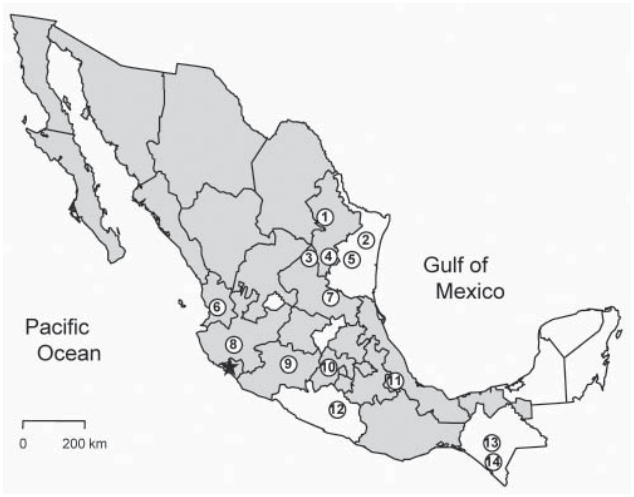


Figure 1. Municipalities in Mexico in which rodents positive for antibodies against hantaviruses were captured: 1) Municipality of Santiago, state of Nuevo León; 2) San Fernando, Tamaulipas; 3) Real de Catorce, San Luis Potosí; 4) Doctor Arroyo, Nuevo León; 5) Soto la Marina, Tamaulipas; 6) Santa María del Oro, Nayarit; 7) Ciudad del Maíz, San Luis Potosí; 8) Autlán de Navarro, Jalisco; 9) Uruapan, Michoacán; 10) Ecatepec de Morelos, México; 11) Perote, Veracruz; 12) Chilpancingo de los Bravo, Guerrero; 13) Ocozocoautla de Espinosa, Chiapas; 14) Mapastepec, Chiapas. The states shaded in gray are those in which members of *Peromyscus maniculatus* have been found (*28*). The star indicates the location in Colima at which rodents infected with Playa de Oro virus were captured in a previous study (*13*).

Sequence nonidentities were equivalent to uncorrected (p) distances. The phylogenetic analyses of nucleotide sequences were conducted with MRBAYES 3.1.2 (*25*) and programs in the computer software package PAUP* (*26*). The Bayesian analyses used the general time reversible + proportion invariant + Γ model and the following options in MRBAYES 3.1.2: two simultaneous runs of 4 Markov chains, 2 million generations, and sample frequency = every 1,000th generation. The first 1,000 trees were discarded after review of the likelihood scores, convergence statistics, and potential scale reduction factors; and a consensus tree (50% majority rule) was constructed from the remaining trees. Probability values in support of the clades were calculated a posteriori, and clades with probability values ≥0.95 were considered supported by the data (*27*).

Results

Antibody (IgG) against hantavirus was found in 35 (4.0%) of 876 rodents captured in 18 states in Mexico (online Appendix Table 1). The antibody-positive rodents were from 16 localities (online Appendix Table 2, wwwnc.cdc.gov/EID/article/18/4/11-1028-TA2.htm) in 14 municipalities in 10 states: Chiapas, Guerrero, Jalisco, México, Michoacán, Nayarit, Nuevo León, San Luis Potosí, Tamaulipas, and Veracruz (Figure 1). None of the rodents captured in Chihuahua (n = 9), Coahuila (n = 16), Guanajuato (n = 8), Oaxaca (n = 64), Puebla (n = 15), Sinaloa (n = 9), Sonora (n = 22), or Tlaxcala (n = 16) were antibody-positive to CADV strain VHV-574.

Hantavirus N protein gene RNA was detected in samples of lung from 24 (68.6%) of the 35 antibody-positive rodents (Table). The Bayesian analyses of the N protein gene sequences separated the 24 Mexican viruses in this study into 4 groups (Figure 2). Group I included CARV, group 2 included HUIV, group III included LSCV and MTNV, and group IV included SNV strains Convict Creek 74, Convict Creek 107, and NM H10.

Hantavirus GPC gene RNA was detected in each of the 11 rodents assayed for GPC gene RNA (Table). The topology of the GPC gene tree (Figure 3) was essentially identical to the topology of the N protein gene tree (Figure 2) with respect to relationships between the viruses from Mexico in this study, CARV, HUIV, MTNV, and the other hantaviruses found in North America. M0040008, CARV, M0040059, H0460041, HUIV, and ELMCV were monophyletic in the Bayesian analyses of the GPC gene sequence data (Figure 3) and N protein gene sequence data (Figure 2).

Nonidentities among the amino acid sequences of the 359-aa fragment of the G_C glycoproteins of the 11

Table. Hantaviruses found in 24 of 35 antibody-positive rodents captured in Mexico in 1998–2008, by state*†

		Rodent					GenBank accession no.	
Virus	Strain	Museum no.	Species	State	Date captured	Antibody titer	S segment	M segment
ELMCV	M0040008	TK93357	*Reithrodontomys sumichrasti*	GR (12)	2000 Jul 20	≥5,120	JN097454	JN097478
ELMCV	M0040010	TK93368	*R. sumichrasti*	GR (12)	2000 Jul 20	≥5,120	JN097455	ND
ELMCV	M0040012	TK93370	*R. sumichrasti*	GR (12)	2000 Jul 20	≥5,120	JN097456	ND
LSCV	M0040047	TK148818	*Peromyscus spicilegus*	JA (8)	2008 Jul 22	≥5,120	JN097457	ND
LSCV	M0040049	TK148820	*P. spicilegus*	JA (8)	2008 Jul 22	≥5,120	JN097458	JN097479
LSCV	B0030007	TK78282	*P. melanotis*	EM (10)	1998 Jun 24	≥5,120	JN097459	ND
LSCV	B0030008	TK78283	*P. hylocetes*	EM (10)	1998 Jun 24	≥5,120	JN097460	JN097480
LSCV	B0030015	TK78290	*P. hylocetes*	EM (10)	1998 Jun 24	≥5,120	JN097461	ND
SNV	H0030065	TK137297	*P. maniculatus*	NL (4)	2005 Aug 6	≥5,120	JN097462	JN097481
SNV	H0030067	TK137312	*P. eremicus*	NL (4)	2005 Aug 6	≥5,120	JN097463	ND
LSCV	H0460002	TK150003	*P. levipes*	NL (1)	2006 Jul 12	≥5,120	JN097464	ND
LSCV	H0460005	TK150006	*P. levipes*	NL (1)	2006 Jul 12	≥5,120	JN097465	ND
LSCV	H0460008	TK150017	*P. levipes*	NL (1)	2006 Jul 12	≥5,120	JN097466	JN097482
SNV	H0020015	TK133396	*P. maniculatus*	SL (3)	2005 Aug 4	≥5,120	JN097467	JN097483
LSCV	H0460017	TK150043	*P. ochraventer*	SL (7)	2006 Jul 13	≥5,120	JN097468	ND
LSCV	H0460023	TK150086	*P. ochraventer*	SL (7)	2006 Jul 13	≥5,120	JN097469	JN097484
SNV	H0030073	TK137359	*P. leucopus*	TM (2)	2005 Aug 8	≥5,120	JN097470	JN097485
ELMCV	M0040059	TK150090	*R. megalotis*	VZ (11)	2006 Jul 15	320	JN097471	JN097486
ELMCV	H0460026	TK150101	*R. megalotis*	VZ (11)	2006 Jul 15	≥5,120	JN097473	ND
ELMCV	H0460029	TK150117	*P. melanotis*	VZ (11)	2006 Jul 16	1,280	JN097472	ND
ELMCV	H0460032	TK150161	*R. megalotis*	VZ (11)	2006 Jul 15	320	JN097474	ND
ELMCV	H0460035	TK150163	*R. megalotis*	VZ (11)	2006 Jul 16	320	JN097475	ND
SNV	H0460038	TK150166	*P. maniculatus*	VZ (11)	2006 Jul 16	≥5,120	JN097476	JN097487
ELMCV	H0460041	TK150182	*R. megalotis*	VZ (11)	2006 Jul 16	1,280	JN097477	JN097488

*Numbers in parentheses indicate locations on the map in Figure 1. S, small; M, medium; ELMCV, El Moro Canyon virus; GR, Guerrero; ND, sequences not determined; LSCV, Limestone Canyon virus; JA, Jalisco; EM, México (state); SNV, Sin Nombre virus; NL, Nuevo León; SL, San Luis Potosí; TM, Tamaulipas; VZ, Veracruz.

†Antibody-positive, hantavirus RNA–negative rodents (species, location, antibody titer): TK78287 (*P. melanotis*, EM, 1,280); TK93383 (*P. megalops*, GR, ≥5,120); TK148439 (*Peromyscus* sp., Nayarit, 1,280); TK148793 (*Baiomys taylori*, JA, 320); TK148836 (*R. microdon*, Michoacán, 1,280); TK148842 (*R. sumichrasti*, Michoacán, 320); TK148845 (*R. sumichrasti*, Michoacán, 1,280); TK148984 (*P. leucopus*, Tamaulipas, ≥5,120); TK150045 (*P. levipes*, SL, ≥5,120); TK150515 (*B. musculus,* Chiapas, 320); and TK150518 (*Oryzomys couesi*, Chiapas, 1,280).

hantaviruses from Mexico in this study, CARV, HUIV, and MTNV ranged from 0% to 18.4% (online Appendix Table 3, wwwnc.cdc.gov/EID/article/18/4/11-1028-TA3.htm). Nonidentities between the sequences of the 359-aa fragment of the G_C glycoproteins of these 14 hantaviruses and the sequences of the homologous fragment of the G_C glycoproteins of the other hantaviruses found in North America ranged from 1.1% (H0030073 and SNV strain Blue River-Oklahoma) to 18.9% (M0040049 and BCCV strain SPB 9408076).

Discussion

The Eighth Report of the International Committee on Taxonomy of Viruses sets forth the criteria for species demarcation in the genus *Hantavirus* (*1*). One of these criteria is that strains of different species must exhibit at least a 7% difference in amino acid sequence identity in comparisons of complete N protein sequences and in comparisons of complete GPC sequences.

ELMCV was first described in 1994 (*10*); LSCV was described in 2001 (*11*); and CARV, HUIV, and MTNV were described in 2011 (*8*). In a previous study (*8*), the amino acid sequence of the N protein of MTNV was 5.8% different from the amino acid sequence of the N protein of LSCV strain 68273, and the amino acid sequence of the N protein of CARV was 3.7% different from the amino acid sequence of the N protein of ELMCV strain RM·97 and 8.4% different from the amino acid sequence of the N protein of RIOSV strain RMx·Costa·1. The amino acid sequence of the N protein of HUIV was 1.4% different from the amino acid sequence of the N protein of ELMCV strain RM·97 and 8.4% different from the amino acid sequence of the N protein of RIOSV strain RMx·Costa·1. Accordingly, MTNV should be considered a strain of LSCV, and CARV and HUIV could be considered strains of ELMCV or RIOSV. Alternatively, CARV and HUIV could be considered members of a species complex that includes ELMCV, RIOSV, and other hantaviruses that are naturally associated with harvest mice (*Reithrodontomys* spp.). There is presumptive evidence for RIOSV or hantavirus(es) that are genetically closely related to RIOSV in Sumichrast's harvest mice, a Mexican harvest mouse (*R. mexicanus*), and a Chiriqui harvest mouse (*R. creper*) captured in Panama (*29*).

Collectively, the results of the Bayesian analyses of N protein gene sequence data (Figure 2), Bayesian analyses of the GPC gene sequence data (Figure 3), and pairwise comparisons of G_C sequences (online Appendix Table 3) indicate that H0020015, H0030065, H0030073, and H0460038 are strains of SNV. The results of these analyses also indicate that M0040008, M0040059, and H0460041 are strains of ELMCV or RIOSV and that B0030008, H0460008, H0460023, and M0040049 are strains of LSCV.

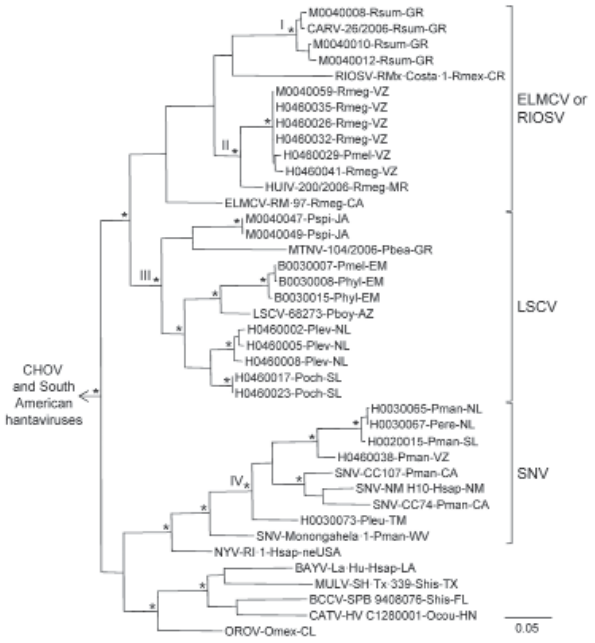


Figure 2. Results of the Bayesian analyses of the nucleotide sequences of a fragment of the nucleocapsid protein genes of the 24 hantaviruses found in Mexico in this study and 22 other hantaviruses naturally associated with members of the Neotominae or Sigmodontinae. An asterisk at a node indicates that the probability values in support of the clade were ≥0.95. Scale bar indicates substitutions per site. The Roman numerals indicate the phylogenetic groups represented by the hantaviruses from Mexico in this study. The branch labels include (in the following order) virus, strain, host species, and state or country. BAYV, Bayou virus, strain LA-Hu (GenBank accession no. L36929); BCCV, Black Creek Canal virus, SPB 9408076 (L39949); CARV, Carrizal virus, 26/2006 (AB620103); CATV, Catacamas virus, HV C1280001 (DQ256126); CHOV, Choclo virus, 588 (DQ285046); ELMCV, El Moro Canyon virus, RM-97 (U11427); HUIV, Huitzilac virus, 200/2006 (AB620106); LSCV, Limestone Canyon virus, 68273 (AF307322); MTNV, Montano virus, 104/2006 (AB620100); MULV, Muleshoe virus, SH-Tx-339 (U54575); NYV, New York virus, RI-1 (U09488); OROV, Playa de Oro virus (EF534079); RIOSV, Rio Segundo virus, RMx-Costa-1 (U18100); and SNV, Sin Nombre virus strains Convict Creek 74 (CC74), Convict Creek 107 (CC107), Monongahela-1, and NM H10 (L33816, L33683, U32591, and L25784, respectively). The viruses found in South America were Andes virus, strain Chile-9717869 (GenBank accession no. AF291702); Caño Delgadito virus, VHV-574 (DQ285566); Laguna Negra virus, 510B (AF005727); Maporal virus, HV 97021050 (AY267347); and Rio Mamoré virus, HTN-007 (FJ532244). Locations: AZ, Arizona; CA, California; CL, Colima; CR, Costa Rica; EM, México (state); FL, Florida; GR, Guerrero; HN, Honduras; JA, Jalisco; LA, Louisiana; MR, Morelos; NL, Nuevo León; NM, New Mexico; neUSA, northeastern United States (New York or Rhode Island); SL, San Luis Potosí; TM, Tamaulipas; TX, Texas; VZ, Veracruz; WV, West Virginia. Species: Hsap, *Homo sapiens*; Pbea, *Peromyscus beatae*; Pboy, *P. boylii*; Pere, *P. eremicus*; Phyl, *P. hylocetes*; Pleu, *P. leucopus*; Plev, *P. levipes*; Pman, *P. maniculatus*; Pmel, *P. melanotis*; Poch, *P. ochraventer*; Pspi, *P. spicilegus*; Ocou, *Oryzomys couesi*; Omex, *O. mexicanus*; Rmeg, *Reithrodontomys megalotis*; Rmex, *R. mexicanus*; Rsum, *R. sumichrasti*; Shis, *Sigmodon hispidus.* The designated outgroup was Andes virus strain Chile-9717869.

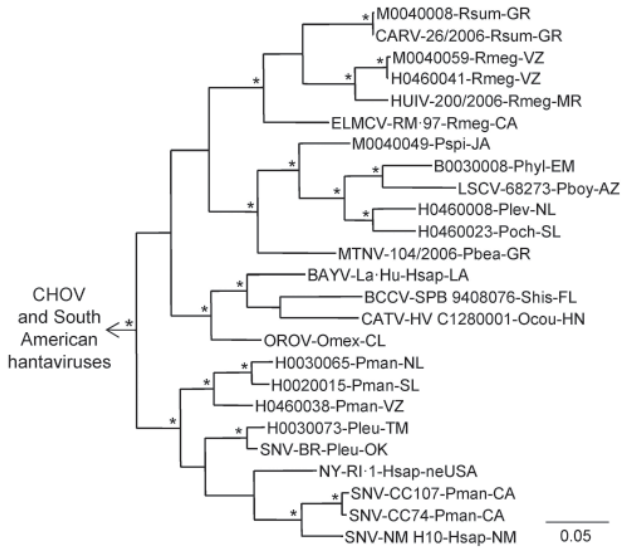


Figure 3. Results of the Bayesian analyses of the nucleotide sequences of a 1,078-nt fragment of the glycoprotein precursor genes of 11 of the 24 hantaviruses found in Mexico in this study and 20 other hantaviruses naturally associated with members of the Neotominae or Sigmodontinae. An asterisk at a node indicates that the probability values in support of the clade were ≥0.95. Scale bar indicates substitutions per site. The branch labels include (in the following order) virus, strain, host species, and state or country. BAYV, GenBank accession no. L36930; BCCV, L39950; CARV, AB620104; CATV, DQ177347; CHOV, DQ285047; ELMCV, U26828; HUIV, AB620107; LSCV, AF307323; MTNV, AB620101; NYV, U36801; OROV, EF534080; SNV—strains BR (AF030552), CC74 (L33684), CC107 (L33474), and NM H10 (L25783). The viruses from South America were Andes virus, GenBank accession no. AF291703; Caño Delgadito virus, DQ284451; Laguna Negra virus, AF005728; Maporal virus, AY363179; and Rio Mamoré virus, FJ608550. The designated outgroup was Andes virus strain Chile-9717869.

Accordingly, the N protein gene RNA–positive rodents in this study (Table) were infected with SNV, ELMCV, RIOSV, or LSCV.

Specific knowledge of the natural host relationships of LSCV previously was limited to LSCV RNA in 6 brush mice (*P. boylii*) captured in northern Arizona (*11*). The results of this study indicate that the transvolcanic mouse (*P. hylocetes*) and the black-eared mouse (*P. melanotis*) in the state of México, the nimble-footed mouse (*P. levipes*) in Nuevo León, the El Carrizo deer mouse (*P. ochraventer*) in San Luis Potosí, and the gleaning mouse (*P. spicilegus*) in Jalisco are natural but not necessarily principal hosts of LSCV.

HPS was first recognized as a distinct clinical entity in the southwestern United States in 1993 (*30*). Through 2009, a total of 510 HPS cases were reported to the National Notifiable Diseases Surveillance System or registered by the Centers for Disease Control and Prevention (*31*). Most of these cases occurred in the southwestern United States, 92 (33.7%) of 273 HPS cases that occurred in the southwestern United States before 2010 were fatal, and all of the cases from the southwestern United States through 2009 were attributed to SNV.

The results of this study indicate that SNV is widely distributed in northeastern Mexico. The geographic distribution of deer mice (*P. maniculatus*) in Mexico includes 23 states (*28*), and ≈20 million persons lived in rural areas in this 23-state region in 2010 (*32*). Yet, to our knowledge, no cases of HPS have been reported from northeastern Mexico or elsewhere in Mexico.

We hypothesize that HPS caused by SNV in Mexico has been confused with other rapidly progressive, life-threatening respiratory diseases (e.g., plague, tularemia, pneumococcal pneumonia, influenza). Alternatively, SNV in Mexico is substantially less virulent than SNV in the western United States, or human contact with SNV-infected rodents in Mexico is less frequent or less intimate than human contact with SNV-infected rodents in the western United States.

Laboratory confirmation of the diagnoses of most HPS cases in the United States before 2010 was based on the results of serologic assays in which ELMCV and LSCV can be highly cross-reactive with SNV (*31*). Thus, in all likelihood, some of the HPS cases from the western United States were actually caused by ELMCV or LSCV, and these viruses as well as SNV are etiologic agents of HPS in Mexico.

Acknowledgments

We thank Robert J. Baker for facilitating the loan of the samples from the Natural Science Research Laboratory, Museum of Texas Tech University; Eleanor R. Deardorff and Avery O. Tatters for assisting with the genetic characterization of the hantaviruses in Mexico.

This study was financially supported by National Institutes of Health grants AI-041435 and AI-067947. Christina Cassetti and Patricia Repik facilitated the grant support for this study.

Ms Milazzo is a senior research associate at the University of Texas Medical Branch, Galveston. Her scientific interests include the epidemiology and ecology of New World rodent-borne RNA viruses.

References

1. Nichol ST, Beaty BJ, Elliott RM, Goldbach R, Plyusnin A, Schmaljohn CS, et al. Family *Bunyaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. Virus taxonomy: eighth report of the International Committee on Taxonomy of Viruses. San Diego (CA): Elsevier Academic Press; 2005, p. 695–716.

2. Musser GG, Carleton MD. Family Cricetidae. In: Wilson DE, Reeder DM, editors. Mammal species of the world: a taxonomic and geographic reference, 3rd ed. Baltimore: Johns Hopkins University Press; 2005. p. 955–1189.
3. Khan AS, Spiropoulou CF, Morzunov S, Zaki SR, Kohn MA, Nawas SR, et al. Fatal illness associated with a new hantavirus in Louisiana. J Med Virol. 1995;46:281–6. http://dx.doi.org/10.1002/jmv.1890460320
4. Khan AS, Gaviria M, Rollin PE, Hlady WG, Ksiazek TG, Armstrong LR, et al. Hantavirus pulmonary syndrome in Florida: association with the newly identified Black Creek Canal virus. Am J Med. 1996;100:46–8. http://dx.doi.org/10.1016/S0002-9343(96)90010-8
5. Nelson R, Cañate R, Pascale JM, Dragoo JW, Armien B, Armien AG, et al. Confirmation of Choclo virus as the cause of hantavirus cardiopulmonary syndrome and high serum antibody prevalence in Panama. J Med Virol. 2010;82:1586–93. http://dx.doi.org/10.1002/jmv.21864
6. Hjelle B, Lee SW, Song W, Torrez-Martinez N, Song JW, Yanagihara R, et al. Molecular linkage of hantavirus pulmonary syndrome to the white-footed mouse, *Peromyscus leucopus*: genetic characterization of the M genome of New York virus. J Virol. 1995;69:8137–41.
7. Ksiazek TG, Peters CJ, Rollin PE, Zaki S, Nichol S, Spiropoulou C, et al. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. Am J Trop Med Hyg. 1995;52:117–23.
8. Kariwa H, Yoshida H, Sánchez-Hernández C, Romero-Almaraz MD, Almazán-Catalán JA, Ramos C, et al. Genetic diversity of hantaviruses in Mexico: identification of three novel hantaviruses from Neotominae rodents. Virus Res. 2011; Epub ahead of print. http://dx.doi.org/10.1016/j.virusres.2011.11.013
9. Milazzo ML, Cajimat MNB, Hanson JD, Bradley RD, Quintana M, Sherman C, et al. *Catacamas virus*, a hantaviral species naturally associated with *Oryzomys couesi* (Coues' oryzomys) in Honduras. Am J Trop Med Hyg. 2006;75:1003–10.
10. Hjelle B, Chavez-Giles F, Torrez-Martinez N, Yates T, Sarisky J, Webb J, et al. Genetic identification of a novel hantavirus of the harvest mouse *Reithrodontomys megalotis.* J Virol. 1994;68:6751–4.
11. Sanchez AJ, Abbott KD, Nichol ST. Genetic identification and characterization of Limestone Canyon virus, a unique *Peromyscus*-borne hantavirus. Virology. 2001;286:345–53. http://dx.doi.org/10.1006/viro.2001.0983
12. Rawlings JA, Torrez-Martinez N, Neill SU, Moore GM, Hicks BN, Pichuantes S, et al. Cocirculation of multiple hantaviruses in Texas, with characterization of the small (S) genome of a previously undescribed virus of cotton rats (*Sigmodon hispidus*). Am J Trop Med Hyg. 1996;55:672–9.
13. Chu Y-K, Owen RD, Sánchez-Hernández C, Romero-Almaraz ML, Jonsson CB. Genetic characterization and phylogeny of a hantavirus from western Mexico. Virus Res. 2008;131:180–8. http://dx.doi.org/10.1016/j.virusres.2007.09.007
14. Hjelle B, Anderson B, Torrez-Martinez N, Song W, Gannon WL, Yates TL. Prevalence and geographic genetic variation of hantaviruses of New World harvest mice (*Reithrodontomys*): identification of a divergent genotype from a Costa Rican *Reithrodontomys mexicanus.* Virology. 1995;207:452–9. http://dx.doi.org/10.1006/viro.1995.1104
15. Ksiazek TG, Nichol ST, Mills JN, Groves MG, Wozniak A, McAdams S, et al. Isolation, genetic diversity, and geographic distribution of *Bayou virus* (*Bunyaviridae*: *Hantavirus*). Am J Trop Med Hyg. 1997;57:445–8.
16. Ceballos G, Arroyo-Cabrales J, Medellín RA. Lista sistemática de las especies. In: Ceballos G, Oliva G, editors. Los mamíferos silvestres de México. Mexico City: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad Fondo de Cultura Económica; 2005. p. 73–95.
17. Childs JE, Ksiazek TG, Spiropoulou CF, Krebs JW, Morzunov S, Maupin GO, et al. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. J Infect Dis. 1994;169:1271–80. http://dx.doi.org/10.1093/infdis/169.6.1271
18. Vincent MJ, Quiroz E, Gracia F, Sanchez AJ, Ksiazek TG, Kitsutani PT, et al. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. Virology. 2000;277:14–9. http://dx.doi.org/10.1006/viro.2000.0563
19. Castro-Arellano I, Suzán G, León RF, Jiménez RM, Lacher TE Jr. Survey for antibody to hantaviruses in Tamaulipas, México. J Wildl Dis. 2009;45:207–12.
20. Mantooth SJ, Milazzo ML, Bradley RD, Hice CL, Ceballos G, Tesh RB, et al. Geographical distribution of rodent-associated hantaviruses in Texas. J Vector Ecol. 2001;26:7–14.
21. Suzán G, Ceballos G, Mills J, Ksiazek TG, Yates T. Serologic evidence of hantavirus infection in sigmodontine rodents in Mexico. J Wildl Dis. 2001;37:391–3.
22. Fulhorst CF, Monroe MC, Salas RA, Duno G, Utrera A, Ksiazek TG, et al. Isolation, characterization and geographic distribution of Caño Delgadito virus, a newly discovered South American hantavirus (family *Bunyaviridae*). Virus Res. 1997;51:159–71. http://dx.doi.org/10.1016/S0168-1702(97)00091-9
23. Morzunov SP, Feldmann H, Spiropoulou CF, Semenova VA, Rollin PE, Ksiazek TG, et al. A newly recognized virus associated with a fatal case of hantavirus pulmonary syndrome in Louisiana. J Virol. 1995;69:1980–3.
24. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W (1.7): improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choices. Nucleic Acids Res. 1994;22:4673–80. http://dx.doi.org/10.1093/nar/22.22.4673
25. Huelsenbeck JP, Ronquist FR. MRBAYES: Bayesian inference of phylogenic trees. Bioinformatics. 2001;17:754–5. http://dx.doi.org/10.1093/bioinformatics/17.8.754
26. Swofford DL. PAUP*: phylogenetic analysis using parsimony (* and other methods), version 4.0b10. Sunderland (MA): Sinauer Associates, Inc.; 2002.
27. Erixon P, Svennblad B, Britton T, Oxelman B. Reliability of Bayesian posterior probabilities and bootstrap frequencies in phylogenetics. Syst Biol. 2003;52:665–73. http://dx.doi.org/10.1080/10635150390235485
28. Ramírez-Pulido J, Sánchez A, Aguilera U, Castro-Campillo A. *Peromyscus maniculatus*. In: Ceballos G, Oliva G, editors. Los Mamiferos Silvestres de Mexico. Mexico City: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad Fondo de Cultura Económica; 2005, p. 748–50.
29. Salazar-Bravo J, Armién B, Suzán G, Armién A, Ruedas LA, Avila M, et al. Serosurvey of wild rodents for hantaviruses in Panama, 2000–2002. J Wildl Dis. 2004;40:103–9.
30. Duchin JS, Koster FT, Peters CJ, Simpson GL, Tempest B, Zaki SR, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. N Engl J Med. 1994;330:949–55. http://dx.doi.org/10.1056/NEJM199404073301401
31. MacNeil A, Ksiazek TG, Rollin PE. Hantavirus pulmonary syndrome, United States, 1993–2009. Emerg Infect Dis. 2011;17:1195–201. http://dx.doi.org/10.3201/eid1707.101306
32. Anuario estadístico de los Estados Unidos Mexicanos, 2010. Instituto Nacional de Estadística y Geografía [cited 2011 Jul 8]. http://www.inegi.org.mx

Address for correspondence: Charles F. Fulhorst, The University of Texas Medical Branch, 301 University Blvd, Galveston, TX 77555-0609, USA; email: cfulhors@utmb.edu

Shiga Toxin–producing *Escherichia coli* Serotype O78:H$^-$ in Family, Finland, 2009

Taru Lienemann, Eeva Salo, Ruska Rimhanen-Finne, Kai Rönnholm, Mari Taimisto, Jari J. Hirvonen, Eveliina Tarkka, Markku Kuusi, and Anja Siitonen

Shiga toxin–producing *Escherichia coli* (STEC) is a pathogen that causes gastroenteritis and bloody diarrhea but can lead to severe disease, such as hemolytic uremic syndrome (HUS). STEC serotype O78:H$^-$: is rare among humans, and infections are often asymptomatic. We detected a sorbitol-fermenting STEC O78:H$^-$*stx*$_{1c}$:*hlyA* in blood and fecal samples of a 2-week-old boy who had bacteremia and HUS and in fecal samples of his asymptomatic family members. The phenotypic and genotypic characteristics and the virulence properties of this invasive STEC were investigated. Our findings demonstrate that contrary to earlier suggestions, STEC under certain conditions can invade the human bloodstream. Moreover, this study highlights the need to implement appropriate diagnostic methods for identifying the whole spectrum of STEC strains associated with HUS.

Diarrheagenic *Escherichia coli* strains, particularly Shiga toxin–producing *E. coli* (STEC), are foodborne and waterborne pathogens that cause a wide spectrum of symptoms, ranging from mild gastroenteritis to severe diseases such as hemorrhagic colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome (HUS) (*1*). STEC has been characterized as a moderately invasive enteric pathogen because it is unable to invade the host cell cytoplasm but secretes phage-encoded Shiga toxin (Stx) that activates the signal pathway, leading to cell death and disease. Several reports have demonstrated the ability of STEC strains to invade epithelial cells in vitro, although in small numbers (*2*,*3*), but no reports of invasion in vivo have been published.

Stx plays a major role in intense inflammatory response and may explain the ability of STEC strains to cause HUS. The *stx* genes are located in a bacteriophage integrated into the bacterial genome, and the production of Stx is linked with the replication cycle of the phage (*4*). Stx has 2 major subfamilies: Stx1 and Stx2. Those producing variants Stx2a, Stx2c, and Stx2d$_{\text{activable}}$ have been associated with more severe illness and HUS, whereas the other variants were often associated with uncomplicated diarrhea and asymptomatic infections (*5*). The colonization mechanism for the cell invasion is not yet fully understood, but the bacterium is known to attach firmly to the epithelial cells through an outer membrane protein called intimin. This protein is encoded by the gene *eae* on a pathogenicity island called the locus of enterocyte effacement, and the bacterial fimbriae are presumed to be involved in the process (*6*).

HUS is characterized by acute onset of microangiopathic hemolytic anemia, renal injury, and low platelet count (*7*). It is primarily a disease of infancy and early childhood because infants and young children are more vulnerable than adults, even for low Stx concentrations; however, humans of all ages can be affected. The reported STEC infections, especially with a linkage to HUS, have been frequently caused by strains of the sorbitol-negative serotype O157:H7 (*8*). However, some sorbitol-positive strains of non–O157 STEC serotypes also cause a similar spectrum of signs and symptoms (*9*).

Infections with STEC of serotype O78:H$^-$ are rare among humans and often linked with asymptomatic infections. We describe a family cluster caused by STEC

Author affiliations: National Institute for Health and Welfare, Helsinki, Finland (T. Lienemann, R. Rimhanen-Finne, M. Kuusi, A. Siitonen); University of Helsinki, Helsinki (E. Salo, K. Rönnholm, E. Tarkka); and Vaasa Central Hospital, Vaasa, Finland (M. Taimisto, J.J. Hirvonen)

DOI: http://dx.doi.org/10.3201/eid1804.111310

serotype O78:H$^-$ associated with neonatal bacteremia and diarrheal (D+) HUS.

Methods

Case Report and Clinical Sampling

The patient, a boy born on October 3, 2009, was the third child of healthy parents. He was breast-fed and healthy. At 2 weeks of age, he became irritable, started feeding poorly, and produced large volumes of watery feces with some blood. At 17 days of age, he was taken to the Vaasa Central Hospital (Vaasa, Finland) for medical care. Blood was collected in a One BacT/Alert pediatric blood culture bottle (bioMérieux, Marcy l'Etoile, France) and incubated in the BacT/Alert automated culturing system at the clinical microbiological laboratory in Vaasa. The blood culture showed a gram-negative rod, which was identified as *E. coli*. Results of a test for the O157 antigen were negative. Because the neonate was severely ill, he was referred to the University Hospital in Pirkanmaa Hospital district, and the s *E. coli* train isolated from his blood was forwarded to the Helsinki University Hospital Laboratory, where the invasive strain from fecal specimens of the neonate and all 4 asymptomatic family members—the mother (31 years of age), father (32 years), sister (3 years), and brother (2 years)—was confirmed by detection of Stx by using the Premier EHEC EIA-test (Meridian Bioscience, Inc., Cincinnati, OH, USA). All the STEC isolates were then sent to the Bacteriology Unit (BU) of the National Institute for Health and Welfare (THL, Helsinki, Finland) for verification and more accurate phenotyping and genotyping. Fecal sampling continued until 3 consecutive STEC-negative results were obtained.

Laboratory examination indicated that the neonate had elevated levels of C-reactive protein (261 g/L [reference <3 mg/L]) and serum creatinine (246 μmol/L [reference 10–56 μmol/L). Later laboratory investigations of the neonate indicated metabolic acidosis, hyponatremia (118 mmol/L [reference 137–145 mmol/L]), and hyperkalemia (8.9 mmol/L [reference 3.3–5.2 mmol/L]). In addition, ultrasound showed enlarged kidneys. The neonate was given intravenous fluids and ceftriaxone in response to presumed sepsis. On October 27, the neonate was referred to the Department of Pediatric Nephrology and Transplantation at the University Hospital for Children and Adolescents in Helsinki for peritoneal dialysis. At admission, the neonate had a history of bloody diarrhea, STEC sepsis, thrombocytopenia, hemolytic anemia (plasma concentrations of lactate hydrogenase and hemoglobin were elevated) with fragmented erythrocytes in peripheral blood and acute uremia. Thus, D+ HUS was diagnosed. A kidney biopsy was not performed.

The neonate had low blood pressure but was anuric and overhydrated. Thus, continuous veno-venous hemodiafiltration with the support of an adrenalin infusion was started. As soon as hemodynamic and clinical conditions improved and the neonate stayed anuric, continuous veno-venous hemodiafiltration was switched to hemodialysis treatment. Because of continuously high C-reactive protein values and signs of abscess in the left kidney, a left-sided nephrectomy was performed on October 28. Histologic investigation of the kidney showed large areas of kidney necrosis, foci of abscesses, and chronic inflammation. The glomeruli of the kidneys were totally destroyed, and no typical early changes of thrombotic microangiopathy changes in HUS were seen. Peritoneal dialysis was continued because of the end-stage kidney disease but was unsuccessful because of continuous problems with hernias (2 inguinal hernia operations) and left-side pleural fluid. Thus, hemodialysis was continued, and the child was discharged on January 19, 2010, in good condition. hemodialysis treatment was performed 3×/week until April 12, 2010, when peritoneal dialysis started again in the University Hospital in Helsinki. The child had regular follow up in the outpatient clinic every 1–2 weeks in the Vaasa Central Hospital and University Hospital and every 3 months in the ward in the Hospital for Children and Adolescents in Helsinki. His nutrition was evaluated at least 1×/month by a pediatric renal nutritionist. The child's neurologic development was normal.

His father was a suitable donor, and a kidney transplantation was performed in April 2011. The operation and posttransplantation period went without complications. After the transplantation, triple immunosuppression (cyclosporine, azatioprine, and methylprednisolone) was used, and no acute rejection episodes occurred. At discharge, the glomerular filtration rate was 91 mL/min. Through the most recent follow-up, kidney function has been stable.

Phenotyping

Biochemical identification of the STEC strains was conducted by using an API 20E test strip (bioMérieux, Marcy l'Etoile, France). The ability to ferment sorbitol was additionally investigated on sorbitol-McConkey agar. By using the agar diffusion method with Müller-Hinton agar, susceptibility was tested for the following 12 antimicrobial agents: ampicillin, chloramphenicol, streptomycin, sulfonamide, tetracycline, ciprofloxacin, trimethoprim, gentamicin, nalidixic acid, cefotaxime, mecillinam, and imipenem (*10*). The production of the Stx1 and Stx2 was investigated by using a reversed passive latex agglutination kit VTEC-RPLA (Oxoid, Basingstoke, UK). Because all 6 strains were O nontypeable by the antiserum available at BU/THL, they were sent to the Statens Serum Institute

(Copenhagen, Denmark) for further serotyping. H-typing was performed at BU/THL. Strains that were not able to migrate through a Graigie tube with semisolid agar were defined as nonmotile (H⁻) (*11*).

Virulence Genes Detection by 16-Plex PCR

Sixteen-plex PCR was used to detect the genes *uidA*, *pic*, *bfpB*, *invE*, *hlyA*, *elt*, *ent*, *escV*, *eaeA*, *ipaH*, *aggR*, stx_1, stx_2, *estIa*, *estIb*, and *astA* by using the primers and PCR conditions as described (*12*). The following control strains were used: RH 4283 (E 2348/69 [(*13*]) for enteropathogenic *E. coli*, RH 4266 (ATCC 35401) for enterotoxigenic *E. coli*, RH 4270 (ATCC 43895) for STEC, RH 6647 (145–46–215, Statens Serum Institute) for enteroinvasive *E. coli*, IH 56822 (patient isolate [*14*]) for enteroaggregative *E. coli*, and RH 6715 (ATCC25922) for *E. coli* negative control.

Pulsed-field Gel Electrophoresis

Pulsed-field gel electrophoresis using *XbaI* as restriction enzyme was performed according to the PulseNet USA protocol for *E. coli* O157:H7 (*15*). The clonal similarity index of the isolates was calculated by using unweighted pair group method with arithmetic mean clustering with the BioNumerics software version 5.10 (Applied Maths, Kortrijk, Belgium).

Sequencing

For sequencing, the whole 1,470-bp fragment of the stx_1 gene (including the −10 and −35 promoter regions) from the 6 STEC strains, isolated from blood and fecal samples from the patient and fecal samples from his asymptomatic family members were amplified by using PCR primers as described (*16*). The PCR products were purified by using a MONTAGE centrifugal filter device kit (Millipore, Billerica, MA, USA). Approximately 10 ng of the PCR product was forwarded to the FIMM Technology Center sequencing laboratory (Helsinki, Finland) for sequencing by using the forward primer (5′-TCGCATGAGATCTGACC-3′) and the ABI3730xl sequencer (Applied Biosystems, Foster City, CA, USA). The sequences were analyzed by using the Bioedit program (www.mbio.ncsu.edu/BioEdit/BioEdit.html) and the homology searches by using the online National Center for Biotechnology Information GeneBlast tool (www.ncbi.nlm.nih.gov/BLAST/).

Results

The 6 strains isolated from the blood and fecal samples of the neonate and from the fecal samples of his asymptomatic parents and 2 siblings showed a sorbitol-fermenting STEC serotype O78:H⁻ that carried the virulence genes stx_1 and *hlyA* (Table). The strains produced Stx1 at a titer that varied from 16 to 32. All strains were susceptible to the 12 antimicrobial drugs tested. In addition, the strains were indistinguishable in the PFGE analysis (Figure). The stx_{1c} sequences obtained in this study were compared by using BLAST alignment with previously sequenced stx_{1c} (AJ312232), stx_{1d} (AY170851), and st_{x1} (M19473) st_{x1} subtypes. The strains in this study were found to be identical to the sequences within the hypervariable gene region of subtype stx_{1c} (AJ312232, in positions 570–598 and 600–627). In these areas, the sequence of the subtype stx_{1c} can be distinguished from the subtypes stx_1, and stx_{1d} (M19473 and AY170851). The sequences have been stored and gene accession codes obtained from the EMBL nucleotide sequence databank (Table).

Discussion

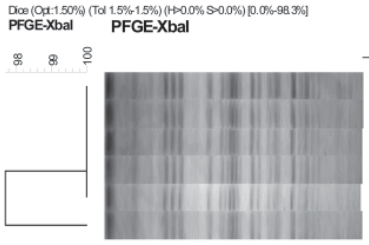
We found clinical evidence for the STEC O78:H⁻ infection leading to bacteremia and D+ HUS. The main cause for uremia in neonates with urosepsis is acute tubular necrosis. However, in this case, the signs fulfilled the criteria for D+ HUS and was associated with a family cluster of STEC O78:H⁻. D+ HUS–causing STEC strains generally are not found in blood; nondiarrheal HUS-causing STEC strains have been reported to cause bacteremic urinary tract infection (*17*).

In previous studies, some *E. coli* O78:H⁻ strains have been linked with septicemia in calves and piglets and in avian extraintestinal infections, such as respiratory infections, colicepticemia, and cellulitis (*18*,*19*). They also have been isolated from humans with extraintestinal infections, such as urinary tract infections, sepsis, and meningitis (*20*). Diarrheagenic *E. coli* strains do not usually cause extraintestinal diseases. STEC O78:H⁻ strains isolated from human gut often are linked with mild diarrhea and asymptomatic infections in humans (*21*–*25*). No invasive STEC strains of serotype O78:H⁻ have been detected (F. Scheutz, pers. comm.).

In STEC infections, the Stx variant produced by the strain is commonly the main risk factor for development

Table. Characteristics of Shiga toxin–producing *Escherichia coli* O78:H⁻:stx_{1c}:*hlyA* strains isolated from family members, Finland, 2009

	Strain characteristics, n = 6				
Family member	Strain no.	Origin	Virulence factor	Gene accession no.	Shiga toxin titer
Neonate	FE94076 and FE94084	Blood and feces	stx_{1c}, *hlyA*	FR875155 and FR875151	16
Brother	FE94098	Feces	stx_{1c}, *hlyA*	FR875153	16
Sister	FE94195	Feces	stx_{1c}, *hlyA*	FR875154	32
Mother	FE94097	Feces	stx_{1c}, *hlyA*	FR875152	16
Father	FE94099	Feces	stx_{1c}, *hlyA*	FR875150	32



Strain	Serotype	PFGE profile	Origin
FE94076	O78:H⁻	O78a	Blood, neonate
FE94084	O78:H⁻	O78a	Stool, neonate
FE94097	O78:H⁻	O78a	Stool, mother
FE94098	O78:H⁻	O78a	Stool, brother
FE94195	O78:H⁻	O78a	Stool, sister
FE94099	O78:H⁻	O78a	Stool, father

Figure. Cluster analysis of *Xba*I pulsed-field gel electrophoresis (PFGE) patterns of Shiga toxin–producing *Escherichia coli* O78:H⁻ strains isolated from blood and feces of a neonate and from feces of his asymptomatic family members, Finland, 2009. Scale bar indicates genotypic similarity of the 6 strains.

of HUS. However, the toxin itself might not be sufficient to cause HUS; other bacterial and patient factors also play a role. The isolated strains produced the toxin variant 1c, which has been linked with mild clinical signs or with asymptomatic carriage (*5*,*23*). A prominent feature of STEC carrying stx_{1c}, which was also found here, is lack of the *eaeA* gene encoding intimin, suggesting the absence of the locus of enterocyte effacement (*23*). On the basis of these findings, the invasive STEC described in this study is likely to have a variety of other still unknown critical virulence factors that affect its pathogenesis and its ability to spread into the bloodstream.

HUS develops in ≈5%–15% of patients <10 years of age in whom *E. coli* O157:H7 infection is diagnosed and occurs 2–14 days after diarrhea onset (*8*). In contrast to the O157-related HUS cases, less information is available about the non–O157-related HUS cases. Some risk factors, including an elevated leukocyte count, administration of antimicrobial drugs, use of antimotility agents, and very young age, are associated with increased risk for HUS (*8*). No specific therapy exists for STEC infections, but antimicrobial drugs, antimotility agents, opioids, and nonsteroidal anti-inflammatory drugs should not be given to acutely infected patients (*8*,*26*). On the other hand, for infants or immunodepressed patients with enteritis, particularly when bacteremia is suspected, antimicrobial drug therapy is fundamental to controlling the disease. Here, ceftriaxone, a third-generation cephalosporin, was given to the neonate. Asymptomatic carriages in some patients, mainly adults, over a 1-year period have been reported (*27*). In this study, the neonate's mother shed STEC bacteria at 21 days, his father at 141 days, his sister at 122 days, and the neonate at 117 days (there are no data regarding shedding for his brother). To prevent further bacterial shedding, probiotics such as *Lactobacillus* spp. and *Saccharomyces boulardi* were given for 1 month, but they had no effect on eliminating carriage.

Ruminants, such as cattle and sheep, are the major reservoir of STEC (*28*). None of the family members, however, had contact with any farm animals, and the family had no pets. One of the family members of the neonate might have been infected with STEC by eating contaminated food, but these food items were not available for investigation. Moreover, because all the family members were asymptomatic, estimating the exact date of their infections is difficult. Secondary infections among family members most likely resulted from person-to-person transmission or from food given to the children with contaminated hands of other family members or from some other cross-contamination. Family clusters have been reported to be common (*29*). In Finland, ≈50% of STEC infections are family related (*30*).

Handwashing practices may be of greater relevance than food as a source of infection in infants and very young children because the infection might result from an infected person or animal in the home. Prolonged excretion of STEC and intimate caring of infants by family members provide a risk for cross-infections. Therefore, to limit the risk for STEC infection, thorough handwashing before touching food or young babies is particularly necessary.

Acknowledgments

We gratefully acknowledge the personnel of the Bacteriology Unit at the National Institute for Health and Welfare, especially Tarja Heiskanen and Anna Liimatainen, for their skillful technical assistance in STEC phenotyping and genotyping. We thank Flemming Scheutz for O-serotyping the O78:H⁻ strains and for the valuable discussion about the occurrence of serotype O78:H⁻ among human isolates in Europe.

This study was supported by the Academy of Finland, ELVIRA project (project no. 117897).

Ms Lienemann is a microbiologist at the National Institute for Health and Welfare, Helsinki, Finland, and attends the Finish Graduate School on Applied Biosciences: Bioengineering, Food & Nutrition and Environment. Her research interests include molecular epidemiology of STEC and *Salmonella* infections and public health.

References

1. Karmali MA, Petric M, Lim C, Fleming PC, Arbus GS, Lior H. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. J Infect Dis. 1985;151:775–82. http://dx.doi.org/10.1093/infdis/151.5.775

2. Cantey JR, Moseley SL. HeLa cell adherence, actin aggregation, and invasion by nonenteropathogenic *Escherichia coli* possessing the *eae* gene. Infect Immun. 1991;59:3924–9.
3. Luck SN, Badea L, Bennett-Wood V, Robins-Browne R, Hartland EL. Contribution of FliC to epithelial cell invasion by enterohemorrhagic *Escherichia coli* O113:H21. Infect Immun. 2006;74:6999–7004. http://dx.doi.org/10.1128/IAI.00435-06
4. Herold S, Karch H, Schmidt H. Shiga toxin–encoding bacteriophages—genomes in motion. Int J Med Microbiol. 2004;294:115–21. http://dx.doi.org/10.1016/j.ijmm.2004.06.023
5. Zhang W, Bielaszewska M, Kuczius T, Karch H. Identification, characterization, and distribution of a Shiga toxin 1 gene variant (stx(1c)) in *Escherichia coli* strains isolated from humans. J Clin Microbiol. 2002;40:1441–6. http://dx.doi.org/10.1128/JCM.40.4.1441-1446.2002
6. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli.* Nat Rev Microbiol. 2004;2:123–40. http://dx.doi.org/10.1038/nrmicro818
7. Karmali MA. Host and pathogen determinants of verocytotoxin-producing *Escherichia coli*–associated hemolytic uremic syndrome. Kidney Int Suppl. 2009; (112):S4–7. http://dx.doi.org/10.1038/ki.2008.608
8. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin–producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet. 2005;365:1073–86. http://dx.doi.org/10.1016/S0140-6736(05)71144-2
9. Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol. 2005;295:405–18. http://dx.doi.org/10.1016/j.ijmm.2005.06.009
10. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard—eighth edition. Wayne (PA): The Institute; 2009 [cited 2011 Aug 1]. http://www.clsi.org/source/orders/free/m07-a8.pdf
11. Ratiner YA. Serotyping of *Escherichia coli* flagellar antigens [in German]. In: Stein G, Fünfstück R, editors. Harnwegsinfektionen. Aktuelle Gesichtspunkte zur Pathogenese, Diagnostik und Therapie. II Wissenschaftliches Symposium, Jena, 1989 Aug 30–Sep 1. Frankfurt am Main: pmi-Verlag GmbH; 1991. p. 47–51.
12. Antikainen J, Tarkka E, Haukka K, Siitonen A, Vaara M, Kirveskari J. New 16-plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples. [PubMed]. Eur J Clin Microbiol Infect Dis. 2009;28:899–908. http://dx.doi.org/10.1007/s10096-009-0720-x
13. Baldini MM, Kaper JB, Levine MM, Candy DC, Moon HW. Plasmid-mediated adhesion in enteropathogenic *Escherichia coli.* J Pediatr Gastroenterol Nutr. 1983;2:534–8. http://dx.doi.org/10.1097/00005176-198302030-00023
14. Keskimäki M, Mattila L, Peltola H, Siitonen A. Prevalence of diarrheagenic *Escherichia coli* in Finns with or without diarrhea during a round-the-world trip. J Clin Microbiol. 2000;38:4425–9.
15. PulseNet. One-day (24–48 h) standardized laboratory protocol for molecular subtyping of *Escherichia coli* O157:H7, non-typhoidal *Salmonella* serotypes, and *Shigella sonnei* by pulsed field gel electrophoresis (PFGE) [cited 2011 Aug 1]. http://www.cdc.gov/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf
16. Paton AW, Beutin L, Paton JC. Heterogeneity of the amino-acid sequences of *Escherichia coli* Shiga-like toxin type-I operons. Gene. 1995;153:71–4. http://dx.doi.org/10.1016/0378-1119(94)00777-P
17. Chiurchiu C, Firrincieli A, Santostefano M, Fusaroli M, Remuzzi G, Ruggenenti P. Adult nondiarrhea hemolytic uremic syndrome associated with Shiga toxin *Escherichia coli* O157:H7 bacteremia and urinary tract infection. Am J Kidney Dis. 2003;41:E4. http://dx.doi.org/10.1053/ajkd.2003.50022
18. Farooq S, Hussain I, Mir MA, Bhat MA, Wani SA. Isolation of atypical enteropathogenic *Escherichia coli* and Shiga toxin 1 and 2f-producing *Escherichia coli* from avian species in India. Lett Appl Microbiol. 2009;48:692–7.
19. Parreira VR, Gyles CL. Shiga toxin genes in avian Escherichia coli. Vet Microbiol. 2002;87:341–52. http://dx.doi.org/10.1016/S0378-1135(02)00084-6
20. Gophna U, Oelschlaeger TA, Hacker J, Ron EZ. Yersinia HPI in septicemic Escherichia coli strains isolated from diverse hosts. FEMS Microbiol Lett. 2001;196:57–60. http://dx.doi.org/10.1111/j.1574-6968.2001.tb10540.x
21. Beutin L, Zimmermann S, Gleier K. Human infections with Shiga toxin–producing Escherichia coli other than serogroup O157 in Germany. Emerg Infect Dis. 1998;4:635–9. http://dx.doi.org/10.3201/eid0404.980415
22. Chérifi A, Contrepois M, Picard B, Goullet P, Orskov I, Orskov F. Clonal relationships among Escherichia coli serogroup O78 isolates from human and animal infections. J Clin Microbiol. 1994;32:1197–202.
23. Friedrich AW, Borell J, Bielaszewska M, Fruth A, Tschäpe H, Karch H. Shiga toxin 1c–producing Escherichia coli strains: phenotypic and genetic characterization and association with human disease. J Clin Microbiol. 2003;41:2448–53. http://dx.doi.org/10.1128/JCM.41.6.2448-2453.2003
24. Shaheen HI, Khalil SB, Rao MR, Abu Elyazeed R, Wierzba TF, Peruski LF Jr, et al. Phenotypic profiles of enterotoxigenic *Escherichia coli* associated with early childhood diarrhea in rural Egypt. J Clin Microbiol. 2004;42:5588–95. http://dx.doi.org/10.1128/JCM.42.12.5588-5595.2004
25. Stephan R, Ragettli S, Untermann F. Prevalence and characteristics of verotoxin-producing *Escherichia coli* (VTEC) in stool samples from asymptomatic human carriers working in the meat processing industry in Switzerland. J Appl Microbiol. 2000;88:335–41. http://dx.doi.org/10.1046/j.1365-2672.2000.00965.x
26. Cimolai N, Morrison BJ, Carter JE. Risk factors for the central nervous system manifestations of gastroenteritis-associated hemolytic-uremic syndrome. Pediatrics. 1992;90:616–21.
27. Kuusi M, Eklund M, Siitonen A, Virkki M, Häkkinen P, Mäkelä R. Prolonged shedding of Shiga toxin–producing *Escherichia coli.* Pediatr Infect Dis J. 2007;26:279. http://dx.doi.org/10.1097/01.inf.0000256733.22690.4d
28. Pennington H. *Escherichia coli* O157. Lancet. 2010;376:1428–35. http://dx.doi.org/10.1016/S0140-6736(10)60963-4
29. Karch H, Bielaszewska M, Bitzan M, Schmidt H. Epidemiology and diagnosis of Shiga toxin–producing *Escherichia coli* infections. Diagn Microbiol Infect Dis. 1999;34:229–43. http://dx.doi.org/10.1016/S0732-8893(99)00031-0
30. Eklund M, Nuorti JP, Ruutu P, Siitonen A. Shigatoxigenic *Escherichia coli* (STEC) infections in Finland during 1998–2002: a population-based surveillance study. Epidemiol Infect. 2005;133:845–52. http://dx.doi.org/10.1017/S0950268805004450

Address for correspondence: Anja Siitonen, National Institute for Health and Welfare, Bacteriology Unit, PO Box 30, Helsinki 00271, Finland; email: anja.siitonen@thl.fi

Identification of Intermediate in Evolutionary Model of Enterohemorrhagic *Escherichia coli* O157

Christian Jenke, Shana R. Leopold, Thomas Weniger, Jörg Rothgänger, Dag Harmsen, Helge Karch, and Alexander Mellmann

Highly pathogenic enterohemorrhagic *Escherichia coli* (EHEC) O157 cause a spectrum of clinical signs that include diarrhea, bloody diarrhea, and hemolytic uremic syndrome. The current evolutionary model of EHEC O157:H7/H$^-$ consists of a stepwise evolution scenario proceeding from O55:H7 to a node (hypothetical intermediate) that then branches into sorbitol-fermenting (SF) O157:H$^-$ and non-SF (NSF) O157:H7. To identify this hypothetical intermediate, we performed single nucleotide polymorphism analysis by sequencing of 92 randomly distributed backbone genomic regions of 40 O157:H7/H$^-$ isolates. Overall, 111 single nucleotide polymorphisms were identified in 75/92 partial open reading frames after sequencing 51,041 nt/strain. The EHEC O157:H7 strain LSU-61 from deer occupied an intermediate position between O55:H7 and both O157 branches (SF and NSF O157), complementing the stepwise evolutionary model of EHEC O157:H7/H$^-$. The animal origin of this intermediate emphasizes the value of nonhuman reservoirs in the clarification of the evolution of human pathogens.

Enterohemorrhagic *Escherichia coli* (EHEC) belongs to the Shiga toxin–producing *E. coli* group and causes clinical signs ranging from watery to bloody diarrhea for most symptomatically infected patients (*1*,*2*). EHEC serotypes O157:H7 and O157:H$^-$ (nonmotile) are the most frequently isolated from patients with severe EHEC-associated diseases, such as bloody diarrhea and hemolytic uremic syndrome. Infections caused by EHEC O157:H7/H$^-$ are major public health threats and require considerable resources for control and prevention (*1*,*3*). Sorbitol-fermenting (SF) EHEC O157:H$^-$, initially found in Germany and later in other countries such as Scotland, Finland, and Australia, are increasingly associated with severe disease (*4*). These strains can ferment sorbitol after overnight incubation on sorbitol MacConkey agar, unlike non-SF (NSF) EHEC O157:H7. Today, SF EHEC O157:H$^-$ strains cause ≈20% of all hemolytic uremic syndrome cases in Germany (*4*–*8*). Classic NSF EHEC O157:H7 are of animal origin and have caused multiple outbreaks through contaminated food (*4*), but SF EHEC O157:H$^-$ are almost exclusively isolated from humans, which suggests that humans are the main reservoir (*5*).

On the basis of multilocus enzyme electrophoresis and multilocus sequence typing (MLST) data (*9*,*10*), the evolutionary model of EHEC O157 suggests that EHEC O157 emerged from *E. coli* O55:H7 by loss and acquisition of virulence and phenotypic traits (*10*). To further explain the evolution from O55:H7, a hypothetical intermediate and putatively extinct clone (missing link) SF O157:H7 emerging from O55:H7 was introduced; theoretically, it is from this intermediate that the 2 branches (NSF O157:H7 and SF O157:H$^-$) diverged (*9*,*10*).

Shaikh and Tarr subdivided NSF O157:H7 into 3 clusters (*11*); in their analysis, the SF O157:H$^-$ branch remained evolutionary conserved and clearly separated from NSF O157:H7, with additional data suggesting a hypothetical intermediate. Recent studies based on whole

Author affiliations: Institute for Hygiene and National Consulting Laboratory on Hemolytic Uremic Syndrome, Münster, Germany (C. Jenke, S.R. Leopold, H. Karch, A. Mellmann); University Hospital Münster Periodontology, Münster (T. Weniger, D. Harmsen); and Ridom GmbH, Münster (J. Rothgänger)

DOI: http://dx.doi.org/10.3201/eid1804.111414

core genome single-nucleotide polymorphisms (SNPs) enabled precise reconstruction of this model (*12*). The *E. coli* O157:H$^-$ strain LSU-61, which was isolated from a deer (*10*,*13*), had been previously discussed by Feng et al. as a potential intermediate, but that hypothesis was rejected because the strain lacked a gene encoding Shiga toxin (*stx*) and had a distinct MLST sequence type (*10*). We used an SNP-based approach to examine isolates from different sources of EHEC O157:H7/H$^-$ to further elucidate the evolutionary model of emergence of this pathogen, paying particular attention to identifying the "missing link" hypothetical intermediate.

Materials and Methods

Bacterial Strains Analyzed

Of the 50 EHEC strains examined (Table), 48 were serotype O157:H7/H$^-$ and 2 were O55:H7. Core or complete genome sequences were available for 8 O157 and 2 O55:H7 strains; these sequences served as a framework of the evolutionary model of EHEC O157. The remaining 40 strains consisted of 13 O157:H7/H$^-$ strains that represented different clusters according to previous multilocus variable-number tandem-repeat analysis (*19*); 26 O157:H7/H$^-$ strains isolated during 1987–2010 that were randomly chosen from our strain collection; and strain LSU-61, which was considered to be an intermediate (*10*).

Identification of EHEC O157 Strains

All 39 EHEC O157 isolates from our laboratory were isolated from stool samples as described (*20*,*21*). Isolates were confirmed to be *E. coli* by the API 20 Etest (bioMérieux, Marcy l'Etoile, France) and serotyped by using antiserum against *E. coli* O antigens 1–181 and H antigens 1–56 (*22*). Subtyping of *fliC* genes in nonmotile isolates by using *Hha*I restriction fragment-length polymorphism of amplicons obtained with primers FSa1 and rFSa1 (*23*,*24*) confirmed the presence of $fliC_{H7}$ in all isolates. All strains were frozen at –70°C until further use.

Isolation of DNA

A single colony from a fresh overnight culture on Columbia blood agar (Heipha, Eppelheim, Germany) was inoculated into a liquid culture of nutrient broth medium (Heipha) and incubated overnight at 37°C. The liquid culture was used to prepare DNA as described (*25*), except that phenol extraction was omitted and the corresponding supernatants were directly precipitated with isopropanol.

Cluster Classification of O157:H7 Strains

Previously determined SNP patterns T/G/T/A or G/T/C/C at Sakai genome positions 337,933 (ECs0320, putative receptor), 1,460,599 (ECs1414, curli production assembly/transport component), 2,370,797 (ECs2397, transport system permease protein), and 5,404,166 (ECs5279, *fimH*–locus) have been shown to be cluster specific (*12*). On this basis, we used Sanger sequencing to group strains into cluster 3 or cluster 1 of subgroup C. Because the prototype strain of cluster 2 shared the SNP pattern with cluster 3, strains of cluster 2 were differentiated by using the published cluster differentiation scheme based on the occupancy of *stx* integration sites and the *stx* genotype (*11*,*15*). SNP pattern T/G/T/C was declared as unknown.

MLST and Sequencing of EHEC O157 Core Genomic Loci

As a first classification, we used MLST to determine the sequence type (ST)for all prototype strains of each subgroup and cluster by sequencing internal fragments of 7 housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) (*26*). Alleles, STs, and clonal complexes were assigned in accordance with the *E. coli* MLST website (http://mlst.ucc.ie/mlst/dbs/Ecoli).

In addition to 10 SNP localizations that were known to differentiate subgroups and clusters (*12*), we randomly selected 82 additional backbone genomic regions for more in-depth SNP analysis. Using the Primer3 algorithm (http://frodo.wi.mit.edu/primer3), we developed 93 primer pairs that generated PCR products of backbone genomic regions ranging from 600 to 700 bp (online Technical Appendix Table 1, wwwnc.cdc.gov/EID/pdfs/11-1414-Techapp.pdf); for open reading frame (ORF) ECs3076, 2 separate primer pairs were designed to cover 2 described SNP localizations (*12*). EHEC O157:H7 strain Sakai served as a reference (GenBank accession no. NC_002655).

PCR was performed in a 14-μL reaction mixture containing 7 μL REDTaq (Sigma Aldrich, St. Louis, MO, USA), ≈6 ng DNA, and 1.5 μL each forward and reverse primer, with a final concentration of 10 μmol/L. The cycling reaction conditions were initial denaturation (2 min at 94°C), 35 cycles of denaturation (45 s at 94°C), annealing (60 s at 60°C), and extension (90 s at 72°C), followed by a final extension (10 min at 72°C). PCR products were purified by using the exonuclease I (New England Biolabs GmbH, Frankfurt-Hoechst, Germany) and shrimp alkaline phosphatase (USB Amersham, Freiburg, Germany) according to methods modified from (*27*). In brief, 7 μL of the PCR product was incubated simultaneously with 1.5 U of each enzyme at 37°C for 45 min, followed by enzyme heat inactivation at 80°C.

For sequencing of both strands, 2 μL of the purified amplicons was mixed with 0.5 μL premix from the ABI Prism BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) plus 1.8 μL Tris-HCl-$MgCl_2$ buffer (400 mmol/L Tris-HCl, 10 mmol/L $MgCl_2$; pH 9) and 2 μL (10 μmol/L)

from the sequencing primer (forward or reverse primer, in a total volume of 10 μL. The cycling reaction conditions were 25 cycles of denaturation (10 s at 96°C) and combined annealing and extension (4 min at 60°C). Finally, the sequencing reaction products were purified by using an alcohol precipitation method as recommended by the manufacturer and loaded onto a 3130xl Genetic Analyzer (Applied Biosystems) for capillary sequencing.

Genotypic Characterization of LSU-61

To further evaluate the genotype of LSU-61 and its potential role in the evolutionary model of EHEC O157,

Table. Fifty strains used for SNP typing of enterohemorrhagic *Escherichia coli* O157*

Strain ID	Year of isolation	Illness	SF status and serotype	Subgroup/cluster†	Reference and/or GenBank accession no.
TB182A‡	1991	D	SF O55:H7	**A**	(*12*)
CB9615‡	2003	D	SF O55:H7	A	NC_013941
493/89‡	1989	HUS	SF O157:H$^-$	**B**	(*16*)
87–14‡	1987	HUS	NSF O157:H7	**C1**	(*12*)
EC4115‡	2006	BD	NSF O157:H7	C1	NC_011353
TW14359‡	2006	BD	NSF O157:H7	**C1**	NC_013008 (*12*,*17*)
TW14588‡	2006	BD	NSF O157:H7	C3	NZ_ABKY00000000.2
86–24‡	1986	HUS	NSF O157:H7	**C2**	(*12*)
Sakai‡	1996	D	NSF O157:H7	**C3**	NC_002695 (*18*)
EDL933‡	1983	NA	NSF O157:H7	**C3**	(*14*)
LSU-61	2001	NA	SF O157:H7	Unknown intermediate	(*10*,*13*)
SNPO157_01	1987	HUS	NSF O157:H7	C1	This study
SNPO157_02	1988	D	NSF O157:H7	C1	This study
SNPO157_03	1988	HUS	SF O157:H$^-$	B	This study
SNPO157_04	1990	HUS	NSF O157:H7	C1	This study
SNPO157_05	1991	HUS	NSF O157:H$^-$	C3	This study
SNPO157_06	1992	HUS	NSF O157:H7	C3	This study
SNPO157_07	1993	HUS	NSF O157:H7	C1	This study
SNPO157_08	1995	HUS	NSF O157:H7	C1	This study
SNPO157_09	1995	HUS	SF O157:H$^-$	B	This study
SNPO157_10	1996	HUS	NSF O157:H$^-$	C1	This study
SNPO157_11	1996	HUS	SF O157:H$^-$	B	This study
SNPO157_12	1996	HUS	SF O157:H$^-$	B	This study
SNPO157_13	1996	HUS	NSF O157:H7	C1	This study
SNPO157_14	1997	HUS	NSF O157:H7	C1	This study
SNPO157_15	1997	HUS	NSF O157:H7	C1	This study
SNPO157_16	1998	HUS	NSF O157:H7	C1	This study
SNPO157_17	1999	HUS	NSF O157:H7	C1	This study
SNPO157_18	1999	HUS	NSF O157:H7	C3	This study
SNPO157_19	2000	HUS	NSF O157:H7	C1	This study
SNPO157_20	2000	D	NSF O157:H7	C3	This study
SNPO157_21	2001	HUS	SF O157:H$^-$	B	This study
SNPO157_22	2001	HUS	NSF O157:H$^-$	C3	This study
SNPO157_23	2002	D	NSF O157:H7	C3	This study
SNPO157_24	2002	A	NSF O157:H7	C1	This study
SNPO157_25	2003	HUS	NSF O157:H7	C3	This study
SNPO157_26	2004	HUS	NSF O157:H7	C1	This study
SNPO157_27	2005	HUS	NSF O157:H7	C3	This study
SNPO157_28	2005	BD	NSF O157:H7	C3	This study
SNPO157_29	2005	HUS	NSF O157:H7	C1	This study
SNPO157_30	2006	HUS	NSF O157:H7	C3	This study
SNPO157_31	2007	HUS	NSF O157:H7	C3	This study
SNPO157_32	2007	HUS	NSF O157:H7	C3	This study
SNPO157_33	2008	HUS	NSF O157:H7	C3	This study
SNPO157_34	2008	D	SF O157:H$^-$	B	This study
SNPO157_35	2008	HUS	SF O157:H$^-$	B	This study
SNPO157_36	2009	D	NSF O157:H7	§	This study
SNPO157_37	2009	HUS	SF O157:H$^-$	B	This study
SNPO157_38	2010	HUS	NSF O157:H7	C3	This study
SNPO157_39	2010	HUS	SF O157:H$^-$	B	This study

*All strains were isolated from humans except strain LSU-61, which was isolated from a deer (*10*), and EDL933, which was isolated from food (*14*). Strains isolated from humans were categorized into 3 subgroups (*11*,*12*); subgroup A represents isolates of serotype O55:H7, subgroup B SF O157:H– isolates, and subgroup C NSF O157:H7. Subgroup C is subdivided into clusters 1–3. SNP, single-nucleotide polymorphism; ID, identification; SF, sorbitol fermenting; D, diarrhea; HUS, hemolytic uremic syndrome; NSF, non-SF; BD, bloody diarrhea; NA, not applicable; A, asymptomatic.
†Subgroup and, if applicable, cluster designation based on 4 SNP loci (Sakai genome positions 337933, 1460599, 2370797, and 5404166) and the occupancy of potential *stx* integration sites in accordance with (*11*,*12*,*15*). **Boldface** indicates cluster designation of prototype strains.
‡Strains were analyzed in silico.
§SNP pattern for NSF O157:H7 grouping resulted in an unknown combination.

we investigated known *stx*-phage integration sites. We used the draft genome sequence of the O157 strain LSU-61 (GenBank accession no. AEUC00000000) (*28*). *yehV*, a known integration site of *stx1*, was screened in silico by using primer pair A/B from (*29*). For analysis of the *wrbA* locus, a site of integration of the *stx2* bacteriophage, we used primer pair C/D from (*29*). The 2 other currently known potential integration sites of *stx2*, *yecE* and *sbcB*, were screened by using primer pairs EC10/EC11, yecD-fwd/yecN-rev, and sbcB1/sbcB2 (*30*).

Data Analysis

Sequence trace files were analyzed and stored by using SeqSphere software version 0.9 beta (Ridom GmbH, Münster, Germany); a minimum-spanning tree was constructed with the integrated minimum-spanning tree algorithm. Gene functions were categorized by using the Pathosystems Resource Integration Center database (www.patricbrc.org/portal/portal/patric/Home) and corresponding Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.jp/kegg) assignments. Overall, genes were grouped into 3 functional categories: metabolism/housekeeping, putative metabolism/housekeeping, and hypothetical protein. If no KEGG phenotype assignment was found, a putative metabolism/housekeeping function was predicted on the basis of BLAST (http://blast.ncbi.mlm.nih.gov/Blast.cgi) results.

Results

Of the 48 EHEC O157 strains studied, 10 were SF serotype O157:H$^-$. Subgrouping and cluster designation of the NSF O157 strains resulted in 18 cluster 1 strains, 1 cluster 2 strain, and 17 cluster 3 strains. For 2 strains, LSU-61 and SNPO157_36, no characteristic SNP pattern was determined (Table). Further characterization by MLST of prototype strains that defined subgroups and clusters resulted in identical STs for all SF and NSF O157 (ST11) and in closely related STs of the O55:H7 strains (ST335).

The 50 strains of serotypes O157:H7/H$^-$ and O55:H7 were further characterized with respect to their SNP prevalence in the core genome. In total, 92 core genomic loci were analyzed, comprising 51,041 bp sequencing information (≈0.9% of the O157:H7 Sakai genome) (Table; online Technical Appendix Table 2). Sequencing demonstrated 111 biallelic variants, an average of 1.2 variants per sequenced locus (online Technical Appendix Table 3). Deletions or insertions were not detected.

Of the 111 SNPs, 53 (47.7%) were synonymous SNPs (sSNPs) and 58 (52.3%) were nonsynonymous SNPs (nsSNPs); 78 (70.3%) SNPs were transitions, and 33 (29.7%) were transversions. Concatenation of all loci resulted in an average of 1 SNP every 460 bp; sSNPs occurred every 963 bp, and nsSNPs every 880 bp. On the level of analyzed partial ORFs, 1 SNP was found in 45 partial ORFs, 2 SNPs in 25 partial ORFs, 3 SNPs in 4 partial ORFs, and 4 SNPs in 1 partial ORF.

To further elucidate the SNP distribution, we categorized the 92 loci into 3 functional groups. Most loci belonged to (putative) metabolism or housekeeping genes because these were chosen solely from backbone regions. If no KEGG assignment was possible, we estimated the function of the corresponding fragment on the basis of BLAST homologies. Defined annotation information regarding the function in metabolism or housekeeping was determined for 25 partial ORFs. A housekeeping/metabolism function was predicted for 58 loci. The remaining 9 loci were hypothetical proteins only (online Technical Appendix).

On the basis of the 111 SNPs, the 50 strains were clustered into 27 SNP genotypes (Figure). The 111 SNPs were able to reconstruct the well-known evolutionary model (*9*–*12*) with the stepwise evolution from O55:H7 (subgroup A) to either SF O157:H$^-$ (subgroup B) or NSF O157:H7/H$^-$ (subgroup C, clusters 1–3). Strain LSU-61 interlinked all 3 subgroups, thereby substituting for the unknown intermediate (Figure). Moreover, the applied SNP scheme differentiated the O55:H7 strains and exhibited 5 genotypes within the 10 SF O157:H$^-$ strains segregated from the SF O157:H7 strain LSU-61. The NSF O157:H7 subgroup C_1 strains comprising 18 isolates were differentiated in 9 SNP genotypes, and the subgroup C_3 strains (n = 17) exhibited 8 SNP genotypes.

To further validate the role of *stx*-negative LSU-61 as a potential intermediate, we investigated each known potential *stx* insertion site in silico to determine the presence or absence of a Shiga toxin–carrying bacteriophage. We conducted BLAST searches within the recently published draft genome sequence of LSU-61 (*28*) by using published primers for the different insertion sites (*29*,*30*). All insertion sites for *stx1* (*yehV*) and *stx*2 (*wrbA*, *yecE*, *sbcB*) were intact.

To investigate the effect of selective pressure on some loci and potential selecting biases, we analyzed sSNP and nsSNP types separately. In each scenario, the phylogenetic reconstruction resulted in comparable branching, with distinct lineages for SF and NSF O157 and strain LSU-61 as an intermediate. Only the number of SNP genotypes differed slightly: 19 sSNP genotypes (13 NSF O157:H7, 3 SF O157:H$^-$, 2 O55:H7, and LSU-61) based on the 53 sSNPs and 22 nsSNP genotypes (16 NSF O157:H7, 3 SF O157:H$^-$, 2 O55:H7, and LSU-61) based on the 58 nsSNPs. This excludes strong selection bias of the different loci.

Discussion

On the basis of SNP analysis of 92 chromosomal backbone regions of EHEC O157, we identified an SF

O157:H7 strain that complements the current model of the stepwise evolution from O55:H7 to EHEC O157 in which the hypothetical intermediate between O55:H7 and SF and NSF O157:H7/H$^-$ has been unknown (*10*,*12*). As with the highly human pathogenic O157:H7 lineage of EHEC, which is known to reside in cattle, deer, and other ruminants, this intermediate strain was isolated from a deer (*13*). These findings support previous observations (*31*,*32*) and suggest an evolution toward an animal reservoir for O157:H7 soon after O157:H$^-$ and O157:H7 divergence. Strain LSU-61 is motile (H-phase 7) and enterohemolysin active (*10*), traits that are typical for NSF O157, further suggesting the intermediate character of LSU-61 between SF and NSF O157. In contrast to the MLST scheme applied from Feng et al. (*10*) and Lacher et al. (*33*), our MLST analysis, using the scheme of Wirth et al. (*26*) that analyzes different genes, further corroborates the intermediate character of LSU-61 because it shares the same ST with SF (subgroup B prototype strain) and NSF O157 (subgroup C prototype strains).

Strain LSU-61 does not carry a *stx* gene, but this fact does not contradict our findings because these genes are encoded on bacteriophages that can be acquired and lost (*30*,*34*,*35*), and we do not have evidence of a progenitor to LSU-61 that contains *stx* genes. Although known potential *stx* phage integration sites in O157 were intact, the possibility of a previous *stx* bacteriophage carriage cannot be excluded. If the SF O157:H7 cluster emerged ≈3,000–4,000 years ago (*12*), certain genetic and phenotypic changes (*10*) occurred well before the first descendants of this cluster were isolated and characterized.

Two previous studies (*31*,*32*) reported isolated comparable strains to LSU-61 from (European) red deer, belonging to the same family (*Cervidae*) as white-tailed deer (North America), with comparable phenotypic and genotypic traits. Some of these were SF O157:H7 strains (*stx* negative or positive, β-glucuronidase positive activity) (*31*,*32*). The proof of the existence of SF O157:H7 in a ruminant (deer) host may indicate transfer into animals soon after the 2 (human pathogenic) O157 subgroups B and C emerged. On the basis of shared characteristics with both O157 branches, we suggest strain LSU-61 as a representative of the intermediate cluster complementing the stepwise evolutionary model of EHEC O157. The phylogeny based on either sSNPs or nsSNPs also resulted in a comparable phylogenetic tree with LSU-61 as a member of the progenitor node, underlining its intermediate role.

On the level of gene categories, a higher percentage of sSNPs, though fewer SNPs overall, were observed in the metabolism/housekeeping category compared with the putative metabolism/housekeeping category. The higher rate of nsSNPs in the latter category, resulting in a higher phenotypic diversity, might be explained by uncertain gene categorization because of currently limited knowledge of gene function. Therefore, SNP typing results may help to find genes involved in host–pathogen interactions rather than in metabolism or housekeeping only. SNP data for hypothetical proteins are difficult to interpret because information about their function is too imprecise to enable estimation of the effect of evolutionary pressure.

The fact that 35 of the 38 O157:H7 strains were subgrouped into either cluster 1 or 3 (17 and 18 strains, respectively) shows a certain persistence of these O157:H7 clusters (*29*), characterized by a successful pathogenicity, for example, outbreaks over a broad time frame (*4*). The preponderance of cluster 1 strains has been noted before, as have the paucity of cluster 2 and the diminished proportion of cluster 3 strains in North America (*29*). We observed a higher number of SNPs within the different NSF O157:H7 clusters compared with the few SNPs within restricted SF

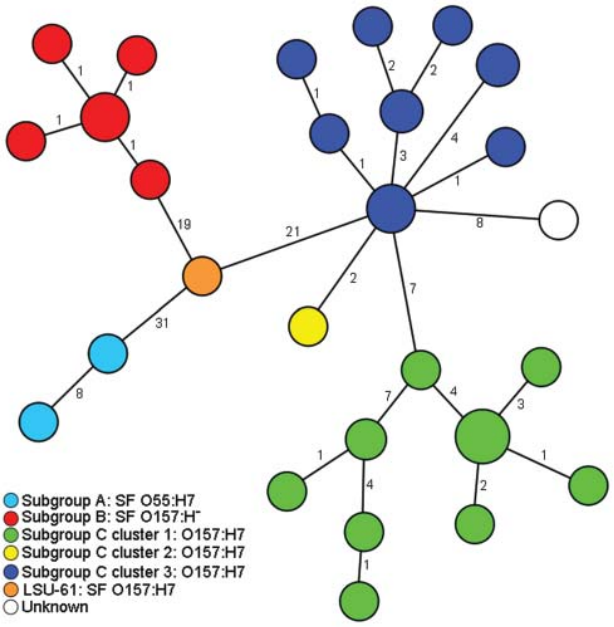


Figure. Minimum-spanning tree based on single-nucleotide polymorphism (SNP) genotypes illustrating the phylogeny of 50 enterohemorrhagic *Escherichia coli* O157:H7/H$^-$ and O55:H7 isolates and the intermediate position of strain LSU-61 during the evolution of O157. Each node represents a unique SNP genotype. The size of each node is proportional to the number of isolates per SNP genotype based on sequence analysis of 51,041 bp comprising 92 partial open reading frames. Numbers on lines between nodes represent distances between the nodes, i.e., the number of SNPs. The node size is proportional to the number of strains sharing the same genotype. Strains are colored according to their classification into subgroups and clusters based on information from Saikh and Tarr (*11*) and Leopold et al. (*12*). Strain LSU-61 represents a potential intermediate interlinking all 3 subgroups. SF, sorbitol fermenting.

O157:H$^-$ genotypes and a maximum pairwise distance of 2 SNPs (Figure). A reason for this phenomenon may be the different animal host origins for the NSF O157:H7 clade, whereas SF O157:H$^-$ are considered to have only 1 main host, humans (*5*,*19*). This high conservation was similarly recognized when multilocus variable-number tandem-repeat analysis was applied (*19*). In this context, certain SNP genotypes may serve to illuminate several strain-specific characteristics, such as increased virulence and other phenotypic traits, as other studies have similarly observed for both SF and NSF O157 (*36*,*37*).

Our results could be interpreted as if C_2 strain 86–24 is an offshoot of cluster 3, which is in contrast to the established stepwise model of O157. However, we believe that this is an artifact caused by sampling bias of the investigated 92 loci because only 11 backbone SNPs have been found to differentiate cluster 2 and 3 within the whole chromosomal backbone (*12*). One strain (SNPO157_36) did not cluster into any known O157:H7 cluster (Figure).

In summary, our identification of an intermediate member of the EHEC 1 clade complements the current evolutionary model of EHEC O157 by using chromosomal backbone SNP data of a spatiotemporally diverse strain collection. The different levels of genotypic conservation within the subgroups and the animal origin of the intermediate underline the great effect of host–pathogen interaction on the evolution of bacterial species. Future studies should focus on this interaction within both human and animal hosts to understand the evolution and persistence in nature of such human pathogens. The survival of the ancestral pathogen until today suggests that its genetic attributes could be informative in identifying fitness and potentially pathogenic loci.

Acknowledgments

We thank Peter Feng for providing strain LSU-61 for this analysis and Phillip I. Tarr for critical reading of the manuscript.

This study was supported by grants from the German Federal Ministry of Education and Research (nos. 0315219A and 01KI0801), from the EU Network ERA-NET PathoGenoMics II (no. 0315443), and from the medical faculty of the University Münster (no. BD9817044).

Dr Jenke is a scientist at the Institute for Hygiene at the University Hospital Münster. His research interests include molecular typing and the epidemiology and phylogeny of Shiga toxin–producing *E. coli*.

References

1. Holtz LR, Neill MA, Tarr PI. Acute bloody diarrhea: a medical emergency for patients of all ages. Gastroenterology. 2009;136:1887–98. http://dx.doi.org/10.1053/j.gastro.2009.02.059
2. Levine MM. *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent. J Infect Dis. 1987;155:377–89. http://dx.doi.org/10.1093/infdis/155.3.377
3. Tarr PI, Gordon CA, Chandler WL. Shiga toxin–producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet. 2005;365:1073–86. http://dx.doi.org/10.1016/S0140-6736(05)71144-2
4. Karch H, Tarr PI, Bielaszewska M. Enterohaemorrhagic *Escherichia coli* in human medicine. Int J Med Microbiol. 2005;295:405–18. http://dx.doi.org/10.1016/j.ijmm.2005.06.009
5. Karch H, Bielaszewska M. Sorbitol-fermenting Shiga toxin–producing *Escherichia coli* O157:H– strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. J Clin Microbiol. 2001;39:2043–9. http://dx.doi.org/10.1128/JCM.39.6.2043-2049.2001
6. Karch H, Mellmann A, Bielaszewska M. Epidemiology and pathogenesis of enterohaemorrhagic *Escherichia coli.* Berl Munch Tierarztl Wochenschr. 2009;122:417–24.
7. Pennington H. *Escherichia coli* O157. Lancet. 2010;376:1428–35. http://dx.doi.org/10.1016/S0140-6736(10)60963-4
8. Werber D, Bielaszewska M, Frank C, Stark K, Karch H. Watch out for the even eviler cousin—sorbitol-fermenting *E coli* O157. Lancet. 2011;377:298–9. http://dx.doi.org/10.1016/S0140-6736(11)60090-1
9. Feng P, Lampel KA, Karch H, Whittam TS. Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. J Infect Dis. 1998;177:1750–3. http://dx.doi.org/10.1086/517438
10. Feng PCH, Monday SR, Lacher DW, Allison L, Siitonen A, Keys C, et al. Genetic diversity among clonal lineages within *Escherichia coli* O157:H7 stepwise evolutionary model. Emerg Infect Dis. 2007;13:1701–6.
11. Shaikh N, Tarr PI. *Escherichia coli* O157:H7 Shiga toxin–encoding bacteriophages: integrations, excisions, truncations, and evolutionary implications. J Bacteriol. 2003;185:3596–605. http://dx.doi.org/10.1128/JB.185.12.3596-3605.2003
12. Leopold SR, Magrini V, Holt NJ, Shaikh N, Mardis ER, Cagno J, et al. A precise reconstruction of the emergence and constrained radiations of *Escherichia coli* O157 portrayed by backbone concatenomic analysis. Proc Natl Acad Sci U S A. 2009;106:8713–8.
13. Dunn JR, Keen JE, Moreland D, Alex T. Prevalence of *Escherichia coli* O157:H7 in white-tailed deer from Louisiana. J Wildl Dis. 2004;40:361–5.
14. Perna NT, Plunkett G III, Burland V, Mau B, Glasner JD, Rose DJ, et al. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. Nature. 2001;409:529–33. http://dx.doi.org/10.1038/35054089
15. Shaikh N, Holt NJ, Johnson JR, Tarr PI. Fim operon variation in the emergence of enterohemorrhagic *Escherichia coli:* an evolutionary and functional analysis. FEMS Microbiol Lett. 2007;273:58–63. http://dx.doi.org/10.1111/j.1574-6968.2007.00781.x
16. Karch H, Wiss R, Gloning H, Emmrich P, Aleksic S, Bockemühl J. Hemolytic-uremic syndrome in infants due to verotoxin-producing *Escherichia coli* [in German]. Dtsch Med Wochenschr. 1990;115:489–95. http://dx.doi.org/10.1055/s-2008-1065036
17. Kulasekara BR, Jacobs M, Zhou Y, Wu Z, Sims E, Saenphimmachak C, et al. Analysis of the genome of the *Escherichia coli* O157:H7 2006 spinach-associated outbreak isolate indicates candidate genes that may enhance virulence. Infect Immun. 2009;77:3713–21. http://dx.doi.org/10.1128/IAI.00198-09
18. Hayashi T, Makino K, Ohnishi M, Kurokawa K, Ishii K, Yokoyama K, et al. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. DNA Res. 2001;8:11–22. http://dx.doi.org/10.1093/dnares/8.1.11

19. Jenke C, Harmsen D, Weniger T, Rothgänger J, Hyytiä-Trees E, Bielaszewska M, et al. Phylogenetic analysis of enterohemorrhagic *Escherichia coli* O157, Germany, 1987–2008. Emerg Infect Dis. 2010;16:610–6.
20. Friedrich AW, Bielaszewska M, Zhang W, Pulz M, Kuczius T, Ammon A, et al. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J Infect Dis. 2002;185:74–84. http://dx.doi.org/10.1086/338115
21. Mellmann A, Bielaszewska M, Zimmerhackl LB, Prager R, Harmsen D, Tschäpe H, et al. Enterohemorrhagic *Escherichia coli* in human infection: in vivo evolution of a bacterial pathogen. Clin Infect Dis. 2005;41:785–92. http://dx.doi.org/10.1086/432722
22. Prager R, Strutz U, Fruth A, Tschäpe H. Subtyping of pathogenic *Escherichia coli* strains using flagellar (H)-antigens: serotyping versus *fliC* polymorphisms. Int J Med Microbiol. 2003;292:477–86. http://dx.doi.org/10.1078/1438-4221-00226
23. Sonntag AK, Prager R, Bielaszewska M, Zhang W, Fruth A, Tschäpe H, et al. Phenotypic and genotypic analyses of enterohemorrhagic *Escherichia coli* O145 strains from patients in Germany. J Clin Microbiol. 2004;42:954–62. http://dx.doi.org/10.1128/JCM.42.3.954-962.2004
24. Zhang Y, Laing C, Steele M, Ziebell K, Johnson R, Benson AK, et al. Genome evolution in major *Escherichia coli* O157:H7 lineages. BMC Genomics. 2007;8:121. http://dx.doi.org/10.1186/1471-2164-8-121
25. Wilson K. Preparation of genomic DNA from bacteria. Curr Protoc Mol Biol. 2001;Chapter 2:Unit 2.4.
26. Wirth T, Falush D, Lan R, Colles F, Mensa P, Wieler LH, et al. Sex and virulence in *Escherichia coli:* an evolutionary perspective. Mol Microbiol. 2006;60:1136–51. http://dx.doi.org/10.1111/j.1365-2958.2006.05172.x
27. Dugan KA, Lawrence HS, Hares DR, Fisher CL, Budowle B. An improved method for post-PCR purification for mtDNA sequence analysis. J Forensic Sci. 2002;47:811–8.
28. Rump LV, Strain EA, Cao G, Allard MW, Fischer M, Brown EW, et al. Draft genome sequences of six *Escherichia coli* isolates from the stepwise model emergence of *Escherichia coli* O157:H7. J Bacteriol. 2011;193:2058–9. http://dx.doi.org/10.1128/JB.00118-11
29. Besser TE, Shaikh N, Holt NJ, Tarr PI, Konkel ME, Malik-Kale P, et al. Greater diversity of Shiga toxin–encoding bacteriophage insertion sites among *Escherichia coli* O157:H7 isolates from cattle than in those from humans. Appl Environ Microbiol. 2007;73:671–9. http://dx.doi.org/10.1128/AEM.01035-06
30. Bielaszewska M, Prager R, Zhang W, Friedrich AW, Mellmann A, Tschäpe H, et al. Chromosomal dynamism in progeny of outbreak-related sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:NM. Appl Environ Microbiol. 2006;72:1900–9. http://dx.doi.org/10.1128/AEM.72.3.1900-1909.2006
31. García-Sánchez A, Sanchez S, Rubio R, Pereira G, Alonso JM, Hermoso de Mendoza J, et al. Presence of Shiga toxin–producing *E. coli* O157:H7 in a survey of wild artiodactyls. Vet Microbiol. 2007;121:373–7. http://dx.doi.org/10.1016/j.vetmic.2006.12.012
32. Díaz S, Vidal D, Herrera-Leon S, Sanchez S. Sorbitol-fermenting, β-glucuronidase–positive, Shiga toxin–negative *Escherichia coli* O157:H7 in free-ranging red deer in south-central Spain. Foodborne Pathog Dis. 2011;8:1313–5. http://dx.doi.org/10.1089/fpd.2011.0923
33. Lacher DW, Steinsland H, Blank TE, Donnenberg MS, Whittam TS. Molecular evolution of typical enteropathogenic *Escherichia coli*: clonal analysis by multilocus sequence typing and virulence gene allelic profiling. J Bacteriol. 2007;189:342–50. http://dx.doi.org/10.1128/JB.01472-06
34. Bielaszewska M, Köck R, Friedrich AW, von Eiff C, Zimmerhackl LB, Karch H, et al. Shiga toxin–mediated hemolytic uremic syndrome: time to change the diagnostic paradigm? PLoS ONE. 2007;2:e1024. http://dx.doi.org/10.1371/journal.pone.0001024
35. Mellmann A, Lu S, Karch H, Xu J, Harmsen D, Schmidt MA, et al. Recycling of Shiga toxin 2 genes in sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:NM. Appl Environ Microbiol. 2008;74:67–72. http://dx.doi.org/10.1128/AEM.01906-07
36. Manning SD, Motiwala AS, Springman AC, Qi W, Lacher DW, Ouellette LM, et al. Variation in virulence among clades of *Escherichia coli* O157:H7 associated with disease outbreaks. Proc Natl Acad Sci U S A. 2008;105:4868–73. http://dx.doi.org/10.1073/pnas.0710834105
37. Zhang W, Qi W, Albert TJ, Motiwala AS, Alland D, Hyytiä-Trees EK, et al. Probing genomic diversity and evolution of *Escherichia coli* O157 by single nucleotide polymorphisms. Genome Res. 2006;16:757–67. http://dx.doi.org/10.1101/gr.4759706

Address for correspondence: Alexander Mellmann, Institut für Hygiene, Universitätsklinikum Münster, Robert-KocH–Strasse 41, 48149 Münster, Germany; email: mellmann@uni-muenster.de

Emergence of Unusual G6P[6] Rotaviruses in Children, Burkina Faso, 2009–2010

Johan Nordgren,[1] Leon W. Nitiema,[1] Sumit Sharma, Djeneba Ouermi, Alfred S. Traore, Jacques Simpore, and Lennart Svensson

To obtain more information about rotavirus (ROTAV) genotypes in Burkina Faso, we characterized 100 ROTAVs isolated from fecal samples of children with acute gastroenteritis in the capital city of Ouagadougou, during December 2009–March 2010. Of note, 13% of the ROTAV-positive samples, including those with mixed infections, were positive for the unusual G6 genotype ROTAV strain. The genotypes identified were G9P[8], G6P[6], G1P[6], G3P[6], G1P[8], and G2P[4]. G9P[8] subgroup (SG) II strains dominated during the beginning of the ROTAV season, but later in the season, other G types associated with P[6] and SGI specificity emerged. This emergence was related to a shift in the overall age of infected children; ROTAV SGII infected younger children and induced more severe symptoms. The finding of a high incidence of G6P[6] strains highlights the need for long-term surveillance of ROTAV strains in Burkina Faso, especially when ROTAV vaccination is being considered in several African countries.

Rotavirus (ROTAV) is a leading cause of severe acute gastroenteritis in infants and young children (*1*). It is estimated that ROTAV is responsible for 527,000 deaths each year; most occur in children from developing countries (*2*). More than 230,000 of these deaths occur in sub-Saharan Africa, and 6 of the 7 countries for which ROTAV diarrhea–related mortality rates are highest are located in Africa (*2*).

The viral protein (VP) 7 and VP4 genes encoding the 2 surface proteins form the basis of the current genotypic characterization of group A ROTAVs into G and P genotypes, respectively. At least 27 G genotypes and 35 P genotypes have been identified (*3*). Of these, 6 ROTAV G-P combinations (G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and G9P[6]) are common worldwide (*4*–*6*); several other combinations occur at low frequencies, and a few others occur mainly in animals (*7*,*8*). On the basis of molecular and immunological analysis, the VP6 gene has been classified into 2 subgroups (SGI and SGII); SGII is the most prevalent among humans (*9*,*10*) and SGI is more commonly found in animals (*11*).

The G-P combinations circulating in Africa often differ from those circulating in other parts of the world (*12*). A review of studies in Africa published during 1997–2006 showed that the most common G and P type combinations were G1P[8] (17.4%), G2P[6] (9.6%), G8P[6] (9.4%), and G3P[8] (7%) (*13*). A study from West Africa, covering 1996–2000, found that G1P[6] was the most common G-P combination, followed by G2P[6] and G9P[8] (*12*). During 1998–2000, the G9P[8] combination was the most prevalent genotype in northern Ghana (*14*). However, many ROTAV strains isolated in Africa still remain to be characterized for G and/or P type (*13*).

Although bovine G6 ROTAV strains are the predominant genotype in cattle in Africa, G6 infection in humans has rarely been described; however, sporadic cases of G6 in humans in combination with P[14] and P[9] have been described (*15*–*21*). P[6] is a commonly observed P type, especially in Africa. However, to our knowledge, the literature reports only once the isolation of strain G6P[6] in Belgium, from a child returning from a trip to Mali (*22*).

In Burkina Faso, previous studies have shown ROTAV to be the main cause of diarrhea in children (*23*–*26*), but limited molecular information exists about the circulating genotypes. The first molecular characterization

Author affiliations: Linköping University, Linköping, Sweden (J. Nordgren, S. Sharma, L. Svensson); and Université de Ouagadougou, Ouagadougou, Burkina Faso (L.W. Nitiema, D. Ouermi, A.S. Traore, J. Simpore)

DOI: http://dx.doi.org/10.3201/eid1804.110973

[1]These authors contributed equally to this article.

of ROTAVs, which was conducted on samples from 1999, showed a predominance of unusual G2P[6]SGI strains (*27*).

In this study, we determined the G and P genotypes of ROTAVs isolated from children with acute gastroenteritis in Ouagadougou, the capital of Burkina Faso, during the cold, dry season of December 2009–March 2010. We found that G6P[6] ROTAV strains were the second most common genotype circulating; incidence was 13%. We discuss the emergence of this most unusual genotype in association with vaccine efficacy and zoonotic transfer.

Materials and Methods

Study Population and Specimens

Fecal specimens were collected from children <5 years of age who sought medical care for acute diarrheal illness, defined as ≥3 liquid stools over a 24-h period. Stool specimens were collected in sterile containers at the microbiology laboratory in Saint Camille Medical Center, in Ouagadougou, Burkina Faso. A 10% (wt/vol) stool suspension was prepared, and 3 aliquots were frozen at –20°C for additional analysis. All ROTAV-positive fecal specimens collected during the ROTAV season (December 2009–March 2010) at the Saint Camille Medical Center and the Biomolecular Research Center Pietro Annigoni, were analyzed. The samples were part of a larger epidemiologic study conducted during May 2009–March 2010 to identify the enteropathogens causing gastroenteritis in children (*26*).

Clinical Assessment

Clinical information was obtained by reviewing the clinical records of case-patients, as described (*26*). In brief, information was obtained regarding age, sex, place of residence, ethnicity, signs and symptoms and their duration (e.g., fever [temperature ≥38°C], nausea, vomiting, loss of appetite, and number of loose stools during the past 24 h), hydration and nutrition status, and whether the children had been given antimicrobial and antiparasitic drugs. All children were clinically evaluated by general practitioners in accordance with a local adaption of the World Health Organization strategy for diarrheal management (*28*). Dehydration was classified as follows, according to the World Health Organization guidelines: severe dehydration, some dehydration, or no dehydration.

Rotavirus Antigen Detection

We used the ProSpect Rotavirus R240396 (Oxoid, Kamstrupvej, Denmark) enzyme immunoassay kit, according to the manufacturer's instructions, to detect group A human ROTAV in fecal specimens. The results were determined visually and confirmed by absorbance readings.

Viral RNA Extraction

Viral RNA was extracted from 10% stool suspensions by using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. A total of 60 μL of viral RNA was collected and stored at –70°C until further use.

Reverse Transcription

Reverse transcription (RT) was performed essentially as described (*29*). Twenty-eight μL of dsRNA was mixed with 2.5 μg of random hexadeoxynucleotides ($pd[N]_6$ primer; GE Healthcare, Uppsala, Sweden), denatured at 97°C for 5 min, and chilled on ice for 2 min. The suspension was then added to 1 RT-PCR bead (GE Healthcare) with RNase-free water to a final volume of 50 μL. The RT reaction was performed for 30 min at 42°C for cDNA synthesis.

Quantification and VP6 Subgrouping by Real-time PCR

All ROTAV-positive specimens were quantified and subgrouped by using the LUX (light-upon-extension) real-time quantitative PCR (qPCR) as described (*30*). This qPCR uses labeled primers with different fluorophores for each VP6 subgroup, and external plasmid standards were used for quantification (*30*). The quantified ROTAV was detected in a range from $\approx 5.0 \times 10^4$ to 7×10^{11} gene equivalents per gram of feces; median concentration was 7.8×10^9.

G and P Typing

G and P genotyping for ROTAV was performed by using seminested type-specific multiplex PCRs that were able to detect 7 G types (G1, G2, G3, G4, G8, G9, G10) and 6 P types (P[4], P[6], P[8], P[9], P[10], P[11]) (*31*–*33*). The PCR products were later visually examined on a 2% agarose gel stained with ethidium bromide and observed in ultraviolet light. The G and P types were determined by the specific sizes of the amplicons on agarose gels.

Sequencing of Untyped Strains and Molecular Characterization of G6P[6] Strains

Sequencing of the PCR amplicons for the VP7 gene of unknown G types was conducted with primers VP7-F and VP7-R (*33*). All G6P[6] strains were further sequenced for VP4 and VP6 genes by using primers Con-2/Con-3 (*32*) and GEN_VP6F/GEN_VP6R (*7*), respectively. Furthermore, to understand the evolution of these G6P[6] ROTAV strains, we randomly selected 4 strains and sequenced the nonstructural protein (NSP) genes (NSP1, NSP2, NSP3, NSP4, and NSP5) by using primers GEN_NSP1F/GEN_NSP1R, MAX-NSP2F/ MAX-NSP2R, MAX-NSP3F/ MAX-NSP3R, GEN_NSP4F/GEN_NSP4R, and MAX-11F/ MAX-11R, respectively (*7*). The following thermal

cycling conditions were used: an initial denaturation step at 94°C for 5 min followed by 40 cycles of amplification (30 s at 94°C, 30 s at 50°C, and 1 min 30 s at 72°C), with a final extension of 7 min at 72°C.

Nucleotide Sequencing

Nucleotide sequencing was performed by Macrogen Inc. (Seoul, South Korea). The sequencing reaction was based on BigDye chemistry, and the sequencing primers were the same primers as those used in the PCR.

Sequence Analysis of Mixed Infections

All chromatograms were visually inspected. Those containing multiple ambiguous base positions were considered to potentially represent multiple ROTAV strains and were analyzed by using the RipSeq mixed DNA interpretation software (iSentio Ltd., Bergen, Norway) (*34*).

Sequence Analysis

Multiple sequence alignment of the obtained nucleotide sequences was performed by using the ClustalW algorithm (www.ebi.ac.uk/Tools/msa/clustalw2/) with default parameters on the European Bioinformatics Institute server. Phylogenetic analysis of the aligned file was performed by using MEGA5 (www.megasoftware.net) with the neighbor-joining method. Phylogenetic distances were measured by using the Kimura 2-parameter model. The statistical significance of the phylogenetic tree was supported by bootstrapping with 1,000 replicates. The sequenced ROTAV strains and GenBank accession numbers are shown in online Technical Appendix Table 1 (wwwnc.cdc.gov/EID/pdfs/11-0973-Techapp.pdf); the nucleotide sequences for sequenced genes can be found in GenBank by using accession nos. JN116505-JN116556 and JQ255029-JQ255033.

Statistical Analysis

Categorical data were analyzed by using the χ^2 test or Fisher exact test with 2-tailed significance. Interval data were analyzed by running an independent *t*-test with 2-tailed significance in SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

Results

High incidence of the Unusual G6P[6] Genotype

The distribution of G and P types is shown in Table 1. We sequenced all unknown G types for which the VP7 amplicon could be generated and found that they belong to genotype G6 with P[6] specificity. Of the 100 ROTAV-positive specimens, 13 contained G6P[6], including 2 with mixed infections with other G types. We detected 5 G types: G1 (23% incidence rate), G2 (2%), G3 (9%), G6 (13%), and G9 (53%). Among the P genotypes, P[8] was most prevalent (61%), followed by P[6] (39%); P[4] was rarely detected (2%). The G-P combinations detected were G9P[8] (48%), G6P[6] (11%), G1P[6] (11%), G3P[6] (8%), G1P[8] (5%), and G2P[4] (2%). Mixed G or P type infections were found in 9% of the total specimens. Among the ROTAV-positive specimens, 6% and 1% could not be assigned a G and P type, respectively (Table 1).

Seasonal Distribution of Circulating ROTAV Strains

In the first part of the ROTAV season (December 2009 and early January 2010), we observed a dominance of G9P[8] strains (Figure 1, panel A). Thereafter, the G9P[8] strains completely disappeared and other G types began to circulate (Figure 1, panel A). All G types observed during this period were associated with P[6] genotype. Moreover, at the end of the ROTAV season, the G6P[6] and G3P[6] strains emerged, and most of the mixed infections were detected during this period. Furthermore, we observed a temporal shift in subgroup specificity (Figure 1, panel B). We subgrouped the VP6 gene by using our qPCR assay, and of the 100 ROTAV-positive specimens, 39 belonged to SGI, 59 belonged to SGII, and 2 specimens contained a mix of both subgroups. In the middle of the ROTAV season, we observed a shift of subgroups, with SGI dominating toward the end of the ROTAV season and SGII dominating in the beginning (Figure 1, panel B).

Table 1. Distribution of G and P types of rotavirus strains detected among children with gastroenteritis, Ouagadougou, Burkina Faso, December 2009–March 2010

	No. (%) strains					
Rotavirus type	P[4]	P[6]	P[8]	P[mix]*	P[nt]	Total
G1	0	11	5	2	0	18 (18)
G2	2	0	0	0	0	2 (2)
G3	0	8	0	0	0	8 (8)
G6	0	11	0	0	0	11 (11)
G9	0	0	48	0	1	49 (49)
Gmix†	0	3	3	0	0	6 (6)
Gnt‡	0	3	2	1	0	6 (6)
Total	2 (2)	36 (36)	58 (58)	3 (3)	1 (1)	100 (100)

*P[6]/P[8].
†G1/G9P[6], G1/G6P[6], and G3/G6P[6] specificity was detected in 1 specimen each; 3 samples were found to be G1/G9P[8] specific.
‡Nontypeable.

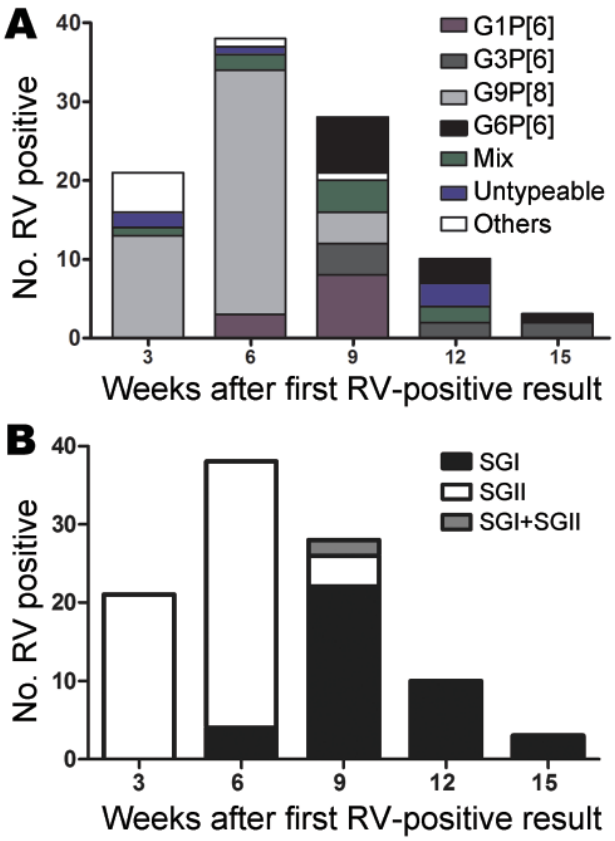


Figure 1. Temporal changes of circulating rotaviruses (ROTAVs) in Burkina Faso during December 2009–March 2010. A) G and P types, with "Others" representing G1P[8] and G2P[4]. B) VP6 subgroups. RV, rotavirus.

SGII ROTAVs More Prevalent in Younger Children and Associated with More Severe Symptoms

All P[8] ROTAVs were associated with VP6 SGII, and all P[6] ROTAVs were associated with VP6 SGI. Children infected with ROTAV SGII had a mean age of 11.8 months (median 11.1 months), compared with a mean age of 15.0 months (median 13.0 months) for children infected with ROTAV SGI ($p < 0.05$). These observations correlate with the shift of VP6 subgroups and P types in the middle of the ROTAV season; children infected later were older than those who were infected early (data not shown). We compared clinical signs and symptoms and found no significant differences, but we did find higher prevalence of vomiting ($p = 0.33$), fever ($p = 0.052$), and severe dehydration ($p = 0.09$) among SGII-infected children (Table 2). We compared the 3 most prevalent G, P, and VP6 constellations (Table 2) and found no significant differences in age profiles; however, we found that ROTAVs of the G9P[8]SGII constellation were more frequent than G1P[6]SGI- and G6P[6]SGI-specific strains among younger children ($p = 0.069$). We also found that G9P[8]SGII infections, compared with SGI-associated G and P type infections, induced significantly more cases of severe dehydration ($p<0.05$). The ROTAV viral load, quantified by using the LUX qPCR, did not correlate with disease severity (data not shown).

Nonspecific Binding of the G9 Genotype Primer to a New Sublineage of G3 Strains

At first, we observed a high number of G3/G9P[6] mixed infections as revealed by multiple bands in the multiplex PCR (data not shown). We sequenced the VP7 gene of these strains (n = 6) and found all 6 to have high nucleotide homology with G3 ROTAVs and to cluster together in a new sublineage (Figure 2, panel A). This sublineage had 96.0%–96.3% nt homology to the most similar G3 strain found in GenBank (Bethesda/DC1730). Furthermore, the G9 primer used in this study (*33*) was highly homologous (16 [80%] of 20 nt) to these G3 strains but less so to other G3 strains available in GenBank (maximum 14 [70%] of 20 nt) (Figure 2, panel B). Furthermore, the 14 bases from the 3′ end of the primer showed a complete match to the G3 strains from Burkina Faso, except at position 9, thereby facilitating unspecific primer annealing and polymerase extension. The VP7 gene of 6 G9P[8] samples was sequenced for verification which proved them to be G9. These findings led us to conclude that G9 ROTAVs appearing as mixed infections with G3P[6], as determined by multiplex G-typing PCR, were nonspecific amplifications resulting in G9 artifact.

Table 2. Clinical differences between subgroup SGI and SGII rotaviruses and the 3 most common G, P, and VP6 type constellations in Burkina Faso, December 2009–March 2010

		Patient characteristics and clinical signs							
	No.	Age		Vomiting		Fever†		Severe dehydration	
Isolate type	specimens*	Mo ± SD	p value	No. (%)	p value	No. (%)	p value	No. (%)	p value
SGI	39	15.0 ± 8.5		30 (77)		14 (36)		5 (13)	
SGII	59	11.8 ± 5.0	0.038‡	50 (85)	0.33‡	33 (56)	0.052‡	16 (27)	0.09‡
G1P[6]SGI	11	15.5 ± 11.0		8 (73)		4 (36)		0 (0)	
G6P[6]SGI	11	16.6 ± 10.2		9 (82)		4 (36)		2 (18)	
G9P[8]SGII	48	11.6 ± 4.6	0.069§	43 (90)	0.320§	24 (50)	0.29§	16 (33)	0.03§

*Two samples positive for both SGI and SGII were excluded.
†Temperature >38°C.
‡SGI versus SGII.
§G9P[8]SGII versus G1P[6]SGI and G6P[6]SGI.

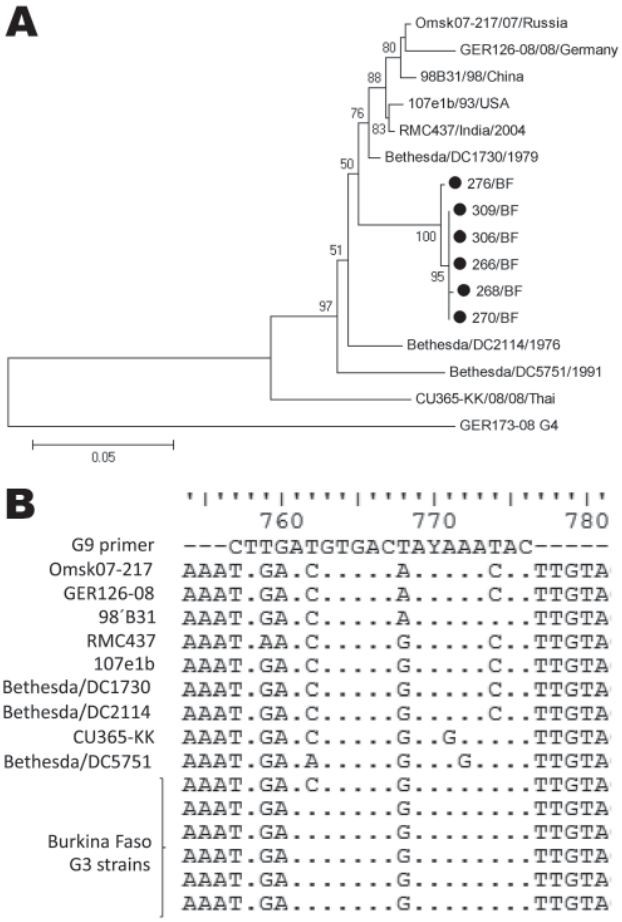


Figure 2. A) Phylogenetic analysis of partial sequences of the viral protein (VP) 7 genes (nt 115–851) for G3 rotavirus strains isolated in Burkina Faso during December 2009–March 2010, with a G4 strain as an outgroup. Scale bar represents number of substitutions per site. Bootstrap values are shown at the branch points (values of <50% are not shown). B) The G9 primer used is homologous at the 3× end to the VP7 gene of the G3 strains in Burkina Faso during December 2009–March 2010. The reference G3 strains were deliberately selected for similarity to the Burkina Faso G3 strains.

Molecular Characterization of the Rare G6P[6] Strains

We further characterized the unusual G6P[6] strains (n = 11) by sequencing the VP7, VP4, VP6, and NSP1–5 genes. All characterized VP4 and VP7 sequences clustered together, demonstrating high homology (Figure 3; Figure 4, panel A). The VP6 genes clustered in different lineages; 4 strains clustered with the B1711 strain, which is the only previously described human ROTAV G6P[6] strain (*22*), and 2 strains clustered closely with bovine strains (Figure 4, panel B). Phylogenetic analysis of the VP6 genes revealed that all strains clustered within lineage I2, which is typical of strains belonging to the DS-1 genogroup (*35*). However, the nucleotide difference between the 2 VP6 clusters was 7.7%–7.8%, strongly indicating different origins of the VP6 genes. To further characterize the G6P[6] strains from Burkina Faso, we sequenced the NSP1–5 genes for 4 of the G6P[6] strains (≥80% nt coverage). All NSP genes of these 4 strains were highly homologous (≥98.6% nt similarity); thus, we chose a representative isolate (272/BF) for comparison with reference strains (comparison results are shown in online Technical Appendix Table 2).

The representative isolate, 272/BF, had a high level of identity with G6 (B1711) and G8 (DRC88) strains belonging to the DS-1 genogroup. The G6P[6] strains were classified as G6-P[6]-I2-A2-N2-T2-E2-H2, strongly suggesting that G6P[6] strains from Burkina Faso belong to the human DS-1 genogroup.

Discussion

We characterized ROTAV strains circulating in Ouagadougou, Burkina Faso, during the cold, dry season (December 2009–March 2010). The strains were assigned a G and P genotype after multiplex RT-PCR or nucleotide sequencing and were subgrouped and quantified by using qPCR. This high incidence (13%) of the rare G6P[6] strain in humans during a ROTAV season is remarkable.

The circulating G and P genotypes showed a dynamic pattern, with G9P[8] strains dominating early in the cold season and then being replaced with various G types, mostly associated with the P[6] genotype. Using our VP6 subgroup-specific qPCR (*30*), we subgrouped all 100 ROTAV-positive specimens. All SGI ROTAV strains were associated with the P[6] or P[4] genotype, and all SGII ROTAV strains were associated with P[8], demonstrating a gene-dependent cosegregation pattern. In the middle of the season, there was a shift from SGII to SGI ROTAV strains, which also corresponded with a slightly older age group of infected children. Herd immunity could be an underlying factor for the differences in age profiles; SGI strains infected older children who may have been immunologically naive to ROTAV SGI but not SGII strains (*36*). Indeed, 3 of 4 children >2.5 years of age were infected with SGI-specific ROTAV strains, and the fourth child was infected with SGI and SGII.

Moreover, we observed that signs and symptoms induced by SGII infections were more severe than those induced by SGI infections; the ROTAV constellation G9P[8]SGII resulted in most cases of severe dehydration (33%). One possible explanation could be that children infected with SGI ROTAV were generally older and thus had an immune memory response from a previous ROTAV infection. It has been reported that children previously infected with ROTAV are protected against severe ROTAV-induced diarrhea during subsequent infections (*37*). Earlier studies also found differences in clinical manifestations between SGI and SGII infections (*36*,*38*). The age-group profile for the SGI- and SGII-infected children in those

studies also differed; thus, it is plausible that results from those studies could also be associated with age differences for children infected with the different subgroups.

The G9 primer was highly homologous to the Burkina Faso G3 ROTAV sublineage. This homology demonstrates the need for continually updating the PCR methods used for assessing G and P type specificity and points out the need for caution when using multiplex PCRs, which could lead to an overestimation of the prevalence of mixed infections or inaccurate genotype assessment (*39*,*40*). In this study, a random selection from each G-P combination was sequenced to verify correct assignment as determined by the multiplex PCR assay.

The major finding of this study was the high proportion (13%) of G6P[6] strains circulating in Ouagadougou, Burkina Faso. A study from Belgium reported the isolation of a G6P[6] strain from a child returning from Mali (*22*), which to our knowledge is the only description published to date of a human infection with G6P[6]. Many ROTAV strains from sub-Saharan Africa remain untypeable, and the high incidence of G6P[6] observed in this study suggests that G6P[6] ROTAV strains might be more widespread than earlier thought. If this was an atypical event in Burkina Faso, or if the G6P[6] strains are more widespread in Africa than previously assumed, more untypeable strains from the region should be investigated by sequencing of the relevant genes. G6 ROTAVs are the most prevalent genotype in cattle worldwide (*8*), but they are rarely found in humans. The high number of G6P[6] sequences found in our study and the fact that all the G6 sequences clustered with the human G6P[6] reported (B1711) indicates a recent introduction. The VP4 gene of the G6 strains was highly homologous to the human B1711 strain and also clustered with a human P[6] strain detected in Ireland (R308). Because bovine G6 strains are highly prevalent in cattle, it is possible that the VP7 gene found in this study is derived from a transmission event between cattle and humans. In the peripheral areas of Ouagadougou, as well as Burkina Faso in general, cattle and humans live in close proximity, thus increasing the possibility of ROTAV transmission between animals and humans. Also, human P[6] ROTAVs are common in Africa (*13*), suggesting that reassortment between a bovine G6 and human P[6] during co-infection could occur, as previously suggested (*22*). We also found 2 cases of mixed infection with G6, 1 with G1, and 1with G3, suggesting that reassortment with the G6 genotype might occur.

We further investigated the VP4 gene of cocirculating G1P[6], G3P[6], and G1/G6P[6] strains. We observed that the VP4 genes of the G3P[6] strains clustered separately as compared with the VP4 gene of the G6P[6] strains (97.5%–97.9% nt identity), whereas the VP4 genes of the G1P[6] and G1G6P[6] were highly similar to the VP4 gene of the G6P[6] strains (99%–100% nt identity), indicating reassortment between the G1P[6] and G6P[6] strains.

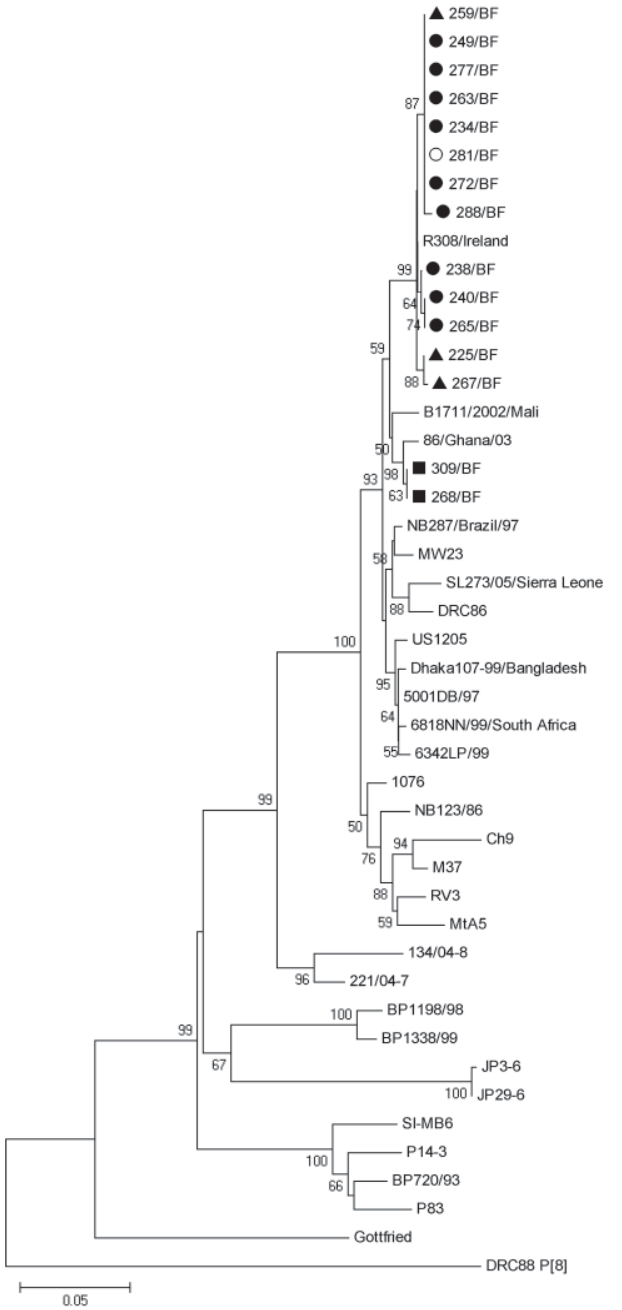


Figure 3. Phylogenetic analysis of partial sequences of viral protein (VP) 8 subunits of the VP4 gene of rotavirus (nt 141–751), with reference strains from all P[6] lineages and with the P[8] DRC88 strain as an outgroup. GenBank accession numbers for VP4 genes of reference strains are available in online Technical Appendix Table 3 (wwwnc.cdc.gov/EID/pdfs/11-0973-Techapp.pdf). Filled circles, G6P[6] rotavirus strains; triangles, G1P[6] strains; squares, G3P[6] strains; open circles, G1G6P[6] strains from Burkina Faso, December 2009–March 2010. Scale bar represents the number of substitutions per site. Bootstrap values are shown at branch nodes (values of <50% are not shown).

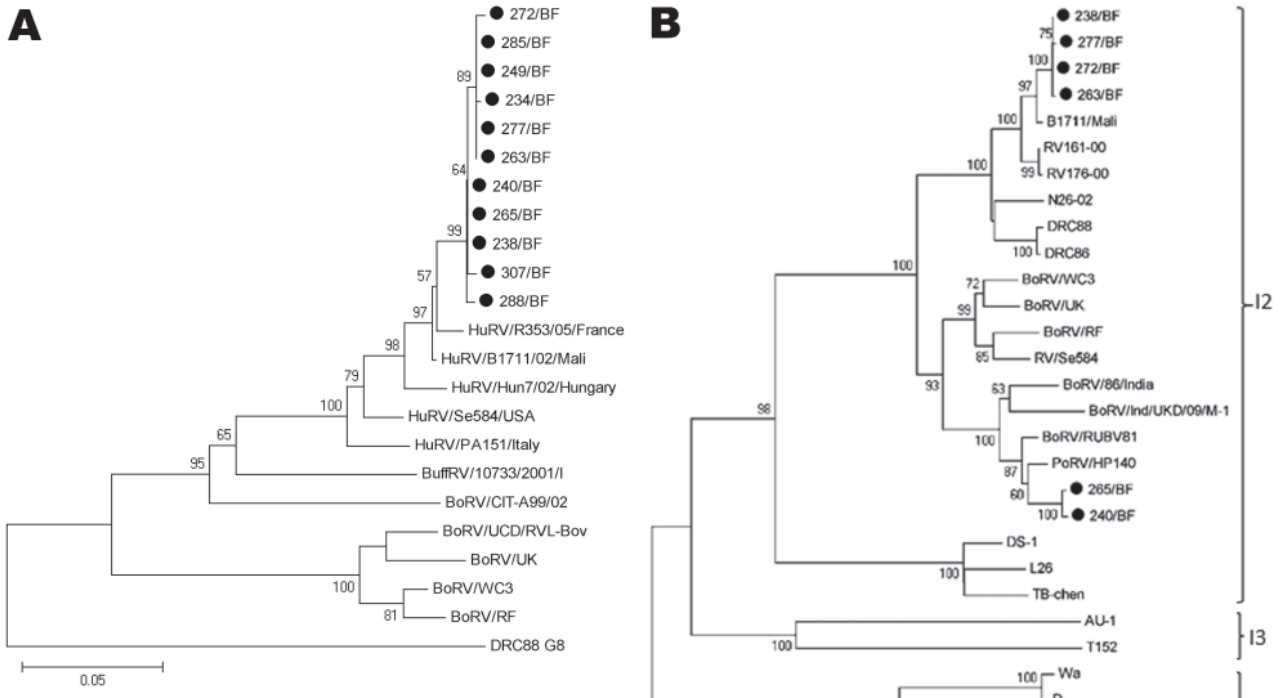


Figure 4. Phylogenetic analysis of partial sequences of A) viral protein (VP) 7 genes of the Burkina Faso G6P[6] rotavirus (ROTAV) strains (nt 135–796), with G8 strain DRC88 as an outgroup. GenBank accession numbers for VP7 genes of reference strains are available in online Technical Appendix Table 4 (wwwnc.cdc.gov/EID/pdfs/11-0973-Techapp.pdf). B) VP6 genes (nt 145–1217) of the G6P[6] ROTAV strains from Burkina Faso, with I2 genotype specificity and reference strains from genotypes I1, I2, and I3. GenBank accession numbers. for VP6 genes of reference strains are available in online Technical Appendix Table 5. Filled circles, Burkina Faso G6P[6] ROTAV strains. Bo, bovine; Buff, buffalo; Hu, human. Po, porcine. Scale bars represent number of substitutions per site. Bootstrap values are shown at the branch nodes (values of <50% are not shown).

Nucleotide sequencing data of the VP6 gene for 6 of the 11 G6P[6] strains showed they had high homology with DS-1–like strains and clustered within the I2 genotype. However, 4 of these had high homology with the B1711 and other human strains belonging to the I2 genotype, and the other 2 shared high nucleotide identity with bovine strains, indicating circulation of 2 subsets of G6P[6] strains in Burkina Faso. Altogether, these findings indicate that animal or animal-derived rotavirus strains circulate in humans in Burkina Faso and that they emerged from different, independent reassortment events. A recent review suggested that 14% of African ROTAV strains had originated in whole or in part from an animal host (*13*).

To gain additional understanding of the evolution of these novel G6P[6] strains, we randomly selected 4 strains (3 with a likely human VP6, 1 with a bovine VP6 gene) and sequenced the nonstructural genes (NSP1–5). Genes NSP1–3 and NSP5 shared high homology with those of strain B1711, but relatively low homology was observed for the G6P[6] NSP4 gene, indicating a different human parental strain than that for B1711. The NSP4 gene shared high homology with the NSP4 gene of a G8P[6] strain (DRC86; 98.5% nt identity) and with a bovine ROTAV strain (UK; 96.3% nt identity). We classified the locally circulating G6P[6] strains as G6-P[6]-I2-A2-N2-T2-E2-H2, belonging to the DS-1 genogroup. We also attempted to sequence the VP1–3 genes, but only short sequence data for VP1 and VP2 could be obtained, which classified these genes as R2 and C2, respectively, also belonging to the DS-1 genogroup. Sequencing data for the genes (VP4, VP6, VP7, NSP1–5) indicated that the G6P[6] strains detected in Burkina Faso have evolved as a result of co-infection with locally circulating subgroup I specific P[6] strains and a bovine strain and/or human G6 strains. The result of these co-infections possibly resulted in generation of 2 types of G6P[6] strains, in 1 of which the VP6 gene is also of bovine

origin. These G6P[6] strains highlight the need to further assess the efficacy of the currently licensed vaccines for such unusual and emerging genotypes.

To conclude, this study describes the diversity of ROTAV strains circulating in Ouagadougou, Burkina Faso, during 2009–2010 and a high incidence of the G6P[6] strain. We found a shift of VP6 G and P types in the middle of the ROTAV season and differences in clinical profiles; G9P[8] SGII strains induced more severe signs and symptoms than SGI strains, which infected older children. Moreover, we found bovine/human reassortant ROTAVs in children. The study highlights the need for continued monitoring of the detection assays and molecular surveillance of ROTAV strains in humans and animals in Burkina Faso. Such monitoring will identify strain diversity and emerging strains. There has not been much evaluation regarding the efficacy of the current rotavirus vaccines against rare ROTAV strains, such as G6P[6]; thus, information about strain diversity and emerging strains would also be useful during post-vaccine follow-up studies to determine vaccine efficacy.

Acknowledgments

We acknowledge the staff of the pediatric unit, the nutritional recuperation center for mothers and children, and the laboratory units of the Saint Camille Medical Center and the Biomolecular Research Center Pietro Annigoni of Ouagadougou for their cooperation.

The study was supported by Swedish Research Council (grant 10392).

Dr Nordgren works primarily with enteric viruses in the Division of Molecular Virology, Linköping University. His main research areas are molecular biology and epidemiology of rotaviruses and noroviruses.

References

1. Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis. 2006;12:304–6. http://dx.doi.org/10.3201/eid1202.050006
2. Parashar UD, Burton A, Lanata C, Boschi-Pinto C, Shibuya K, Steele D, et al. Global mortality associated with rotavirus disease among children in 2004. J Infect Dis. 2009;200(Suppl 1):S9–15. http://dx.doi.org/10.1086/605025
3. Matthijnssens J, Ciarlet M, McDonald SM, Attoui H, Banyai K, Brister JR, et al. Uniformity of rotavirus strain nomenclature proposed by the Rotavirus Classification Working Group (RCWG). Arch Virol. 2011;156:1397–413. http://dx.doi.org/10.1007/s00705-011-1006-z
4. Gentsch JR, Laird AR, Bielfelt B, Griffin DD, Banyai K, Ramachandran M, et al. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. J Infect Dis. 2005;192(Suppl 1):S146–59. http://dx.doi.org/10.1086/431499
5. Gentsch JR, Hull JJ, Teel EN, Kerin TK, Freeman MM, Esona MD, et al. G and P types of circulating rotavirus strains in the United States during 1996–2005: nine years of prevaccine data. J Infect Dis. 2009;200(Suppl 1):S99–105. http://dx.doi.org/10.1086/605038
6. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. Rev Med Virol. 2005;15:29–56. http://dx.doi.org/10.1002/rmv.448
7. Matthijnssens J, Ciarlet M, Heiman E, Arijs I, Delbeke T, McDonald SM, et al. Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1–like and bovine rotavirus strains. J Virol. 2008;82:3204–19. http://dx.doi.org/10.1128/JVI.02257-07
8. Martella V, Bányai K, Matthijnssens J, Buonavoglia C, Ciarlet M. Zoonotic aspects of rotaviruses. Vet Microbiol. 2010;140:246–55. http://dx.doi.org/10.1016/j.vetmic.2009.08.028
9. Iturriza Gómara M, Wong C, Blome S, Desselberger U, Gray J. Molecular characterization of VP6 genes of human rotavirus isolates: correlation of genogroups with subgroups and evidence of independent segregation. J Virol. 2002;76:6596–601. http://dx.doi.org/10.1128/JVI.76.13.6596-6601.2002
10. Greenberg H, McAuliffe V, Valdesuso J, Wyatt R, Flores J, Kalica A, et al. Serological analysis of the subgroup protein of rotavirus, using monoclonal antibodies. Infect Immun. 1983;39:91–9.
11. Tang B, Gilbert JM, Matsui SM, Greenberg HB. Comparison of the rotavirus gene 6 from different species by sequence analysis and localization of subgroup-specific epitopes using site-directed mutagenesis. Virology. 1997;237:89–96. http://dx.doi.org/10.1006/viro.1997.8762
12. Armah GE, Steele AD, Esona MD, Akran VA, Nimzing L, Pennap G. Diversity of rotavirus strains circulating in west Africa from 1996 to 2000. J Infect Dis. 2010;202(Suppl):S64–71. http://dx.doi.org/10.1086/653571
13. Todd S, Page NA, Duncan Steele A, Peenze I, Cunliffe NA. Rotavirus strain types circulating in Africa: review of studies published during 1997–2006. J Infect Dis. 2010;202(Suppl):S34–42. http://dx.doi.org/10.1086/653555
14. Armah GE, Steele AD, Binka FN, Esona MD, Asmah RH, Anto F, et al. Changing patterns of rotavirus genotypes in Ghana: emergence of human rotavirus G9 as a major cause of diarrhea in children. J Clin Microbiol. 2003;41:2317–22. http://dx.doi.org/10.1128/JCM.41.6.2317-2322.2003
15. Griffin DD, Nakagomi T, Hoshino Y, Nakagomi O, Kirkwood CD, Parashar UD, et al. Characterization of nontypeable rotavirus strains from the United States: identification of a new rotavirus reassortant (P2A[6],G12) and rare P3[9] strains related to bovine rotaviruses. Virology. 2002;294:256–69. http://dx.doi.org/10.1006/viro.2001.1333
16. Palombo EA, Bishop RF. Genetic and antigenic characterization of a serotype G6 human rotavirus isolated in Melbourne, Australia. J Med Virol. 1995;47:348–54. http://dx.doi.org/10.1002/jmv.1890470410
17. Bányai K, Gentsch JR, Griffin DD, Holmes JL, Glass RI, Szücs G. Genetic variability among serotype G6 human rotaviruses: identification of a novel lineage isolated in Hungary. J Med Virol. 2003;71:124–34. http://dx.doi.org/10.1002/jmv.10462
18. Yamamoto D, Kawaguchiya M, Ghosh S, Ichikawa M, Numazaki K, Kobayashi N. Detection and full genomic analysis of G6P[9] human rotavirus in Japan. Virus Genes. 2011;43:215–23. http://dx.doi.org/10.1007/s11262-011-0624-6
19. De Grazia S, Martella V, Rotolo V, Bonura F, Matthijnssens J, Bányai K, et al. Molecular characterization of genotype G6 human rotavirus strains detected in Italy from 1986 to 2009. Infect Genet Evol. 2011;11:1449–55. http://dx.doi.org/10.1016/j.meegid.2011.05.015

20. Matthijnssens J, Potgieter CA, Ciarlet M, Parreno V, Martella V, Bányai K, et al. Are human P[14] rotavirus strains the result of interspecies transmissions from sheep or other ungulates that belong to the mammalian order Artiodactyla? J Virol. 2009;83:2917–29. http://dx.doi.org/10.1128/JVI.02246-08
21. De Grazia S, Ramirez S, Giammanco GM, Colomba C, Martella V, Lo Biundo C, et al. Diversity of human rotaviruses detected in Sicily, Italy, over a 5-year period (2001–2005). Arch Virol. 2007;152:833–7. http://dx.doi.org/10.1007/s00705-006-0874-0
22. Rahman M, De Leener K, Goegebuer T, Wollants E, Van der Donck I, Hoovels LV, et al. Genetic characterization of a novel, naturally occurring recombinant human G6P [6] rotavirus. J Clin Microbiol. 2003;41:2088–95. http://dx.doi.org/10.1128/JCM.41.5.2088-2095.2003
23. Djeneba O, Karou D, Ilboudo D, Nadembega WMC, Pietra V, Belem A, et al. Prevalence of rotavirus, adenovirus and enteric parasites among pediatric patients attending Saint Camille Medical Centre in Ouagadougou. Pak J Biol Sci. 2007;10:4266–70. http://dx.doi.org/10.3923/pjbs.2007.4266.4270
24. Simpore J, Ouermi D, Ilboudo D, Kabre A, Zeba B, Pietra V, et al. Aetiology of acute gastro-enteritis in children at Saint Camille Medical Centre, Ouagadougou, Burkina Faso. Pak J Biol Sci. 2009;12:258–63. http://dx.doi.org/10.3923/pjbs.2009.258.263
25. Bonkoungou IJ, Sanou I, Bon F, Benon B, Coulibaly SO, Haukka K, et al. Epidemiology of rotavirus infection among young children with acute diarrhoea in Burkina Faso. BMC Pediatr. 2010;10:94. http://dx.doi.org/10.1186/1471-2431-10-94
26. Nitiema LW, Nordgren J, Ouermi D, Dianou D, Traore AS, Svensson L, et al. Burden of rotavirus and other enteropathogens among children with diarrhea in Burkina Faso. Int J Infect Dis. 2011;15:e646–52. http://dx.doi.org/10.1016/j.ijid.2011.05.009
27. Steele AD, Page N, de Beer M, Sawadogo S. Antigenic and molecular characterization of unusual rotavirus strains in Burkina Faso in 1999. J Infect Dis. 2010;202(Suppl):S225–30. http://dx.doi.org/10.1086/653574
28. United Nations Children's Fund /World Health Organization. Diarrhoea: why children are still dying and what can be done. New York: The Fund/The Organization; 2009.
29. Bucardo F, Karlsson B, Nordgren J, Paniagua M, Gonzalez A, Amador JJ, et al. Mutated G4P[8] rotavirus associated with a nationwide outbreak of gastroenteritis in Nicaragua in 2005. J Clin Microbiol. 2007;45:990–7. http://dx.doi.org/10.1128/JCM.01992-06
30. Nordgren J, Bucardo F, Svensson L, Lindgren PE. Novel light-upon-extension real-time PCR assay for simultaneous detection, quantification, and genogrouping of group A rotavirus. J Clin Microbiol. 2010;48:1859–65. http://dx.doi.org/10.1128/JCM.02288-09
31. Gouvea V, Glass RI, Woods P, Taniguchi K, Clark HF, Forrester B, et al. Polymerase chain reaction amplification and typing of rotavirus nucleic acid from stool specimens. J Clin Microbiol. 1990;28:276–82.
32. Gentsch JR, Glass RI, Woods P, Gouvea V, Gorziglia M, Flores J, et al. Identification of group A rotavirus gene 4 types by polymerase chain reaction. J Clin Microbiol. 1992;30:1365–73.
33. Iturriza-Gómara M, Kang G, Gray J. Rotavirus genotyping: keeping up with an evolving population of human rotaviruses. J Clin Virol. 2004;31:259–65. http://dx.doi.org/10.1016/j.jcv.2004.04.009
34. Kommedal O, Karlsen B, Saebo O. Analysis of mixed sequencing chromatograms and its application in direct 16S rRNA gene sequencing of polymicrobial samples. J Clin Microbiol. 2008;46:3766–71. http://dx.doi.org/10.1128/JCM.00213-08
35. Nakagomi O, Nakagomi T, Akatani K, Ikegami N. Identification of rotavirus genogroups by RNA-RNA hybridization. Mol Cell Probes. 1989;3:251–61. http://dx.doi.org/10.1016/0890-8508(89)90006-6
36. Steele AD, Bos P, Alexander JJ. Clinical features of acute infantile gastroenteritis associated with human rotavirus subgroups I and II. J Clin Microbiol. 1988;26:2647–9.
37. Velázquez FR, Matson DO, Calva JJ, Guerrero L, Morrow AL, Carter-Campbell S, et al. Rotavirus infections in infants as protection against subsequent infections. N Engl J Med. 1996;335:1022–8. http://dx.doi.org/10.1056/NEJM199610033351404
38. Uhnoo I, Svensson L. Clinical and epidemiological features of acute infantile gastroenteritis associated with human rotavirus subgroups 1 and 2. J Clin Microbiol. 1986;23:551–5.
39. Aladin F, Nawaz S, Iturriza-Gómara M, Gray J. Identification of G8 rotavirus strains determined as G12 by rotavirus genotyping PCR: updating the current genotyping methods. J Clin Virol. 2010;47:340–4. http://dx.doi.org/10.1016/j.jcv.2010.01.004
40. Simmonds MK, Armah G, Asmah R, Banerjee I, Damanka S, Esona M, et al. New oligonucleotide primers for P-typing of rotavirus strains: strategies for typing previously untypeable strains. J Clin Virol. 2008;42:368–73. http://dx.doi.org/10.1016/j.jcv.2008.02.011

Address for correspondence: Johan Nordgren, Division of Molecular Virology, Department of Clinical and Experimental Medicine, Medical Faculty, Linköping University, SE-581 85 Linköping, Sweden; email: johan.nordgren@liu.se

Comparison of *Escherichia coli* ST131 Pulsotypes, by Epidemiologic Traits, 1967–2009

James R. Johnson, Marie-Hélène Nicolas-Chanoine, Chitrita DebRoy, Mariana Castanheira, Ari Robicsek, Glen Hansen, Scott Weissman, Carl Urban, Joanne Platell, Darren Trott, George Zhanel, Connie Clabots, Brian D. Johnston, Michael A. Kuskowski, and the MASTER Investigators[1]

Escherichia coli sequence type 131 (ST131), an emerging disseminated public health threat, causes multidrug-resistant extraintestinal infections. Among 579 diverse *E. coli* ST131 isolates from 1967–2009, we compared pulsotypes (≥94% similar *Xba*I pulsed-field gel electrophoresis profiles) by collection year, geographic origin, source, and antimicrobial drug–resistance traits. Of 170 pulsotypes, 65 had ≥2 isolates and accounted for 85% of isolates. Although extensively dispersed geographically, pulsotypes were significantly source specific (e.g., had little commonality between humans vs. foods and food animals). The most prevalent pulsotypes were associated with recent isolation, humans, and antimicrobial drug resistance. Predominant pulsotype 968 was associated specifically with fluoroquinolone resistance but not with extended-spectrum β-lactamase production or $bla_{CTX\text{-}M\text{-}15}$. Thus, several highly successful antimicrobial drug–resistant lineages within *E. coli* ST131 have recently emerged and diffused extensively among locales while maintaining a comparatively restricted host/source range. Identification of factors contributing to this behavior of ST131 could help protect public health.

Author affiliations: Veterans Affairs Medical Center, Minneapolis, Minnesota, USA (J.R. Johnson, C. Clabots, B.D. Johnston, M.A. Kuskowski); University of Minnesota, Minneapolis (J.R. Johnson, B.D. Johnston, M.A. Kuskowski); Hôpital Beaujon, Clichy, France (M.-H. Nicolas-Chanoine); The Pennsylvania State University, College Park, Pennsylvania, USA (C. DebRoy); JMI Laboratories, North Liberty, Iowa, USA (M. Castanheira); NorthShore University HealthSystem, Evanston, Illinois, USA (A. Robicsek); Hennepin County Medical Center, Minneapolis (G. Hansen); University of Washington, Seattle, Washington, USA (S. Weissman); New York Hospital Queens, Flushing, New York, USA (C. Urban); New York University School of Medicine, New York, New York, USA (C. Urban); University of Queensland, Brisbane, Queensland, Australia (J. Platell); University of Adelaide, Adelaide, South Australia, Australia (D. Trott); and University of Manitoba, Winnipeg, Manitoba, Canada (G. Zhanel)

DOI: http://dx.doi.org/10.3201/eid1804.111627

The prevalence of resistance to fluoroquinolones and extended-spectrum cephalosporins in *Escherichia coli* has increased dramatically over the past decade. This increase is largely the result of the widespread emergence of a single disseminated *E. coli* clonal group, designated sequence type (ST) 131 according to multilocus sequence typing (MLST) (*1*,*2*). *E. coli* ST131 is characterized by serotype O25b:H4 and often produces CTX-M-15 or other extended-spectrum β-lactamases (ESBLs) (*3*–*5*). Unlike most other antimicrobial drug–resistant *E. coli*, ST131 derives from virulence-associated phylogenetic group B2 and typically exhibits multiple virulence factors, including adhesins, siderophores, toxins, and group 2 capsule (*1*–*7*). It thereby poses the dual threat of extensive antimicrobial drug resistance plus virulence.

By definition, ST131 is homogeneous with respect to housekeeping gene sequence across the 7 MLST loci; however, within-lineage genetic variation has been noted since ST131 was first described (*3*–*5*). Specifically, diversity of pulsed-field gel electrophoresis (PFGE) profiles has provided insights into the ecology of ST131. For example, the presence of ST131 isolates with similar PFGE profiles in widely dispersed locales and of isolates with quite different profiles in the same locale has suggested rapid and

[1]MASTER (Macrorestriction Analysis of ST131 for Epidemiologic Research) Investigators who contributed data are listed at the end of this article.

ongoing global dissemination of ST131 (*3*,*8*). Likewise, recovery of ST131 isolates with similar PFGE profiles from multiple household members (*9*–*12*) and from food animals (or retail meats) and humans (*13*) has suggested host-to-host or foodborne transmission, respectively, as potential mechanisms for dissemination of ST131.

However, relevant studies to date have included relatively few isolates, locales, and sources and limited time periods (*2*,*6*). In addition, the idiosyncratic nature of PFGE analysis precludes across-study comparisons. Thus, we analyzed 579 ST131 isolates from diverse sources according to a standardized PFGE protocol and then compared PFGE profiles with other characteristics, including geographic origin, time of collection, ecologic source, and antimicrobial drug–resistance traits.

Materials and Methods

Isolates

The 579 ST131 study isolates, some previously published (*3*,*9*–*12*,*14*–*19*), were compiled as a series of convenience samples from collaborators in diverse locales. The isolates came already identified as ST131 or as generic *E. coli* in need of screening for ST131 status. They derived mostly from collections assembled by investigators or reference laboratories on the basis of specific resistance phenotypes, O antigens, geographic origins, and/or clinical syndromes of interest. Some isolates were from cases or case series involving infected humans or animals with distinctive signs and symptoms and/or predisposing conditions (*9*–*12*,*14*,*15*).

Isolates were accompanied by data regarding date of isolation (or receipt in the reference laboratory), ecologic source (i.e., host species, food, or water), and locale of origin. For some isolates, data were available regarding resistance-associated characteristics, i.e., fluoroquinolone resistance; ESBL production; and presence of $bla_{\text{CTX-M-15}}$, which encodes the CTX-M-15 ESBL variant. If not provided, this information was newly generated.

ST131 Status

Of the 579 study isolates, 34 (5.9%) came already defined as ST131 by 7-locus MLST (http://mlst.ucc.ie/mlst/dbs/Ecoli). The remaining 545 (94.1%) were presumptively identified as ST131 in the study laboratory by PCR-based screening for ST131-specific single-nucleotide polymorphisms (SNPs) in *gyrB* and *mdh* (*17*). Full MLST was done de novo for 57 (10.5%) of these presumptive ST131 isolates, which represented diverse time periods, locales, sources, and resistance characteristics; in each instance, presumptive ST131 status was confirmed (SNP PCR specificity 100%; 95% CI 94%–100%). Thus, 91 (16%) isolates were directly confirmed by MLST to be ST131. Another 301 (52%) isolates represented pulsotypes with ≥1 MLST-confirmed ST131 isolate, which indirectly confirmed their ST131 status. Therefore, in total, 392 (68%) isolates were directly or indirectly confirmed by MLST to be ST131. Of the 31 earliest isolates (1967–1997), including the 5 earliest isolates (1967–1986), 32% were directly confirmed by MLST to be ST131.

PFGE Analysis

PFGE analysis of *Xba*I-restricted total DNA of isolates was performed according to a standardized protocol (*20*) by a single observer in 1 laboratory. Profiles were captured and analyzed digitally by using BioNumerics software version 6.6 (Applied Maths, Austin, TX, USA). Marker lanes in each gel (*E. coli* O157:H7 strain g5244) enabled normalization within and across gels. Band positions were assigned manually, with computer assistance. The band tolerance setting, as derived empirically from analysis of multiple same-isolate profiles, was 1.15%.

Pairwise Dice similarity coefficients were used to define pulsotypes. Isolates exhibiting ≥94% profile similarity (≈3-band difference) to the index isolate for an established pulsotype, implying genetic similarity (*21*), were assigned to that pulsotype; others became the index isolate for a new pulsotype. Newly encountered pulsotypes were numbered sequentially. A PFGE profile dendrogram was constructed according to the unweighted pair group method for 87 (15%) of the isolates (selected randomly after inclusion of 2 representatives of each pulsotype with ≥6 members) plus the earliest isolated (1967) and earliest published (1985) isolates (*8*).

Susceptibility Testing

Disk diffusion testing for ciprofloxacin susceptibility and ESBL production was performed on isolates of unknown fluoroquinolone or ESBL phenotype as described (*22*,*23*). Fluoroquinolone resistance was defined as nonsusceptibility to ciprofloxacin.

Statistical Analyses

Geographic origin was categorized as United States (with 4 subregions—West, Midwest, South, and Northeast—as defined by the US Census Bureau [www.census.gov/geo/www/us_regdiv.pdf]), Canada, and other international locales combined. Ecologic source was categorized as human, companion animal, food animal, other animal, food, and water. Year of isolation/submission was assessed both continuously and categorically (e.g., pre-1990 vs. later).

Comparisons of proportions were tested by using 2-tailed Fisher exact (unpaired comparisons) and McNemar (paired comparisons) tests. Comparisons involving continuous variables were tested by using the Mann-

Whitney U test (2-tailed). Other variables were assessed as independent predictors of selected pulsotype categories by using multivariable logistic regression analysis. The significance criterion was $p<0.05$.

Results

Isolate Origins and Characteristics

The 579 ST131 study isolates were derived from humans (486 [84%]), animals (77 [13%]), environmental sources (15 [3%]), and an unknown source (n = 1). Animal uses included companion (22 [4%]), food (45 [8%]), and other (10 [2%]). Environmental sources included food (6 [1%]) and water (9 [2%]). Geographic origins included the United States (446 [77%]), Canada (53 [9%]), and other international locales (80 [14.1%]). The US isolates were from the West (59 [10%]; 9 centers, 4 states), Midwest (180 [31%] centers, 11 states), South (98 [17%] centers, 9 states), and Northeast (109 [19%] centers, 7 states). Isolates from Canada were from 9 centers in 8 provinces. Other international isolates were from 11 centers in 11 countries (Australia, Chile, France, South Korea, Lebanon, India, Italy, Peru, Portugal, Spain, Switzerland). Dates of isolation/submission ranged from 1967 through 2009 (median year 2007). Twenty (4%) isolates were isolated/submitted during 1967–1989, the 5 earliest during 1967, 1982, 1983, 1985, and 1986; 22 (4%) were isolated/submitted during 1990–1999; and 537 (93%) were isolated/submitted during 2000–2009. Overall, 462 (80%) isolates were fluoroquinolone-resistant, 272 (47%) were ESBL-producers, and 188 (33%) had $bla_{\text{CTX-M-15}}$.

Pulsotypes

*Xba*I PFGE analysis resolved 170 distinct pulsotypes, each accounting for 1 isolate (105 pulsotypes) to 136 isolates (1 pulsotype, type 968). The 105 single-isolate pulsotypes collectively accounted for 105 isolates (18% of total); the 65 multiple-isolate pulsotypes accounted for the remaining 474 isolates (82% of total). Among the multiple-isolate pulsotypes, 12 contained ≥6 isolates each (i.e., >1% of the population), collectively accounting for 327 isolates (56% of total). The multiple-isolate pulsotypes contained 62 clusters of isolates (each comprising 2–5 isolates; total 150 isolates) with indistinguishable profiles.

Temporal Patterns

Pulsotypes varied significantly by temporal occurrence. The 65 multiple-isolate pulsotypes and 12 high-prevalence pulsotypes were significantly associated with more recent dates of isolation/submission, relative to the low-prevalence and single-isolate pulsotypes (Table 1). Temporal variation was also evident among the 12 high-prevalence pulsotypes; 4 were significantly associated with later and 1 (type 955) with earlier occurrence (Table 1).

Table 1. Association of year of isolation/submission with pulsotype and other characteristics for 579 *Escherichia coli* sequence type isolates, 1967–2009*

Associated characteristic, specific trait†‡	Characteristic absent		Characteristic present		p value†
	No. isolates	Year, median (range)	No. isolates	Year, median (range)	
Pulsotype					
High-prevalence	252	2007 (1967–2009)	327	2007 (1987–2009)	<0.001
Multiple-isolate	105	2007 (1967–2009)	474	2007 (1982–2009)	<0.001
968	443	2007 (1967–2009)	136	2008 (1992–2009)	<0.001
812	547	2007 (1967–2009)	32	2007 (2005–2009)	0.03
987	568	2007 (1967–2009)	11	2008 (1998–2008)	0.004
955	572	2007 (1967–2009)	7	2002 (1993–2005)	0.001
1160	573	2007 (1967–2009)	6	2008 (2004–2009)	0.04
Resistance					
FQ	117	2003 (1967–2009)	462	2007 (2000–2009)	<0.001
ESBL	307	2007 (1967–2009)	272	2007 (2000–2009)	0.001
$bla_{\text{CTX-M-15}}$	391	2007 (1967–2009)	188	2007 (2000–2009)	0.048
Source§					
Human	92	2003 (1982–2009)	486	2007 (1985–2009)	<0.001
Pet	556	2007 (1982–2009)	22	2008 (2002–2009)	<0.001
Food animal	533	2007 (1985–2009)	45	1997 (1982–2009)	<0.001
Food/water	563	2007 (1982–2009)	15	2003 (1993–2007)	<0.001
Region					
United States	133	2005 (1998–2009)	446	2007 (1967–2009)	0.007
West	520	2007 (1967–2009)	59	2007 (1982–2007)	<0.001
Midwest	399	2007 (1967–2009)	180	2008 (1986–2009)	<0.001
Canada	526	2007 (1967–2009)	53	2004 (1998–2004)	<0.001
International	499	2007 (1967–2009)	80	2008 (2002–2009)	<0.001

*ST, sequence type; FQ, fluoroquinolone; ESBL, extended-spectrum β-lactamase; $bla_{\text{CTX-M-15}}$, gene encoding the CTX-M-15 ESBL; high-prevalence, pulsotypes with ≥6 isolates each; multiple-isolate, pulsotypes with ≥2 isolates each; international, non-US locales other than Canada.
†Characteristics shown are those that yielded $p<0.05$ (Mann-Whitney U test, 2-tailed).
‡High-prevalence pulsotypes 800, 905, 1202, 807, 919, 595, and 797; other animal source; and origin from the US South or US Northeast did not exhibit a significant association with year.
§Total for all source variables is 578 rather than 579 because source was unknown for 1 isolate.

Analysis of temporal prevalence trends (Figure) showed that the 12 high-prevalence pulsotypes accounted collectively for only 5% of 20 isolates during the earliest period (1967–1989) but for 58% of isolates during subsequent years ($p<0.001$). Three of these pulsotypes (988, 800, 812) were the top 1, 2, or 3 most prevalent, overall and within each interval from 1990 forward. These 3 types appeared sequentially by overall pulsotype prevalence (i.e., in 1990–1999 for type 968, in 2000–2002 for type 800, and in 2005 for type 812) and, except for type 800 in 2003, were detected continuously after first appearing. After it appeared, type 968 maintained a consistently high prevalence (≥19%), whereas types 800 and 812 exhibited early prevalence spikes followed by sizeable drops. In contrast, the 9 other high-prevalence types appeared intermittently, which, depending on the pulsotype, was mostly in earlier years, later years, or sporadically throughout (Figure).

A temporal trend was also evident in the PFGE dendrogram (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/1/11-1627-FA1.htm), which extended to 67% similarity. The more highly similar PFGE profiles in the upper region of the tree involved mostly recent isolates and higher prevalence pulsotypes, whereas the more basal, dissimilar profiles toward the lower region of the tree involved more older isolates (including isolates from 1967, 1982, 1985, and 1986) and low-prevalence pulsotypes.

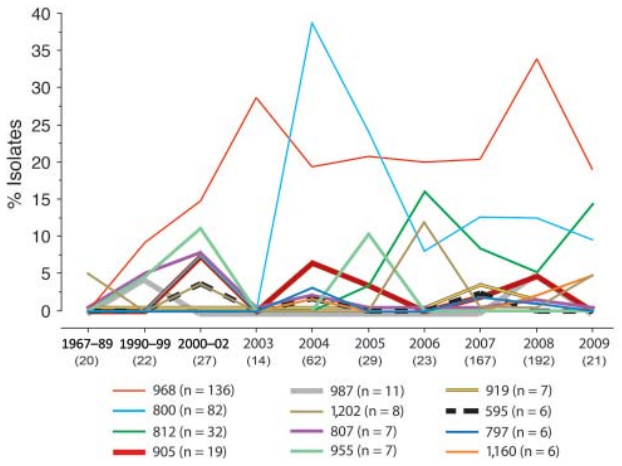


Figure. Prevalence over time of 12 high-prevalence *Xba*I pulsotypes among 579 *Escherichia coli* ST131 isolates. High-prevalence pulsotypes are those with ≥6 isolates (≥1% of population) each. Years before 2003 are combined into 3 groups because of the small numbers of isolates. On the x-axis, the number of isolates for the particular period is shown in parentheses below the dates. y-axis prevalence values are based on the total number of isolates in the particular period.

Geographic Distribution

The pulsotypes primarily exhibited a broad geographic distribution, yet there was some geographic segregation. Table 2 shows the number of mutually exclusive geographic regions (among 6 total) in which each of the 65 multiple-isolate pulsotypes were found. Only 18 of 65 multiple-isolate pulsotypes were limited to a single geographic region ($p<0.001$ for occurrence in 1 vs. multiple regions, McNemar test). Moreover, these 18 pulsotypes included only 2 (13 pulsotypes), 3 (4 pulsotypes), or 4 (1 pulsotype) isolates each and represented <50% of pulsotypes within their size category. In contrast, all pulsotypes including ≥5 isolates were found in multiple geographic regions, and 3 of the 6 pulsotypes comprising ≥8 isolates spanned all 6 geographic regions (Table 2). Table 3 shows the overlap among regions by the number of shared pulsotypes and by the number of isolates in these pulsotypes. Each region overlapped partially with every other region (Table 3).

Against this background of broad geographic distribution, substantial geographic segregation of pulsotypes was evident. For example, at the isolate level, each region was negatively associated with at least 1 other region; the US West and non-Canadian international sites exhibited the greatest number of such negative associations, suggesting somewhat locale-specific pulsotype populations in these regions (Table 3). The only positive association between nonoverlapping regions involved the US Midwest and Canada.

Table 2. Geographic origin and ecologic source of 65 pulsotypes among 579 *Escherichia coli* ST131 isolates, 1967–2009*

No. isolates per pulsotype	No. pulsotypes	No. pulsotypes with isolate from indicated no. geographic regions†						No. pulsotypes with isolates from indicated no. ecologic sources‡			
		1	2	3	4	5	6	1	2	3	4
2	28	13	15	NA	NA	NA	NA	26	2	NA	NA
3	12	4	8	0	NA	NA	NA	10	2	0	NA
4	10	1	3	5	1	NA	NA	9	0	0	1
5	3	0	2	1	0	0	NA	1	2	0	0
6	3	0	0	0	2	1	0	2	1	0	0
7	3	0	0	0	1	2	0	2	0	1	0
≥8	6	0	1	1	0	1	3	1	3	2	0

*ST, sequence type; NA, not applicable (no. regions or source groups exceeds no. isolates in pulsotype).
†The 6 geographic regions included the US West, Midwest, South, and Northeast; Canada; and other international sites (collectively).
‡The 5 source groups included humans, companion animals, food animals, other animals, and food and water combined.

Table 3. Segregation, by geographic region, of multiple-isolate pulsotypes and of *Escherichia coli* ST131 isolates from the pulsotypes, 1967–2009*

Source region	Multiple-isolate pulsotype group: Total no. pulsotypes in group (65)	Total no. isolates in group (474)	No. isolates, by geographic region, from pulsotype group (474): US (358)	W (47)	MW (139)	S (84)	NE (88)	CAN (52)	INT (64)	No. pulsotypes, by geographic region, among isolate group (65): US (61)	W (24)	MW (38)	S (23)	NE (31)	CAN (15)
US	61	463	NA	47	**139**†	84	88	50	***55***‡	NA					
West	24	348	***272***†	NA	105	62	58	41	***35***‡	24	NA				
MW	38	408	309	***33***†	NA	74	***63***‡	47	52	**38**†	15	NA			
South	23	350	272	***23***‡	108	NA	***57***†	43	***35***‡	23	10	17	NA		
NE	31	368	***287***†	***23***‡	106	68	NA	43	***38***‡	31	12	17	12	NA	
CAN	15	279	***201***†	***10***‡	**92**†	52	47	NA	***26***§	14	5	11	7	8	NA
INT	20	336	***231***‡	***17***‡	102	58	***54***†	41	NA	17	14	14	8	10	5

*Values in parentheses are n values. **Boldface** indicates significant associations; ***boldface italics*** indicate negative associations. Blank spaces indicate duplicated comparisons (mirror image of matrix). ST, sequence type; US, United States; W, West; MW, Midwest; S, South; NE, Northeast; CAN, Canada; INT, international (non-US locales other than Canada); NA, not applicable.
†p≤0.05 for indicated region vs. all other regions (Fisher exact test).
‡p≤0.001 for indicated region vs. all other regions (Fisher exact test).
§p≤0.01 for indicated region vs. all other regions (Fisher exact test).

Table 4 provides a pulsotype-level analysis of these geographic associations. For example, the high-prevalence and multiple-isolate pulsotypes were collectively significantly overrepresented in Canada, and the high-prevalence pulsotypes were also significantly underrepresented in the United States, specifically, in the US West. Among individual high-prevalence pulsotypes, 968 was overrepresented in the US Midwest and underrepresented in the US West; 800 was over-represented in Canada and under-represented in the United States and the US West; 812 was over-represented in the US South; 987 was over-represented internationally (specifically in Australia, data not shown) and under-represented in the United States; and 1202 was over-represented in the US West (Table 4).

Source Distribution

In contrast with the generally broad geographic distribution of pulsotypes, the source distribution was more restricted, and source-specific segregation predominated over across-source commonality. For example, only 13 of 65 multiple-isolate pulsotypes spanned multiple sources ($p<0.001$, McNemar test); most of these included only 2 sources each, and none included >4 (of 6 possible) sources (Table 2).

Likewise, the by-source distribution of pulsotypes (Table 5) showed less overall commonality than did geographic distribution. Still, it showed multiple positive and negative associations at the isolate and the pulsotype level. Specifically, isolates from humans were associated positively with pulsotypes comprising isolates from water and negatively with pulsotypes comprising isolates from companion animals, food animals, or food. Isolates from companion animals were associated positively with pulsotypes containing isolates from other animals, and isolates from food animals were associated positively with pulsotypes containing isolates from food (Table 5). In addition, pulsotypes containing isolates from food animals were associated negatively with pulsotypes containing isolates from humans, but they were associated positively with pulsotypes containing isolates from food (Table 5).

Significant by-source segregation also was evident for individual pulsotypes (Table 6). Collectively, the multiple-isolate and high-prevalence pulsotypes were associated positively with humans and negatively with

Table 4. Distribution, by geographic region, of *Escherichia coli* ST131 isolates from different pulsotype groups and from individual pulsotypes, 1967–2009*

Region	Total isolates, N = 579	High-prevalence pulsotypes, n = 327	Multiple-isolate pulsotypes, n = 474	Individual high-prevalence pulsotypes: 968, n = 136	800, n = 82	812, n = 32	987, n = 11	1202, n = 8
US	446 (77)	**239 (73)**†	358 (80)	107 (79)	**55 (67)**‡	25 (78)	**1 (9)**§	8 (100)
West	59 (10)	**22 (7)**†	47 (10)	**4 (7)**§	**3 (4)**‡	5 (16)	0	**4 (50)**†
MW	180 (31)	56 (31)	139 (29)	**53 (39)**‡	24 (29)	7 (22)	1 (9)	2 (25)
South	98 (17)	64 (20)	84 (18)	26 (19)	17 (21)	**8 (25)**†	0	2 (25)
NE	109 (19)	53 (16)	88 (19)	24 (18)	11 (13)	5 (16)	0	0
CAN	53 (9)	**42 (13)**§	**52 (11)**§	14 (10)	**20 (24)**§	0	0	0
INT	80 (14)	46 (14)	64 (14)	15 (11)	7 (9)	7 (23)	**10 (91)**§	0

*Values are no (%) isolates. **Boldface** indicates p<0.05 in comparison with all other isolates (Fisher exact test). Pulsotypes are those among the 12 high-prevalence pulsotypes that exhibited a significant association with ≥1 geographic region. ST, sequence type; US, United States; MW, US Midwest; NE, US Northeast; CAN, Canada; INT, international (non-US locales other than Canada).
†p≤0.01 for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).
‡ p<0.05 for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).
§p≤0.001 for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).

Table 5. Segregation, by ecologic source, of multiple-isolate pulsotypes and of *Escherichia coli* ST131 isolates from the pulsotypes, 1967–2009*

Ecologic source	No. pulsotypes, n = 65	Total no. isolates, n = 474	No. isolates						No. pulsotypes comprising isolates				
			HU, n = 412	CA, n = 21	FA, n = 28	OA, n = 7	Food, n = 4	Water, n = 2	HU, n = 57	CA, n = 8	FA, n = 13	OA, n = 2	Food, n = 2
HU	57	453	NA	19	***12***†	7	***1***†	2	NA				
CA	8	216	***178***‡	NA	8	**6**§	3	0	7	NA			
FA	13	43	***7***†	3	NA	1	**4**†	0	***5***†	2	NA.		
OA	2	140	118	**13**‡	**1**†	NA	1	0	2	1	1	NA	
Food	2	11	***1***†	2	**3**§	1	NA	0	1	1	**2**§	1	NA
Water	2	84	**82**†	***0***§	***0***‡	0	0	NA	2	0	0	0	0

*Blank spaces indicate duplicated comparisons (mirror image of matrix). **Boldface** indicates significant associations; ***boldface italics*** indicate negative associations. ST, sequence type; HU, human; CA, companion animal; FA, food animal; OA, other animal; NA, not applicable.
†$p \leq 0.001$ for indicated source vs. all other sources (Fisher exact test).
‡$p \leq 0.01$ for indicated source vs. all other sources (Fisher exact test).
§$p \leq 0.05$ for indicated source vs. all other sources (Fisher exact test).

food animals and environmental sources, and high-prevalence pulsotypes were associated with companion animals. Furthermore, 5 high-prevalence pulsotypes were individually significantly distributed by source: type 968 was associated positively with pets and other animals and negatively with food animals and environmental sources, 800 was associated positively with humans and negatively with food animals, 812 was associated positively with humans, 1202 was associated negatively with humans and positively with food animals, and 955 was associated negatively with humans and positively with pets and environmental sources (Table 6). Five additional pulsotypes, comprising 2 food animal isolates each, were significantly associated with food animals ($p = 0.004$ for each; data not shown).

Antimicrobial Drug Resistance

Fluoroquinolone resistance, ESBL production, and $bla_{\text{CTX-M-15}}$ also segregated significantly by pulsotype in varied patterns (Table 7). The high-prevalence and multiple-isolate pulsotypes collectively and type 968 were associated positively with fluoroquinolone resistance but indifferently with ESBL production and $bla_{\text{CTX-M-15}}$. In contrast, type 800 was associated positively with fluoroquinolone resistance but negatively with ESBL production and $bla_{\text{CTX-M-15}}$, whereas types 905, 812, and 919 were associated positively with all 3 traits, and type 987 was associated negatively with all 3 traits (Table 7).

Multivariable Analysis

All 3 resistance traits, plus several source groups and geographic regions, exhibited significant associations with year of isolation/submission (Table 1), suggesting possible confounding by temporal correlations among variables. Thus, we used multivariable logistic regression analysis to assess for independent associations of selected predictor variables with pulsotype. Separate models were constructed for the 3 most prevalent pulsotypes, the high-frequency pulsotypes, and the multiple-isolate pulsotypes, by using as candidate predictor variables 1 representative from each epidemiologic or resistance category (year, ecologic source, locale, fluoroquinolone phenotype, and ESBL status). ESBL status was a significant predictor in all 5 resulting models, as was fluoroquinolone resistance in 4 models (fluoroquinolone resistance was excluded from the fifth model because of its 100% prevalence in pulsotype 812), year in 3 models, and human source in 2 models (Table 8). In contrast, US origin (the representative geographic variable) was not a significant predictor in any model.

Table 6. Distribution, by ecologic source, of *Escherichia coli* ST131 isolates among different pulsotype groups and individual pulsotypes, 1967–2009*

Ecologic source	Total isolates, N = 579	No. (%) isolates						
		High-prevalence pulsotypes, n = 327	Multiple-isolate pulsotypes, n = 474	Individual high-prevalence pulsotypes				
				968, n = 136	800, n = 82	812, n = 32	1202, n = 8	955, n = 7
Human	486 (84)	**290 (89)**†	**412 (87)**†	117 (86)	**81 (99)**†	**31 (97)**‡	**1 (13)**†	0 (0)
Comp. animal	22 (4)	**19 (6)**#	21 (4)	**13 (10)**†	0	1 (3)	1 (8)	**2 (29)**§
Food animal	45 (8)	**8 (2)**†	**28 (6)**†	**0**†	**0**†	0	**6 (75)**†	2 (29)
Other animal	10 (1.7)	6 (2)	7 (1.5)	**6 (4)**§	0	0	0	0
Food/water	15 (2.6)	**4 (1)**‡	**6 (1)**†	**0**‡	1 (1)	0	0	3 (43)
Food	6 (1.0)	3 (0.9)	4 (0.8)	0	0	0	0	**3 (43)**†
Water	9 (1.6)	**1 (0.3)**§	**2 (0.4)**†	0	1 (1)	0	0	0

*__Boldface__ indicates $p<0.05$ in comparison with all other isolates. Pulsotypes are those, among the 12 high-prevalence pulsotypes, that exhibited a significant association with ≥1 source group. ST, sequence type; comp., companion.
†$p<0.001$ for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).
‡$p \leq 0.05$ for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).
§$p \leq 0.01$ for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).

Table 7. Distribution, by antimicrobial drug resistance trait, of *Escherichia coli* ST131 isolates among different pulsotype groups and individual pulsotypes, 1967–2009*

Antimicrobial drug resistance trait	No. (%) isolates											
	Total no. isolates, N = 579	High-prevalence pulsotypes, n = 327	Multiple-isolate pulsotypes, n = 474	Individual high-prevalence pulsotypes								
				968, n = 136	800, n = 82	812, n = 32	905, n = 19	987, n = 11	1202, n = 8	919, n = 7	955, n = 7	797, n = 6
FQ-R	462 (80)	**293 (90)**†	**401 (85)**†	**133 (98)**†	**81 (99)**†	**32 (100)**†	**19 (100)**‡	**0**†	**1 (13)**†	**7 (100)**§	**1 (14)**†	**0**†
ESBL	272 (47)	144 (44)	219 (46)	62 (46)	**12 (15)**†	**30 (94)**†	**17 (90)**†	**0**†	3 (38)	**7 (100)**§	**2 (29)**§	**0**‡
bla$_{CTX-M-15}$	188 (33)	102 (31)	156 (33)	38 (28)	**5 (6)**†	**28 (88)**†	**16 (84)**†	**0**‡	**0**‡	**6 (86)**§	0	0

*__Boldface__ indicates p <0.05 in comparison with all other isolates. Pulsotypes shown are those, among the 12 high-prevalence pulsotypes, that exhibited a significant association with ≥1 resistance trait. ST, sequence type; FQ-R, fluoroquinolone resistance; ESBL, extended-spectrum β-lactamase production; *bla*$_{CTX-M-15}$, gene encoding CTX-M-15.
†p≤0.001 for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).
‡p<0.05 for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).
§p≤0.01 for indicated pulsotype group or pulsotype vs. all others (Fisher exact test).

Indistinguishable PFGE Profile Isolates

We also assessed associations with other variables for the 7 largest clusters of isolates with indistinguishable PFGE profiles; each cluster contained 4–5 isolates. Of the 31 constituent isolates, 28 were recent (2007–2009) and the other 3 were from 2002 or 2004. Of the 7 clusters, 6 included isolates from multiple locales, from multiple continents in 3 instances. In contrast, only 3 clusters came from multiple host species. Whereas each cluster was internally homogeneous for fluoroquinolone phenotype (6 all-resistant clusters, 1 all-susceptible cluster), 4 were internally heterogeneous according to ESBL and/or *bla*CTX-M-15 status.

Discussion

We used PFGE analysis to define population structure among 579 diverse *E. coli* ST131 isolates and then assessed temporal, geographic, ecologic, and resistance trait associations for the various pulsotypes, i.e., presumed sub-ST genetic lineages. Our findings support 4 main conclusions. First, although ST131 is highly diverse at the pulsotype level, a small number of high-frequency pulsotypes predominate, and pulsotype 968 accounts for 24% of the population. Second, pulsotypes differ in prevalence over time; high-prevalence pulsotypes tend to occur in more recent years, consistent with recent emergence and expansion, implying greater fitness. Third, whereas broad geographic distribution predominates over locale-specific segregation, implying widespread dispersal

Table 8. Results of multivariable logistic regression analysis for predictors of selected pulsotype categories among 579 *Escherichia coli* ST131 isolates, 1967–2009*

Outcome variable, significant predictor variables†	Odds ratio (95% CI)	p value	Nagelkerke R^2 for model‡
Pulsotype 968			0.18
Human source	0.20 (0.09–0.46)	<0.001	
FQ-R	55.04 (10.63–285.03)	<0.001	
ESBL production	0.63 (0.41–0.90)	0.03	
Pulsotype 800			0.31
Human source	10.61 (1.32–85.48)	0.03	
FQ-R	46.50 (3.75–576.74)	.003	
ESBL production	0.10 (0.05–0.19)	<0.001	
Pulsotype 812			0.21
Year of isolation/submission	1.47 (1.07–2.03)	0.02	
ESBL production	17.53 (4.02–76.07)	<0.001	
High-prevalence pulsotypes			0.16
Year of isolation/submission	1.09 (1.03–1.55)	0.003	
FQ-R	4.04 (2.22–7.34)	<0.001	
ESBL production	0.47 (0.32–0.70)	<0.001	
Multiple-isolate pulsotypes			0.13
Year of isolation/submission	1.07 (1.02–1.13)	0.009	
FQ-R	3.42 (1.76–6.66)	<0.001	
ESBL production	0.42 (0.24–0.71)	0.001	

*ST, sequence type; FQ-R, fluoroquinolone resistance/resistant; ESBL, extended-spectrum β-lactamase; high-prevalence pulsotypes, 12 pulsotypes that contained ≥6 isolates (≥1% of population) each; multiple-isolate pulsotypes, 65 pulsotypes that contained ≥1 isolate each.
†Variables shown are those that yielded p values of <0.05. With 1 exception, each model included the following candidate predictor variables: human source (vs. other sources), US origin (vs. Canada or other international locale origin), year of isolation/submission, FQ-R, and ESBL production. All pulsotype 812 isolates were FQ-R; thus, FQ-R could not be included in that model.
‡Nagelkerke R^2 provides an estimate of the total amount of variance accounted for by the model, i.e., the model's explanatory power. Values range from 0 (no explanatory power) to 1.0 (complete prediction).

rather than localized endemicity, segregation by ecologic source predominates over across-source commonality, implying niche adaptation rather than broad host-range capability and interspecies transmission. Fourth, resistance traits (i.e., fluoroquinolone resistance, ESBL production, and $bla_{\text{CTX-M-15}}$) are highly pulsotype-specific, suggesting predominantly subclonal distribution.

The striking prevalence disparities among pulsotypes suggest that certain pulsotypes, especially the exceptionally successful pulsotype 968, possess fitness advantages over others. In retrospect, pulsotype 968 accounted for all previously reported household clusters, 3 of which involved serious or fatal disease in ≥1 household members (*9*–*12*). A possible founder effect for type 968 is unlikely because the pulsotypes that were detected earliest were mostly low-prevalence types; higher prevalence pulsotypes appeared only later, seemingly outcompeting the low-prevalence types. In regard to possible fitness advantages, ESBL production and $bla_{\text{CTX-M-15}}$ were significantly associated with several high-prevalence pulsotypes and were present in most or all of their members (Table 7). However, they were not significantly associated with (predominant) pulsotype 968 and so are unlikely the main explanation for the recent expansion of ST131, of which pulsotype 968 was the single main component (Figure). In contrast, fluoroquinolone resistance was significantly associated with each of the 4 most prevalent pulsotypes and collectively with the 12 high-prevalence and 65 multiple-isolate pulsotypes. Thus, fluoroquinolone resistance may have made a major contribution to the recent expansion of ST131.

Although the predominant pattern was broad dispersal of pulsotypes, localized segregation also occurred. These trends imply considerable ongoing dissemination and intermixing of ST131 lineages among locales (sufficient to largely preclude establishment of locale-specific populations) but with variable degrees of intermixing versus segregation by locale and pulsotype. For example, the US Midwest and Canada shared pulsotypes more extensively than did other regions. Conversely, non-Canadian international locales and the US West had less pulsotype commonality with other regions (i.e., had more highly locale-specific populations) than did other locales. Several high-prevalence pulsotypes similarly exhibited distinct patterns of distribution and were variably concentrated in specific regions. Similar patterns have been described previously for ST131 but in lesser detail and without statistical analysis (*3*,*6*,*8*,*17*,*24*,*25*). The undefined mechanisms for the ongoing dispersal of ST131, possibly including international travel and commerce, wild bird migration, and foodborne or waterborne transmission, and its limited locale-specific segregation by pulsotype warrant study.

The associations of specific pulsotypes with different ecologic sources are relevant to the dispersal mechanisms of ST131. In contrast to the striking geographic dissemination of pulsotypes, we also found some evidence of niche segregation. Positive associations for niche segregation were found between humans and water, companion animals and other animals, and food animals and food. In contrast, negative associations were found between humans and most other sources, companion animals or other animals and food or food animals, and water and companion animals or food animals. These findings implicate humans as the source for ST131 isolates found in water and implicate food animals as the source for isolates found in food. In contrast, and consistent with findings in most, but not all, previous studies (*13*,*26*–*28*), these findings indicate that food animals and food are not major sources of ST131 for humans. They also suggest no special pet–human commonality of ST131 pulsotypes, notwithstanding some well-documented overlap (*29*). This argues against pets and the food supply as major vehicles for dissemination of ST131 strains among humans. Indeed, in 1 study, 7% of healthy humans were found to be colonized intestinally with an ST131 strain (*30*); thus, humans may be the main reservoir for human-associated strains. A larger, more current, and more systematically assembled study population is needed to confirm the findings of the present study.

Until now, the earliest reported isolate of ST131 was from a patient with urosepsis in 1985 (*8*). Here, we report 3 earlier isolations, from 1967, 1982, and 1983, none of which were from a high-prevalence pulsotype. This finding documents the presence of ST131 decades before its emergence as a disseminated human pathogen and suggests an opportunity to compare early isolates with recent isolates for characteristics that might confer enhanced fitness, possibly contributing to the emergence of ST131.

Our study had limitations. First, the population was a convenience sample with multiple possible sources of bias. Second, despite considerable diversity, the population was not balanced; it predominantly comprised recent isolates from human in the United States, reducing both generalizability and power for comparisons involving other times, sources, and regions. Third, minimal associated data (especially clinical details) were available for many isolates, limiting the possible epidemiologic analyses. Fourth, PFGE profiles reflect genetic relationships only indirectly and require subjective interpretation. Fifth, the multiple comparisons could have produced spurious associations by chance alone. However, the proportion of comparisons yielding a significant p value was much greater, and the associated p values much smaller, than should occur by chance alone. Last, the 94% PFGE similarity pulsotype criterion was somewhat arbitrary and possibly suboptimal; however, an alternate 100% similarity criterion yielded qualitatively similar conclusions.

Our study also had strengths. The population was the largest reported to date for ST131 (*2*) and the most extensively distributed by time, source, and region. The PFGE analysis was conducted by 1 experienced observer in 1 laboratory by using software that enabled concurrent comparisons for all isolates. Diverse univariable and multivariable statistical approaches were used, pulsotypes were analyzed collectively and individually, and PFGE profiles were assessed by using 2 similarity thresholds (94% and 100%) and in a dendrogram.

Thus, within a large, diverse collection of *E. coli* ST131 isolates, we documented extensive PFGE profile diversity and a predominance of certain high-prevalence pulsotypes (particularly pulsotype 968, 24% overall) that exhibited distinctive temporal patterns of emergence. Notwithstanding some geographic localization, pulsotypes were extensively dispersed by region. In contrast, they were more highly source specific; in particular, isolates from humans exhibited almost no commonality with isolates from food animals or foods. Pulsotype 968 was much more closely associated with fluoroquinolone resistance than with ESBL production or $bla_{\text{CTX-M-15}}$, suggesting a greater role for fluoroquinolone resistance than ESBLs in the expansion of this dominant pulsotype and ST131 in general. These findings considerably advance our understanding of the genetic structure, ecology, geographic distribution, and emergence of this widely disseminated antimicrobial drug–resistant pathogen, which represents a growing public health threat.

The MASTER (Macrorestriction Analysis of ST131 for Epidemiologic Research) Investigators who contributed data include Jo-Ellyn Abraham (Abbott-Northwestern Hospital, Minneapolis, Minnesota, USA), Javier Adachi (The University of Texas MD Anderson Cancer Center, Houston, Texas, USA), Aristides Assimacopoulos (University of South Dakota, Souix Falls, South Dakota, USA), Robert L. Bergsbaken (Health Partners and Regions Medical Center, St. Paul, Minnesota, USA), Jorge Blanco (University of Santiago de Compostela, Lugo, Spain), Michael Cooperstock (University of Missouri School of Medicine, Columbia, Missouri, USA), Paul Edelstein (Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania, USA), Peter T. Ender (St. Luke's Hospital and Health Network, Bethlehem, Pennsylvania, USA), Nancy Hansen (Creighton University, Omaha, Nebraska, USA), John Holter (Veterans Affairs Medical Center, Minneapolis), Thomas M. Hooton (University of Miami, Miami, Florida, USA), Michelle Hulse (Childrens Hospital, Minneapolis), James H. Jorgensen and James S. Lewis II (University Health System, San Antonio, Texas, USA), Karen Lolans (Rush Medical School, Chicago, Illinois, USA), Sybille Miller (Leesburg Veterinary Internal Medicine, Leesburg, Virginia, USA), Rob Owens (Cubist Pharmaceuticals, Falmouth, Maine, USA), Elizabeth Palavecino (Wake Forest University Baptist Medical Center, Winston-Salem, North Carolina, USA), David Paterson (University of Pittsburgh, Pittsburgh, Pennsylvania, USA, and Queensland University, Brisbane, Queensland, Australia), Johann D. D. Pitout (Calgary Laboratory Services, Calgary, Alberta, Canada), John P. Quinn (Pfizer Global Research, Groton, Connecticut, USA), James Rice (The Scripps Research Institute, La Jolla, California, USA), Daniel F. Sahm (Eurofins-Medinet, Chantilly, Virginia, USA), and Karen Vigil (University of Texas Health Sciences Center at Houston, Houston)

This material is based on work supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs (J.R.J.). Dave Prentiss (Minneapolis VA Medical Center) prepared the figures.

J.R.J. received research funding from Merck and Rochester Medical Group; J.S.L. received research funding and/or honoraria from Merck, Ortho-McNeil, and Pfizer; J.P.Q. is an employee of Astra-Zeneca and a shareholder in Pfizer Global Research.

Dr Johnson is professor of medicine and senior associate director of the infectious diseases fellowship program at the University of Minnesota and head of the molecular epidemiology laboratory at the Minneapolis VA Medical Center. His research focuses on the molecular epidemiology, ecology, evolution, and virulence of extraintestinal pathogenic and antimicrobial drug–resistant *E. coli*.

References

1. Peirano G, Pitout JDD. Molecular epidemiology of *Escherichia coli* producing CTX-M β-lactamases: the worldwide emergence of clone ST131 O25:H4. Int J Antimicrob Agents. 2010;35:316–21. http://dx.doi.org/10.1016/j.ijantimicag.2009.11.003
2. Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother. 2011;66:1–14. http://dx.doi.org/10.1093/jac/dkq415
3. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Caniça MM, et al. Intercontinental emergence of *Escherichia coli* clone O25:H4–ST131 producing CTX-M-15. J Antimicrob Chemother. 2008;61:273–81. http://dx.doi.org/10.1093/jac/dkm464
4. Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, et al. The CTX-M-15producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J Antimicrob Chemother. 2008;61:1024–8. http://dx.doi.org/10.1093/jac/dkn084
5. Coque TM, Novais Â, Carattoli A, Poirel L, Pitout J, Peixe L, et al. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. Emerg Infect Dis. 2008;14:195–200. http://dx.doi.org/10.3201/eid1402.070350
6. Gibreel TM, Dodgson AR, Cheesbrough J, Fox AJ, Bolton FJ, Upton M. Population structure, virulence potential and antibiotic susceptibility of uropathogenic *Escherichia coli* from northwest England. J Antimicrob Chemother. 2012;67:346–56. http://dx.doi.org/10.1093/jac/dkr451

7. Totsika M, Beatson SA, Sarkar S, Phan MD, Petty NK, Bachmann N, et al. Insights into a multidrug resistant *Escherichia coli* pathogen of the globally disseminated ST131 lineage: genome analysis and virulence mechanisms. PLoS ONE. 2011;6:e26578. http://dx.doi.org/10.1371/journal.pone.0026578
8. Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States (2007). Clin Infect Dis. 2010;51:286–94. http://dx.doi.org/10.1086/653932
9. Johnson JR, Anderson JT, Clabots C, Johnston B, Cooperstock M. Within-household sharing of a fluoroquinolone-resistant *Escherichia coli* sequence type ST131 strain causing pediatric osteoarticular infection. Pediatr Infect Dis J. 2010;29:473–5. http://dx.doi.org/10.1097/INF.0b013e3181c89bd7
10. Ender PT, Gajanana D, Johnston B, Clabots C, Tamarkin FJ, Johnson JR. Transmission of extended-spectrum beta-lactamase-producing *Escherichia coli* (sequence type ST131) between a father and daughter resulting in septic shock and emphysematous pyelonephritis. J Clin Microbiol. 2009;47:3780–2. http://dx.doi.org/10.1128/JCM.01361-09
11. Owens RC, Johnson JR, Stogstill P, Yarmus L, Lolans K, Quinn J. Community transmission in the United States of a CTX-M-15-producing sequence type ST131 *Escherichia coli* strain resulting in death. J Clin Microbiol. 2011;49:3406–8. http://dx.doi.org/10.1128/JCM.00993-11
12. Johnson JR, Miller S, Johnston B, Clabots C. DebRoy C. Sharing of *Escherichia coli* sequence type ST131 and other multidrug-resistant and urovirulent *E. coli* strains among dogs and cats within a household. J Clin Microbiol. 2009;47:3721–5. http://dx.doi.org/10.1128/JCM.01581-09
13. Platell JL, Johnson JR, Cobbold RN, Trott DJ. Multidrug-resistant extraintestinal pathogenic *Escherichia coli* of sequence type ST131 in animals and foods. Vet Microbiol. 2011;153:99–108. http://dx.doi.org/10.1016/j.vetmic.2011.05.007
14. Johnson JR, Johnston B, Clabots C, Kuskowski MA, Pendyala S, DebRoy C, et al. *Escherichia coli* sequence type ST131 as an emerging fluoroquinolone-resistant uropathogen among renal transplant recipients. Antimicrob Agents Chemother. 2010;54:546–50. http://dx.doi.org/10.1128/AAC.01089-09
15. Vigil KJ, Johnson JR, Johnston BD, Kontoyiannis DP, Mulanovich VE, Raad II, et al. *Escherichia coli* pyomyositis: an emerging entity among patients with hematologic malignancies. Clin Infect Dis. 2010;50:374–80. http://dx.doi.org/10.1086/649866
16. Urban C, Mariano N, Bradford PA, Tuckman M, Segal-Maurer S, Wehbeh W, et al. Identification of CTX-M β-lactamases in *Escherichia coli* from hospitalized patients and residents of long-term care facilities. Diagn Microbiol Infect Dis. 2010;66:402–6. http://dx.doi.org/10.1016/j.diagmicrobio.2009.11.012
17. Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002–2004. Antimicrob Agents Chemother. 2009;53:2733–9. http://dx.doi.org/10.1128/AAC.00297-09
18. Sidjabat HE, Paterson DL, Adams-Haduch JM, Ewan L, Pasculle AW, Muto CA, et al. Molecular epidemiology of CTX-M–producing *Escherichia coli* isolates at a tertiary medical center in western Pennsylvania. Antimicrob Agents Chemother. 2009;53:4733–9. http://dx.doi.org/10.1128/AAC.00533-09
19. Lewis JS II, Herrera M, Wickes B, Patterson JE, Jorgensen JH. First report of the emergence of CTX-M–type extended-spectrum beta-lactamases (ESBLs) as the predominant ESBL isolated in a US health care system. Antimicrob Agents Chemother. 2007;51:4015–21. http://dx.doi.org/10.1128/AAC.00576-07
20. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. Foodborne Pathog Dis. 2006;3:59–67. http://dx.doi.org/10.1089/fpd.2006.3.59
21. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995;33:2233–9.
22. Clinical Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests; approved standard—tenth edition. M02–A10. Wayne (PA): The Institute; 2009.
23. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. M100–S19. Wayne (PA): The Institute; 2009.
24. Blanco M, Alonso MP, Nicolas-Chanoine MH, Dahbi G, Mora A, Blanco JE, et al. Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases in Lugo (Spain): dissemination of clone O25b:H4–ST131 producing CTX-M-15. J Antimicrob Chemother. 2009;63:1135–41. http://dx.doi.org/10.1093/jac/dkp122
25. Manges AR, Tabor H, Tellis P, Vincent C, Tellier P-P. Endemic and epidemic lineages of *Escherichia coli* that cause urinary tract infections. Emerg Infect Dis. 2008;14:1575–83. http://dx.doi.org/10.3201/eid1410.080102
26. Dhanji H, Murphy NM, Doumith M, Durmus S, Lee SS, Hope R, et al. Cephalosporin resistance mechanisms in *Escherichia coli* isolated from raw chicken imported into the UK. J Antimicrob Chemother. 2010;65:2534–7. http://dx.doi.org/10.1093/jac/dkq376
27. Mora A, Herrera A, Mamani R, López C, Alonso MP, Blanco JE, et al. Recent emergence of clonal group O25b:K1:H4–B2-ST131 *ibeA* strains among *Escherichia coli* poultry isolates, including CTX-M-9producing strains, and comparison with clinical human isolates. Appl Environ Microbiol. 2010;76:6991–7. http://dx.doi.org/10.1128/AEM.01112-10
28. Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, et al. Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis. 2010;16:88–95. http://dx.doi.org/10.3201/eid1601.091118
29. Platell JL, Cobbold RN, Johnson JR, Heisig A, Heisig P, Clabots C, et al. Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal *Escherichia coli* isolates from humans and companion animals in Australia. Antimicrob Agents Chemother. 2011;55:3782–7. http://dx.doi.org/10.1128/AAC.00306-11
30. Leflon-Guibout V, Blanco J, Amaqdouf K, Mora A, Guize L, Nicolas-Chanoine MH. Absence of CTX-M enzymes but high prevalence of clones, including clone ST131, among fecal *Escherichia coli* isolates from healthy subjects living in the area of Paris, France. J Clin Microbiol. 2008;46:3900–5. http://dx.doi.org/10.1128/JCM.00734-08

Address for correspondence: James R. Johnson, VA Medical Center, Infectious Diseases (111F), 1 Veterans Dr, Minneapolis, MN 55417, USA; email: johns007@umn.edu

Lessons Learned during Dengue Outbreaks in the United States, 2001–2011

Amesh A. Adalja, Tara Kirk Sell, Nidhi Bouri, and Crystal Franco

Since 2001, three autochthonous dengue fever outbreaks have occurred in the United States: in Hawaii (2001); Brownsville, Texas (2005); and southern Florida (2009–2011). We sought to characterize and describe the response to these outbreaks from the perspectives of public health and vector control officials. By conducting a medical literature review through PubMed and news media searches through Google, we identified persons involved in managing each outbreak; 26 persons then participated in qualitative, semistructured interviews. After analyzing the 3 outbreaks, we found the following prominent themes in the response efforts: timely detection of illness; communication of up-to-date, correct information; and development of a rapid response that engages the community. We therefore recommend that public health authorities involve the clinical and laboratory community promptly, provide accurate information, and engage the local community in vector control and case identification and reporting.

Dengue is a mosquito-borne viral disease, endemic to tropical regions. In the United States, most dengue infections have been limited to travelers returning from dengue-endemic regions; the last outbreak in the continental United States occurred in 1945 (*1*). However, epidemic dengue remains a threat to US areas that have competent mosquito vector populations and host large numbers of travelers from dengue-endemic regions, as evidenced by the return of dengue to Florida (*1*).

Practical experience with dengue in the United States is decades old, and mitigation measures used decades ago may not be fully applicable today. As the threat of dengue grows, the risks for an outbreak and the responses needed must be understood.

Author affiliation: University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA

DOI: http://dx.doi.org/10.3201/eid1804.110968

In this article, we describe the responses to 3 recent US dengue outbreaks (in Hawaii, 2001; Brownsville, Texas, 2005; and southern Florida, 2009–2011) from the perspectives of public health and vector control officials at local, state, and federal levels. We conducted a retrospective analysis to assess mitigation strategies used during each outbreak and identify policy implications for public health departments, vector control agencies, and clinicians in areas vulnerable to dengue and other mosquito-borne diseases. The goal of this study was to help improve community responses to future dengue outbreaks. The analysis concludes with recommendations for practitioners and policy makers.

Methods

To understand the outbreaks and identify contacts involved in managing each outbreak, the research team reviewed the medical literature through PubMed and also searched Google to identify names of outbreak management officials. Researchers then asked these persons to participate in qualitative, semistructured interviews. Additional participants were also identified and added throughout the interview process. In total, 26 persons were interviewed (9 from the Hawaii outbreak, 10 from the Florida outbreak, and 7 from the Texas outbreak). The interviewees from each outbreak included heads of local health departments, personnel from the US Centers for Disease Control and Prevention (CDC), vector control officers, and state and local health department staff.

During the interviews, the research team posed open-ended questions aimed at eliciting an understanding of how each outbreak was discovered and the outbreak management techniques, case-finding methods, and public outreach/engagement strategies that were used (online Technical Appendix, wwnc.cdc.gov/EID/pdfs/11-0968-Techapp.pdf). In addition, questions were posed regarding

the usefulness of specific interventions, including the interactions between city, county, state, and federal public health authorities, and outreach to physicians. As a final question, each interview participant was asked to describe what he or she would have done differently or what activities he or she would recommend for a future outbreak.

Outbreak Findings

Hawaii, 2001

Initial Case

The Hawaii outbreak was discovered in September 2001 by a non-island physician temporarily employed in the rural region of Hana on Maui. Before 2001, autochthonous dengue infections had last been definitively reported in Hawaii in 1944. (Two suspected cases were reported to have occurred in German travelers to Hawaii in 1995 [*2*]. In March 2011, however, 4 cases of dengue were confirmed on Oahu. Although travel-related dengue is not an uncommon diagnosis in Hawaii, these cases were acquired locally [autochthonous] [*3*].) Therefore, dengue was not considered in the differential diagnosis for persons without a travel history who sought treatment. The physician made a clinical diagnosis on the basis of the initial patient's symptoms and alerted the Hawaii Department of Health, prompting an investigation that uncovered additional suspected autochthonous cases. However, laboratory confirmation was delayed because of the events of September 11, 2001, after which air travel was suspended and specimens could not be shipped to CDC. The outbreak was not officially confirmed until September 21, 2001 (*4*).

Outbreak

In all, 122 laboratory-confirmed cases were identified through 2002 (92 on Maui, 26 on Oahu, and 4 on Kauai). All isolates were typed as dengue virus type 1 (DENV-1) and had a specific envelope glycoprotein sequence, indicating that the strain was likely imported by travelers from French Polynesia, where a large DENV-1 epidemic caused by the same genotype was occurring (*4*). This outbreak was unique because it involved the less competent dengue mosquito vector *Aedes albopictus* (some interviewees speculated that this may have caused the outbreak to end relatively quickly). Cases began in a rural region of Maui, and subsequent cases were centered in areas with thick vegetation and heavy precipitation. Infections on the other islands represented local transmission. In 1 study, case-patients were found to be ≈7 times more likely to live in homes with birds, chickens, or both, than in homes without these animals (*5*). The response was managed at the state level, with state district health officials in the chain of command. CDC provided technical assistance. Interviewees noted that during the outbreak, tension existed between responding parties over jurisdictional issues that largely remained unresolved.

In addition to the epidemiologic and vector control response to the outbreak, officials also had to address issues that were politically and publicly sensitive. Because the Hawaii economy depends on tourism, the response had to balance the need for protective action on the part of local residents and tourists with the need to avoid discouraging tourism. Additionally, although some members of the public were concerned about the negative effects of pesticide use, others demanded that spraying be conducted around schools (which had questionable utility in combating the outbreak). Finally, community engagement practices had to be tailored to the needs of specific localities. For example, on 1 island, attendance at town hall meetings was high, but on another island, attendance at similar meetings was low. However, public health officials believed that residents of this second island were more receptive to receiving information from fliers distributed in general stores.

Mitigation and Response

After dengue cases in Maui were discovered, state health department officials began an aggressive campaign of public engagement involving town hall meetings, door-to-door campaigns to identify case-patients and educate the public about mosquito abatement, and media messaging (television, radio, and Internet). A public relations agency was hired to help manage questions from the public. The state health department announced daily case counts at press conferences, and highway checkpoints were established for distribution of mosquito repellent. In addition, health officials engaged car rental agencies and hotels to distribute educational brochures for travelers and tourists.

Upon request, CDC deployed Epidemiologic Intelligence Service officers as well as vector experts to Hawaii. *Ae. albopictus* mosquitoes were soon found to be propagating the outbreak. Vector control activities included spraying to kill adult mosquitoes (adulticiding) within a 200-m radius around homes of case-patients, breeding-site control activities such as trash collection and elimination of standing water, and door-to-door campaigns to educate the public about eliminating mosquitoes around homes.

Persons who were interviewed emphasized that during vector control activities, they focused on addressing mosquito breeding sites and not on potential building code violations or the farming of prohibited plants. Outreach to clinicians included grand rounds presentations, visits with clinicians, and encouragement and support for clinicians to conduct testing of suspected case-patients.

An unpublished communications study was conducted by the Hawaii Department of Health during the outbreak

to assess the general public's response to key public health messages. Some key conclusions from this study included the finding that 16 (40%) of 90 residents surveyed stated that they took action to prevent dengue. Of those who took action, 74% eliminated stagnant water outside their homes and 63% took action to prevent mosquitoes from entering their homes (Hawaii Department of Health, unpub. data). Conclusions drawn from Hawaii's response to the outbreak are listed in the Table.

Brownsville, Texas, 2005

Initial Case

In July 2005, a diagnosis of dengue hemorrhagic fever (DHF) was made for a woman who had become ill with symptoms consistent with dengue in June. She had traveled from Brownsville to Mexico for treatment and received a clinical diagnosis of dengue in Mexico. She returned to the United States and was hospitalized as symptoms progressed. At that time, although she was given a diagnosis of murine typhus in Texas, doctors conducted serologic testing for dengue virus. The collection of blood samples was facilitated by CDC's Border Infectious Disease Surveillance project in conjunction with other CDC programs. The woman had no history of travel to Mexico in the 2 months before her illness, and her current dengue infection (presumably not her first, given the occurrence of DHF) had occurred in Texas (*6*,*7*).

Outbreak

Limited outbreaks of locally acquired dengue have occurred sporadically since 1980 in areas of Texas that border Mexico (*7*,*8*). During the 2005 Brownsville outbreak, 25 cases of dengue were found, 3 autochthonous cases and 22 in persons who had traveled to Mexico. This outbreak was part of an epidemic that included 1,251 cases of dengue in the bordering Mexican state of Tamaulipas during August 2005 (*7*). The outbreak was managed by using city, county, and state resources; CDC conducted Border Infectious Disease Surveillance project work and serosurveys. Laboratory testing for cases was performed by the state department of health. Serosurveys indicated evidence of recent dengue virus infection in 4% of the population of Brownsville (*6*. In a risk factor analysis, Brownsville residents with properties smaller than the median lot size were 15 times more likely to be seropositive for dengue, whereas non–US-born residents were 3 times more likely to be seropositive (*6*).

Mitigation and Response

Because of the risk of acquiring dengue in the regions of Texas bordering dengue-endemic Mexico, officials at the Texas State Department of Health had conducted a series of workshops in 2004 to develop mitigation and response tools, including plans for community clean-up days as well as a school play to educate schoolchildren about dengue. After the initial case was identified in 2005, health officials expanded case-finding activities through direct contact with clinicians, medical record reviews, and serosurveys.

Once the outbreak was confirmed, health officials undertook additional education efforts, including town hall meetings, visits to physicians' offices, and media messaging (including the media in Mexico). The main thrust of their efforts was to increase discovery, diagnosis, and reporting of cases. This effort included facilitating collection of blood samples and testing them at the state health department. Increased vector control activities, including sampling of mosquitoes for dengue virus, were also conducted. However, some interviewees questioned the use of the latter because it was thought to divert resources and have poor predictive power. Health officials stressed that in areas of Brownsville with clusters of "fevers of unknown

Table. Lessons learned during US dengue outbreaks, 2001–2011*

Location, year	Lessons learned
Hawaii, 2001	Populations are not completely homogeneous, and messages should be tailored to specific locales. Tourism concerns must be balanced with public health response. Community engagement activities are palatable to the public when nonpunitive, actionable initiatives are undertaken by public health agencies. A communication study validates the community engagement approach, with substantial numbers of residents aware of the outbreak and those taking actions performing the correct action. A lack of in-state testing capacity delays confirmation of the outbreak. Although the *Aedes albopictus* mosquito is a competent vector, its involvement may limit this outbreak in a rural Hawaii setting, especially with prompt outbreak control efforts.
Brownsville, Texas, 2005	Nearby foci of endemicity make dengue a continual threat, including the possibility of dengue hemorrhagic fever. Involving CDC/BIDS facilitates fast identification of the index case. Pre-outbreak awareness of and preparation for the potential threat of dengue enhances the ability to respond to an actual outbreak.
Florida, 2009–2011	An aggressive multimodal campaign engages the public. Door-to-door vector control activities are essential; the ability to inspect property without homeowner permission improves coverage. Clear communication with tourism officials diminishes the possibility of opposing viewpoints.

*CDC, Centers for Disease Control and Prevention; BIDS, Border Infectious Disease Surveillance.

origin" (possibly representing unrecognized dengue cases), vector control was crucial.

Currently, Brownsville ranks dengue as a top priority and maintains ongoing efforts to combat it, including reducing mosquito breeding sites and increasing public awareness of dengue symptoms through a federal Environmental Protection Agency grant (e.g., encouraging use of reusable shopping bags with dengue information, direct mailings, and television interviews with health authorities).

The Brownsville outbreak also highlights the need for ongoing surveillance for vector-borne diseases, especially as decreases in funding for these activities are anticipated. The conclusions we drew from Brownsville's response are listed in the Table.

Florida, 2009–2011

Initial Case

In September 2009, a physician in New York notified Florida's Monroe County Health Department of a diagnosis of dengue fever in a traveler returning from Key West (the patient had not traveled to other locations), heralding the first autochthonous dengue case in Florida since 1934. After identification of the first case, enhanced case-finding activities uncovered more autochthonous cases in Key West (*1*).

Outbreak

Ultimately, in Key West, Monroe County, 90 cases were identified as part of the outbreak (27 cases in 2009 and 63 in 2010) (*1*,*9*). A 2009 serosurvey indicated that 5.4% of Key West residents had evidence of recent dengue virus infection (*1*). In 2010–2011, autochthonous dengue fever was also discovered in 5 other Florida counties: Broward (1 case), Hillsborough (1 case), Martin (1 case), Palm Beach (2 cases), and Miami-Dade (3 cases). In at least 2 of these instances, the dengue serotype recovered was distinct from the Monroe County serotype, indicating >1 introduction of dengue into Florida (*9*–*11*). Interviewees from Key West stated that their cases were centered in the Old Town area of Key West, a tourist area and where the so-called Key West lifestyle is common. Interviewees stated that this lifestyle, which involves spending a high proportion of time outdoors and keeping house windows open, was thought to be responsible for the transmission of the virus.

Mitigation and Response

At the time of discovery of the outbreak, the priority of Monroe County Health Department was to prevent deaths from dengue. The health department began a campaign in collaboration with the Florida Keys Mosquito Control District to control the outbreak. Response strategies used in Key West included town hall meetings, door-to-door visits/inspections, a public information telephone line, tourism council press releases, editorials, dispatching a biologist to schools, and the use of a television program (called Mosquito TV). Outreach to clinicians was performed through visits from health department personnel. Interviewees also stated that a frank discussion with tourism officials was held, which created an environment in which public health, vector control, and tourism officials could work together. Many of the materials and response activities developed by Monroe County were used in other counties in Florida that experienced dengue cases.

Vector control activities focused on door-to-door visits at residences to assess the prevalence of mosquito breeding sites so authorities could intervene if mosquitoes were found. When breeding sites were discovered, residents were asked to participate in inspections of their property and were instructed on how to eliminate breeding sites. In some cases, when properties proximate to dengue case-patients were not easily accessible (because of a resident's absence), vector control officers had to scale fences. Although Florida law sanctions these actions and authorizes fines for those who hinder mosquito control, officials did not issue fines. Vector control officials reported that often the same properties had repeat violations, highlighting the difficulties in changing behavior. In Florida, funding for vector control activities varies at the county level; for example, Monroe County is funded by a dedicated property tax (which covers vector as well as nuisance mosquitoes), while Broward and Miami-Dade vector control activities are funded through general funds. The variance in funding sources for vector control efforts affects the annual amount of funding allocated because budgets may allocate funds for vector control differently each year.

In January 2011, the Monroe County Health Department launched an initiative called ABCD (Action to Break the Cycle of Dengue), which is designed to "draw more members of the public into the fight against" dengue. The program has performed such activities as encouraging cemeteries to dump standing water, a "Fight the Bite" poster contest, continued posting of door hangers with dengue information, neighborhood cleanups of mosquito breeding sites, and creating cartoon characters to communicate information about dengue to children (and adults) (Figures 1, 2). Conclusions drawn from Florida's response to the outbreak are listed in the Table.

3 Key Recommendations

Involve Clinical and Laboratory Community Promptly

First, we recommend that state and local public health agencies in areas at risk for autochthonous dengue engage the clinical community and develop capacity to better

Figure 1. Example of an activity to engage the public in controlling dengue outbreaks, Florida, USA, 2009–2011.

ensure prompt clinical diagnosis and laboratory detection of this disease. Early recognition and identification of dengue, a nationally reportable disease since 2010, are critical for successful response. In 2 of the outbreaks (Hawaii and Florida), clinicians from outside the outbreak area made the diagnosis. After suspected dengue cases were reported, local health department and mosquito control officials were able to act on this information to initiate their response.

These scenarios highlight the value of physician awareness of the signs, symptoms, and diagnostic testing related to dengue, especially in areas most at risk for autochthonous dengue (those areas with 1 or both of the competent mosquito vectors and a population of travelers from disease-endemic regions). Public health departments should dedicate time and effort to engage the clinical community in issues related to dengue. Clinicians in areas at risk for dengue—as well as those in areas that receive travelers from dengue-endemic regions—should know the signs and symptoms of the disease and the requirements for laboratory testing and confirmation and should report suspected and confirmed cases to public health departments.

In areas at risk for dengue, laboratories capable of doing on-site testing should be identified beforehand, and plans for sample collection should be predetermined. In localities where testing is not available, alternative plans for rapid and efficient testing should be developed. This information should be disseminated. In Hawaii, testing was not available in any laboratory within the state, and confirmation of the outbreak was delayed because of the need to ship samples to CDC. In the other outbreaks, testing could not be done locally but was done at the state level. Interviewees stated that the ability to confirm dengue fever—which may be difficult to distinguish clinically from influenza—is hampered when access to testing is not available locally.

In these 3 outbreaks, public health laboratories conducted most testing. However, private laboratories also demonstrated the ability to perform dengue serologic testing (and in Florida were first to confirm dengue [*1*]), illustrating that laboratory capacity need not be solely the responsibility of public health authorities. In fact, commercial laboratory chains offer serologic testing, and an IgM serologic test (cleared by the US Food and Drug Administration) is available that will enhance the confidence of public health departments in the assay (*12*) because the test result will clearly reflect current infection. However, infection with other flaviviruses may produce false-positive serologic results. In addition, IgM results can be negative in a proportion of samples from persons with secondary dengue infections (*13*). PCR and serotyping work would still require the use of CDC or university laboratories.

Provide Accurate Information

Second, we recommend that public health agencies involved in responding to an outbreak of dengue

Figure 2. Cartoon character used in public relations campaign to control dengue outbreaks, Florida, USA, 2009–2011.

commit themselves to providing accurate and up-to-date information to the public, other public health and vector control jurisdictions, policy makers, and the clinical community. During the 3 dengue outbreaks, communication flowed in many directions. Ensuring the flow of information among health jurisdictions, the public, and mosquito control personnel is essential for managing an outbreak. Because dengue is a mosquito-borne disease, it necessarily will encompass a wide variety of entities in outbreak management and inclusion of a more extensive group of stakeholders than a disease not involving a vector.

Once dengue is detected, no delay should occur in telling the public about the outbreak or in disseminating strategies to minimize risk. Open communication will enhance public trust and make persons and communities more likely to participate in response and mitigation activities. In Hawaii, daily press conferences were critical to updating the public on the status of the outbreak. In other outbreaks, using door hangers and other media to relay information about dengue was instrumental. Honest communication, tailored to public needs and to the ways that a population best receives information, is the most effective way to gain the public's trust and cooperation in outbreak response (*14*). Public messaging should also provide specific actions that members of the public can take to protect themselves, their families, and their communities.

Interagency communication to political leadership about the risks and benefits of action is also an integral part of dengue outbreak response. Although, in general, relations between agencies and political leadership were constructive in each of the 3 outbreaks analyzed here, delayed initiation of coordination between government entities occasionally expended valuable time or led to contradictory public messages. Local public health and mosquito control agencies in areas with competent vector mosquito species should establish lines of communication with one another, with local and state governments, and with CDC before an outbreak. Concerns about the effects on tourism should be considered but should not interfere with effective public health management.

Engage Affected Community in Vector Control, Case Identification, and Case Reporting

Third, we recommend that the public health response to an outbreak of dengue in the United States focus on engaging the affected community in vector control activities, case identification, and case reporting. The chief means of combating dengue is reducing mosquito populations. Given that dengue mosquito vectors are peridomestic and have breeding sites close to human dwellings, often in backyards, public engagement in mosquito abatement is essential for controlling a dengue outbreak (*15*). Each of the 3 US outbreaks mobilized the public to combat mosquitoes. The door-to-door efforts of mosquito control and public health personnel to educate residents and facilitate their engagement in the fight against dengue were deemed by interviewees as some of the most effective mitigation activities undertaken to control the outbreaks. All localities noted that one-on-one contact with the public played a key role in their outbreak response efforts. These activities will still require population-level evaluation to fully validate their effectiveness, however. Other mosquito abatement activities, such as aerial spraying, were often considered superfluous by interviewees.

Public health and vector control officials should engage directly with residents to identify case-patients and remove mosquito breeding sites from their properties. Simple tasks such as dumping standing water are essential and easy to perform. In addition, the focus of response should be on community engagement measures to control dengue, rather than on punitive measures (i.e., pest control citations or citations for other code violations). A positive approach to public engagement for dengue response will help avoid conflict with residents, will make residents more apt to participate in mosquito control and case reporting, and will build trust with local public health officials. An integrated response directed by vector control and public health officials that melds with community efforts may be the optimal approach (*16*).

Dr Adalja is an associate at the Center for Biosecurity and adjunct instructor in the Department of Medicine's Division of Infectious Diseases at the University of Pittsburgh School of Medicine and University of Pittsburgh Medical Center. His research interests include bioterrorism and other public health emergencies.

References

1. Centers for Disease Control and Prevention. Locally acquired dengue—Key West, Florida, 2009–2010. MMWR Morb Mortal Wkly Rep. 2010;59:577–81.
2. Jelinek T, Dobler G, Nothdurft HD. Evidence of dengue virus infection in a German couple returning from Hawaii. J Travel Med. 1998;5:44–5. http://dx.doi.org/10.1111/j.1708-8305.1998.tb00458.x
3. Hawaii State Department of Health. DOH investigates cases of dengue fever on Oahu and asks public and medical community to be vigilant. March 24, 2011 [cited 2011 May 11]. http://hawaii.gov/health/about/pr/2011/11-028.pdf
4. Effler PV, Pang L, Kitsutani P, Vorndam V, Nakata M, Ayers T, et al. Dengue fever, Hawaii, 2001–2002. Emerg Infect Dis. 2005;11:742–9.
5. Hayes JM, Rigau-Perez JG, Reiter P, Effler PV, Pang L, Vorndam V, et al. Risk factors for infection during a dengue-1 outbreak in Maui, Hawaii, 2001. Trans R Soc Trop Med Hyg. 2006;100:559–66. http://dx.doi.org/10.1016/j.trstmh.2005.08.013
6. Ramos MM, Mohammed H, Zielinski-Gutierrez E, Hayden MH, Lopez JL, Fournier M, et al. Epidemic dengue and dengue hemorrhagic fever at the Texas–Mexico border: results of a household-based seroepidemiologic survey, December 2005. Am J Trop Med Hyg. 2008;78:364–9.

7. Centers for Disease Control and Prevention. Dengue hemorrhagic fever—U.S.–Mexico border, 2005. MMWR Morb Mortal Wkly Rep. 2007;56:785–9.
8. Rawlings JA, Hendricks KA, Burgess CR, Campman RM, Clark GG, Tabony LJ, et al. Dengue surveillance in Texas, 1995. Am J Trop Med Hyg. 1998;59:95–9.
9. Florida State Department of Health. Florida Arbovirus surveillance. January 1, 2011 [cited 2011 Jun 11]. http://www.myfloridaeh.com/medicine/arboviral/pdfs/2010/2010Week52ArbovirusReport_1_1_2011.pdf
10. Florida Department of Health. Florida Arbovirus surveillance week 43: October 23–October 29, 2011 [cited 2011 Nov 1]. http://www.doh.state.fl.us/Environment/medicine/arboviral/pdfs/2011/2011Week43ArbovirusReport_10-29-2011.pdf
11. Florida Department of Health. Broward County Health Department issues health advisory regarding mosquito-borne disease. August 12, 2010 [cited 2011 May 11]. http://browardchd.org/LinkClick.aspx?fileticket=umNFwE6KQfl%3d&tabid=113
12. InBios. DENV Detect™ IgM Capture ELISA [cited 2011 May 18]. http://www.inbios.com/elisas/denv-detect-igm-ELISA
13. Centers for Disease Control and Prevention. Laboratory guidance and diagnostic testing [cited 2011 Nov 1]. http://www.cdc.gov/dengue/clinicalLab/laboratory.html
14. World Health Organization Outbreak communication. Best practices for communicating with the public during an outbreak. Geneva: The Organization; 2005 [2011 May 11]. http://www.who.int/csr/resources/publications/WHO_CDS_2005_32web.pdf
15. Toledo ME, Baly A, Vanlerberghe V, Rodríguez M, Benitez JR, Duvergel J, et al. The unbearable lightness of technocratic efforts at dengue control. Trop Med Int Health. 2008;13:728–36. http://dx.doi.org/10.1111/j.1365-3156.2008.02046.x
16. Gubler DJ, Clark GG. Community involvement in the control of *Aedes aegypti*. Acta Trop. 1996;61:169–79. http://dx.doi.org/10.1016/0001-706X(95)00103-L

Address for correspondence: Amesh A. Adalja, Center for Biosecurity of UPMC, 621 E Pratt St, Suite 210, Baltimore, MD 21202, USA; email: ameshaa@aol.com

Malaria in Highlands of Ecuador since 1900

Lauren L. Pinault and Fiona F. Hunter

A recent epidemic of malaria in the highlands of Bolivia and establishment of multiple *Anopheles* species mosquitoes in the highlands of Ecuador highlights the reemergence of malaria in the Andes Mountains in South America. Because malaria was endemic to many highland valleys at the beginning of the 20th century, this review outlines the 20th century history of malaria in the highlands of Ecuador, and focuses on its incidence (e.g., geographic distribution) and elimination from the northern highland valleys of Pichincha and Imbabura and the role of the Guayaquil to Quito railway in creating highland larval habitat and inadvertently promoting transportation of the vector and parasite. Involvement of control organizations in combating malaria in Ecuador is also outlined in a historical context.

Some authors have speculated that *Anopheles* mosquitoes may begin transmitting malaria parasites (*Plasmodium* spp.) at higher altitudes in the South American Andes because of climate change (*1*,*2*). In contrast, highland malaria in Africa has more often been attributed to land use alterations, malaria treatment resistance, changes in vector control measures, and human migration into foothill and mountainous regions (*3*). Before 2004, a short-lived epidemic of *P. vivax* malaria was recorded in a village in Bolivia at an altitude of 2,300 m that was transmitted by *Anopheles pseudopunctipennis* Theobald mosquitoes (*4)*. Multiple anopheline malaria vectors have also become established in the highlands of Ecuador (*5*).

In this review, we summarize documented cases of highland malaria that occurred in Ecuador during the early 20th century. We define the term highland malaria to mean all malaria that occurs in regions with steep topography. Using geographic information systems (ArcGIS version 10; ESRI, Redlands, CA, USA) and tabulated data from historical sources, we reconstruct the geographic extent of malaria incidence during several periods of interest. We also outline malaria control efforts and attempts at malaria elimination for Ecuador during the 20th century and at the beginning of the 21st century.

Author affiliation: Brock University, St. Catharines, Ontario, Canada

DOI: http://dx.doi.org/10.3201/eid1804.111267

Malaria in Ecuador at the Beginning of the 20th Century

Although malaria was prevalent on the coast of Ecuador at the beginning of the 20th century, it was considered by public health officials to be a minor problem (*6*). Until 1908, Guayaquil on the coast of Ecuador was affected by the constant menace of mosquitoes transmitting yellow fever, and flea-borne bubonic plague reoccurred regularly in all areas of the city and surrounding countryside (*6*–*8*). Because Guayaquil had an image of being an unhealthy major port city, officials in Ecuador signed an international sanitation convention in 1906 to combat outbreaks of yellow fever, bubonic plague, and cholera (*9*). Under terms of the convention, officials were to take measures to prevent ongoing transmission of these diseases, including the use of mosquito screens on windows and doors of hospitals (*9*). During that time, officials also experimented with the use of mosquito larvae–eating fish as a biocontrol method in an attempt to control yellow fever (*8*).

In 1908, the public health movement became active in Ecuador, and a special sanitary commission was formed in Guayaquil (*7*,*8*). At that time, malaria was still considered a minor health problem and many residents allowed mosquitoes to bite them to provide them with long-term immunity to malaria (*10*). Malaria prevention measures included bed nets, window and door screens, and anopheline larval habitat destruction (*8*,*10*). Several medical entomologists became active during this period

in Ecuador. These entomologists included the French entomologist Paul Rivet, and the Ecuadorian entomologists F.R. Campos, Luis León, and J. Rodríguez (*11*).

In 1919, many physicians in Ecuador began to receive training in foreign countries, particularly in the United States through grants from the Rockefeller Foundation, in an attempt to eliminate yellow fever and malaria from Ecuador (*6*,*8*,*12*). With the elimination of yellow fever in 1920, attention inevitably turned to malaria, which still accounted for a large percentage of deaths on the coast of Ecuador (*13*). By 1940, malaria still remained a priority and was the second leading cause of death in Ecuador after whooping cough (*7*). At the time, it was recommended that a campaign against malaria should be initiated throughout the entire country (*7*).

Highland Malaria during Construction of Guayaquil to Quito Railway (1890–1945)

In 1886, construction began on the railway that was to link some of the low-altitude regions of the country near Guayaquil to highland regions and eventually Quito (altitude 2,800 m) (*14*). The railway was constructed on a route that began in Guayaquil (at sea level), passed through Milagro, and followed the valley bottom up toward Huigra, in Chimborazo (altitude 1,250 m). After Huigra, the railway continued higher toward Alausí, Chimborazo (altitude 2,340 m), after climbing the infamous Devil's Nose switchbacks (*14*) (Figure 1).

When construction of the railway reached an elevation of 200 m, workers reported bites from an unidentified bush-dwelling, flying insect and many subsequently died of high fevers (*15*). At higher altitudes, workers began to report fevers attributed to malaria (*14*). In 1906, the Guayaquil newspaper *Grito del Pueblo* reported that railway workers affected by fevers were removed from the construction site and brought to the highland village of Chasqui, Pichincha, for recovery (*16*). The following quotation from Daniel Barragán, one of the engineers for the railway, provides strong evidence that mosquitoes (Culicidae) were present at worksites: "The mosquitoes were our eternal companions, during all of the night, their melodious and incessant humming many times did not let us find sleep" (translated into English by L.L.P.) (*14*).

Meitzner described treating many of the railway workers for malaria during 1911 (*10*). In the winter of that year, the incidence of malaria was so great among workers that construction halted completely (*10*). Patients were usually brought to higher altitude towns such as Huigra for treatment because there were insufficient medical facilities at lower altitudes (*10*). Patients with malaria among the railway workers were treated by Meitzner by using a combination of castor oil and quinine and a diet that excluded meat (*10*). Before operation of the railway,

Figure 1. Ecuador showing elevation (red shading), provinces (thin gray lines and **boldface**), country border (thick gray line), and 15 cities/valleys (black dots). Approximate location of the historic railway between Guayaquil and Alausí is indicated by black railroad tracks, and increasing altitude is indicated by darker shades of red. Map was constructed by using ArcGIS version 10 (ESRI, Redlands, CA, USA).

transportation between the coast and highland regions was limited. Therefore, during the early operation of the railway to Quito, the malaria parasite could have been repeatedly introduced by infected passengers and workers to higher altitude regions, including the valleys around the city of Quito (*10*).

The presence of malaria rather than other similar febrile illnesses in railway workers is further supported by multiple collections of *An. pseudopunctipennis* larvae (the highland malaria vector) in the Chiripungo Valley, near Alausí, Chimborazo (altitude <2,400 m) (*17*). As early as 1911, Meitzner made recommendations to railway engineers to construct drainage ditches along the sides of the tracks to prevent establishment of additional larval habitats in the pools that formed there (*10*). Despite the efforts of Meitzner, *An. pseudopunctipennis* mosquitoes remained in highland valleys of Chimborazo along the railway at least into the mid 1940s. In 1943, Levi Castillo collected *An. pseudopunctipennis* larvae along railway tracks up to

an altitude of 1,250 m, and in 1944, he collected larvae in pools associated with rivers in the towns of Huigra and Sibambe, Chimborazo (*17*). These entomologic collections are consistent with the epidemiology of continued malaria transmission; in 1944, a total of 154 cases of malaria among 864 residents were documented in Huigra (*18*). Aside from habitats associated with the railway line, as shown in Figure 2, partial blockage of the river below the newly built tracks along Devil's Nose would probably have produced suitable pools for *An. pseudopunctipennis* larval habitat.

Trains were a likely carrier for continued introduction of anopheline mosquitoes into highland regions from the coast (*17*,*19*). At Milagro station, adult mosquitoes were observed to fill train cars bound for higher elevations (*19*). Levi Castillo found pools of water in ceiling portions of trains, which served as mobile larval habitats for anopheline mosquitoes (*17*). At higher elevations (>1,100 m), *An. pseudopunctipennis* mosquitoes were documented as the sole vector, although *An. albimanus* Wiedemann larvae were collected at lower altitudes along the railway (*18*). Trains likely served to introduce anopheline mosquitoes to highland regions until the 1960s and 1970s, when the railway fell into disuse (*15*).

Highland Malaria Foci in Ecuador (1900–1950)

Malaria in highland regions of the northern Andes was not exclusive to Ecuador and was found in Colombia in the Cauca, Manizales, Cali, and Medellín Valleys, and in Peru in the Rimac, Urubamba, and Laurin-Orcocota Valleys (*20*). Although malaria became more readily studied and possibly more widespread in Ecuador during the 1940s, the vector mosquito *An. pseudopunctipennis* was likely present for a much longer period in highland regions (*21*). The malaria parasite is believed to have been introduced to a handful of highland valleys in the 1800s because there are no records of it before that time (*22*).

Figure 2. Railway construction at base of the Devil's Nose switchbacks, Ecuador, showing railway on the left and stone-lined riverbed on the right, where several pools can be seen (likely formed by falling rocks from construction), which would likely have provided suitable habitat for *Anopheles pseudopunctipennis* larvae. Photograph: Historical Archive of Banco Central and García Idrovo (*14*).

In 1905, students at the University of Guayaquil listed the following highland valleys in Ecuador to which malaria was endemic: Imbabura, Chota and Pinampiro valleys in Pichincha; Tumbaco and Guayllabamba Valleys in Tungurahua; the Patate Valley; and the Yunguilla Valley in Azuay (*23*) (Figure 1). All of these valleys except Patate were regarded as regions to which malaria was endemic into the 1940s (*24*). In almost every highland valley, *Plasmodium vivax* was implicated as the only malaria parasite with *An. pseudopunctipennis* mosquitoes as vectors (*11*,*24*).

Highland malaria was widespread in the early 1940s when it appeared to reach its widest distribution (*24*). In addition to the valleys listed above and highland valleys in Chimborazo associated with the railway, malaria transmission was observed in Imbabura (Mira Valley and Salinas), Pichincha (widespread in all highland valleys), Cañar (all valleys at an elevation <2,500 m), Chimborazo (Pallatanga Valley), Azuay (Yunguilla Valley), and Loja (Catamayo Valley) (*11*,*24*) (Figure 1).

In 1938, Hanson and Montalvan documented a new epidemic of *P. vivax* and *P. falciparum* malaria in Balzapamba, Bolívar (population 700), in an orange-growing region at an elevation of 650 m (*25*) (Figure 1). Residents had reportedly never experienced malaria until 1935, although they lived near (≈10 km away) the malaria-endemic coastal plain. In 1935, an earthquake and associated landslides diverted the course of the main river, and an open canal was constructed to provide the town with drinking water (*25*). Throughout their search, Hanson and Montalvan were able to locate only *An. pseudopunctipennis* larvae in the open canal and in the algae-covered pools, which formed on the edges of the newly-diverted river (*25*). This epidemic highlights the scarcity of available larval habitat in steep topography regions and the probable role of river pools and human-made canals as habitat for anopheline larvae in highland regions.

Although *An. albimanus* mosquitoes have traditionally been considered low-altitude (<300 m) vectors, they were identified as the main malaria vector in an epidemic in the Yunguilla Valley in Azuay (altitude ≈1,500 m) in the late 1940s (*17*,*18*). *An. pseudopunctipennis* mosquitoes were collected from higher-altitude towns in Azuay, such as Santa Isabel, during the 1940s (*18*). DDT was just beginning to be used at that time in Ecuador and was successfully applied in the 1940s to the Yunguilla Valley (*19*). During that time, 5% DDT in a solution of kerosene was applied inside homes and to larval habitats (*26*).

Little research was conducted in southern Ecuador, although there were confirmed malaria cases in the Chota and Pinampiro Valleys in in Imbabura; the Tumbaco and

Guayllabamba Valleys in Pichincha; the Patate Valley in Tungurahua; and the Yunguilla Valley in Azuay (*18*). Montalvan reported his unconfirmed belief that the main vector in Catamayo might be *An. punctimacula* Dyar mosquitoes, which were otherwise not implicated in highland malaria transmission during the 1940s in Ecuador (*18*). Similarly, there are few reports of malaria in highland parts of the Amazonian side of the Andes during the early 20th century, likely because the region was sparsely settled. Even in the lower altitude Ecuadorian Amazon communities such as Puyo and Napo (presently Puerto Napo) (altitude 700–900 m), the residents reported no cases of malaria (*27*) (Figure 1).

Highland Malaria in Northern Valleys (Pichincha and Imbabura) (1940–1950)

Highland malaria in the northern valleys of Ecuador was well documented during its most widespread period (1940–1950) (*18*,*24*). Malaria was reported from valleys in Imbabura and Pichincha Provinces, although it never reached the city of Quito (altitude 2,800 m) (*24*). On the basis of valleys affected and maximum altitudes recorded for anopheline species, the probable extent of highland malaria in the northern valleys during its peak is shown in Figure 3.

Before the widespread occurrence of malaria in the 1940s, Guayllabamba, Pichincha, was considered an area to which malaria was endemic (*18*,*21*,*22*). Gradually, the vector and parasite spread to other valleys, reaching Tingo and Alangasi by 1917 (*21*) (Figures 1, 3). The spread of malaria may have been in part caused by an exodus of citizens from Guayllabamba during the maximum incidence of the disease (*28*). When malaria became more widespread in the 1940s, the government in Ecuador brought in the US malariologist Henry Hanson, who identified *An. pseudopunctipennis* mosquitoes as the only vectors (*28*). The maximum altitude of the species was estimated to be 2,500 m–2,700 m, although they have since been observed at 3,200 m (*11*,*21*,*26*). *An. pseudopunctipennis* larvae were collected from clean, sunlit, rocky pools associated with rivers, springs of water, irrigation ditches, and hoof-prints from horses (*17*,*18*,*20*,*21*). Entomologists also noted a strong larval association with spirogyra algae (*18*,*21*,*26*).

Although the expansion of *Anopheles* mosquito distribution is often attributed to land use change, highland valleys of northern Ecuador have been cleared and continuously farmed since pre-Colombian times (*29*). The reported use of river edges as habitats also makes land use change unlikely to be the sole explanation (*17*,*18*). Spread of the parasite and vector may also be attributed to meteorologic causes, especially an increase in minimum temperatures, which might otherwise limit parasite or vector development. Increases of 0.5°C in average daily temperature and 1.3°C in minimum nightly temperature were observed in Quito during 1900–1930 (Figure 4). Therefore, meteorologic factors may have caused the increased range of highland malaria before 1940.

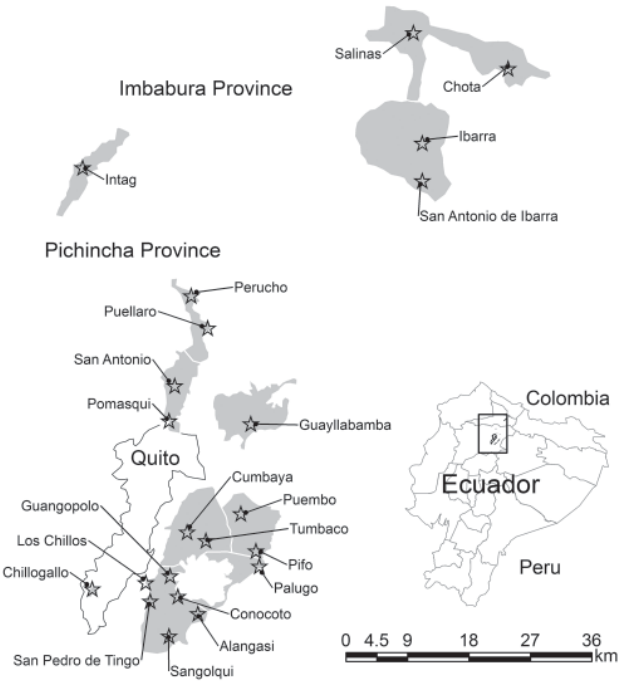


Figure 3. Probable extent of highland valley malaria incidence (shaded areas) during the early 1940s in Ecuador. Stars indicate approximate location of original towns to which malaria was reported as endemic, judged by the presence of the historical town square in Google Earth satellite imagery (Google, 2010). Shading was determined by the valley bottom with an affected town up to an altitude of 2,500 m. Inset: Approximate location of region in Ecuador. Data were obtained from Levi Castillo (*17*), Montalvan (*18*), and Levi Castillo *(21)*.

A widespread campaign began in 1940 to eliminate malaria from highland valleys of Pichincha and Imbabura (*28*). Malaria was eliminated in the Los Chillos Valley by the *Servicio Antipalúdico del Valle de los Chillos*, led by Jaime Rivadeneira, and assisted by Carlos A. Marín and Benjamín Wandemberg (*11*,*17*,*21*,*24*). A field laboratory was set up in San Pedro de Tingo to lead an initial systematic larval habitat inspection of the entire valley (*21*). All pools of water used by anopheline larvae were drained by the construction of dikes and sprayed with crude oil mixed with kerosene and occasionally DDT (*17*,*21*,*28*). Smaller pools were filled with earth, and residents were provided with chemical repellent for personal use (*17*,*20*). The campaign was deemed a success and malaria did not return in subsequent years (*21*).

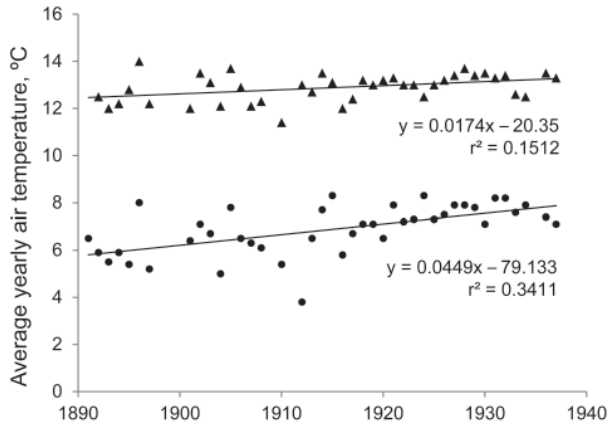


Figure 4. Increase in yearly average daily temperature during a 24-hour period (▲) and average minimum nightly temperature (●) in Quito, Ecuador, 1891–1937, leading up to years of observation of highland malaria in valleys surrounding Quito. Although average temperature only increased at a rate of 0.017°C/year, minimum nightly temperature, which may be more essential for survival of *Anopheles* spp. species, increased at a rate of 0.045°C/year. Data were obtained from the Astronomical and Meteorological Observatory of Quito (*30*).

Human Colonization of Coastal Foothill Tropical Forests (1950–1970)

A map of malaria incidence published in 1950 shows the greatest incidence in Ecuador to be in the northern coastal region and *An. albimanus*, *An. pseudopunctipennis*, and *An. punctimacula* mosquitoes to be the most common vectors (*31*). The foothills of the northern coast were sparsely populated and land was not substantially developed before 1950, because the region was covered in dense tropical forest with limited access (*32*). However, roads were built linking Quito to the coast in the late 1940s, and settlers moved into the region, forming Santo Domingo (presently Santo Domingo de los Tsáchiles) (altitude ≈500 m) (*32*). Settlers converted wide swathes of forest to maize, rice, cocoa, and coffee plantations for trade in Quito and in port cities (*32*). Seasonal workers from the highlands migrated into the region to work during summers (*32*). The first major epidemic of malaria was reported in 1958 (*32*). Land conversion likely provided sunlit habitat, which may have been more suitable for some species of *Anopheles* mosquitoes. Also, the immigration of large groups of highlanders lacking immunity likely contributed to an epidemic in 1958 and to subsequent epidemics.

History of Malaria Elimination/Control Efforts in Ecuador

Although there were several regional public health organizations addressing malaria on the coast of Ecuador, the National Institute of Hygiene and Tropical Medicine Leopoldo Izquieta Pérez was formed in 1940 (*33*). In 1944, Ecuador had the largest available hospital facilities of any country in Latin America. Physicians were trained by the Pan American Sanitary Bureau with funding from the Rockefeller Foundation, and new public health laboratories were constructed (*34*,*35*). In 1948, the *Servicio Nacional Antimalárico* was formed to campaign against malaria, especially on the coast, and to organize DDT spray operations twice a year (*22*). In the early 1950s, US organizations led efforts to eradicate malaria from malaria-endemic countries, although insecticide resistance was beginning to appear and slow the eradication progress (*36*). By 1956, Ecuador was considered to be in an early attack phase of an eradication program (*36*).

After a recommendation from the Pan American Sanitation Committee, the *Sistema Nacional de Eradicación de Malaria* (SNEM) was founded on July 21, 1956 (*13*,*22*). Its focus was to prevent insect-borne diseases through vector control, mainly through use of chemical insecticides and larval habitat elimination, and public education through school visits, interviews, and community meetings (*13*). In addition to malaria, SNEM has monitored and controlled Chagas disease, dengue fever, onchocerciasis, yellow fever (in Amazonia), and leishmaniasis in Ecuador (*13*).

Success of the SNEM in combating malaria has been closely associated with its variable levels of funding. During 1957–1959, dieldrin was sprayed inside houses on a continuous schedule, but was regularly underdosed and therefore not effective (*37*). During 1961–1965, DDT was applied to houses under the direction of the US Agency for International Development (USAID) and the Pan American Health Organization with greater success (*37*). Funding for medical entomology research was so poor and unreliable that R. Levi Castillo, who had previously documented many cases of highland malaria, renounced his post at the University of Guayaquil and burned his books in protest (*33*). By the late 1960s, USAID funding had decreased substantially, resulting in a subsequent epidemic (*37*). Azuay and Cañar, and to a lesser extent Pichincha and Chimborazo, had a small increase of malaria cases in low-lying valleys (Figure 5). Similarly, in 1969 during another peak year, these highland provinces and most areas of the coast of Ecuador were affected by malaria (Figure 6, panel A).

USAID funding was reinstated in 1973 through a reinvestment with the SNEM (*22*,*37*). However, by 1980, the SNEM was considered operational and no longer relied on international funding (*37*). During an assessment in 1983, the SNEM was deemed to be a capable department but with some financial concerns, such as having an aging fleet of boats and jeeps, and a residual house spray schedule of 3 times a year rather than the recommended 4 times (*37*).

Through the latter half of the 1980s, some malaria cases were reported in highland provinces of Pichincha,

Cotopaxi, Bolívar, and to a lesser extent, Chimborazo and Loja (Figure 5). Tungurahua, Carchi, and Imbabura Provinces reported only occasional malaria cases (Figure 5). During 1 of the peak years (1990), there was widespread malaria along the coast, in Amazonia, and in highland provinces (although perhaps only in lower-altitude regions of these provinces) (Figure 5; Figure 6, panel B). Again in 2000, widespread malaria was observed in the coastal and Amazonian areas of Ecuador (Figure 6, panel C), but only Pichincha and to a lesser extent Cotopaxi and Bolívar observed an increase in cases in the highlands (Figures 5, 6). However, despite occasional cases of malaria, the SNEM reported a steady decrease in malaria in Ecuador during the past 20 years, likely as a response to efforts of various programs that have been more recently implemented in the country (*13*).

Conclusions

Malaria became more widespread in northern highland regions of Ecuador during 1900–1940 but was subsequently eliminated from these regions through habitat elimination and use of chemical insecticides (*21*). In Chimborazo

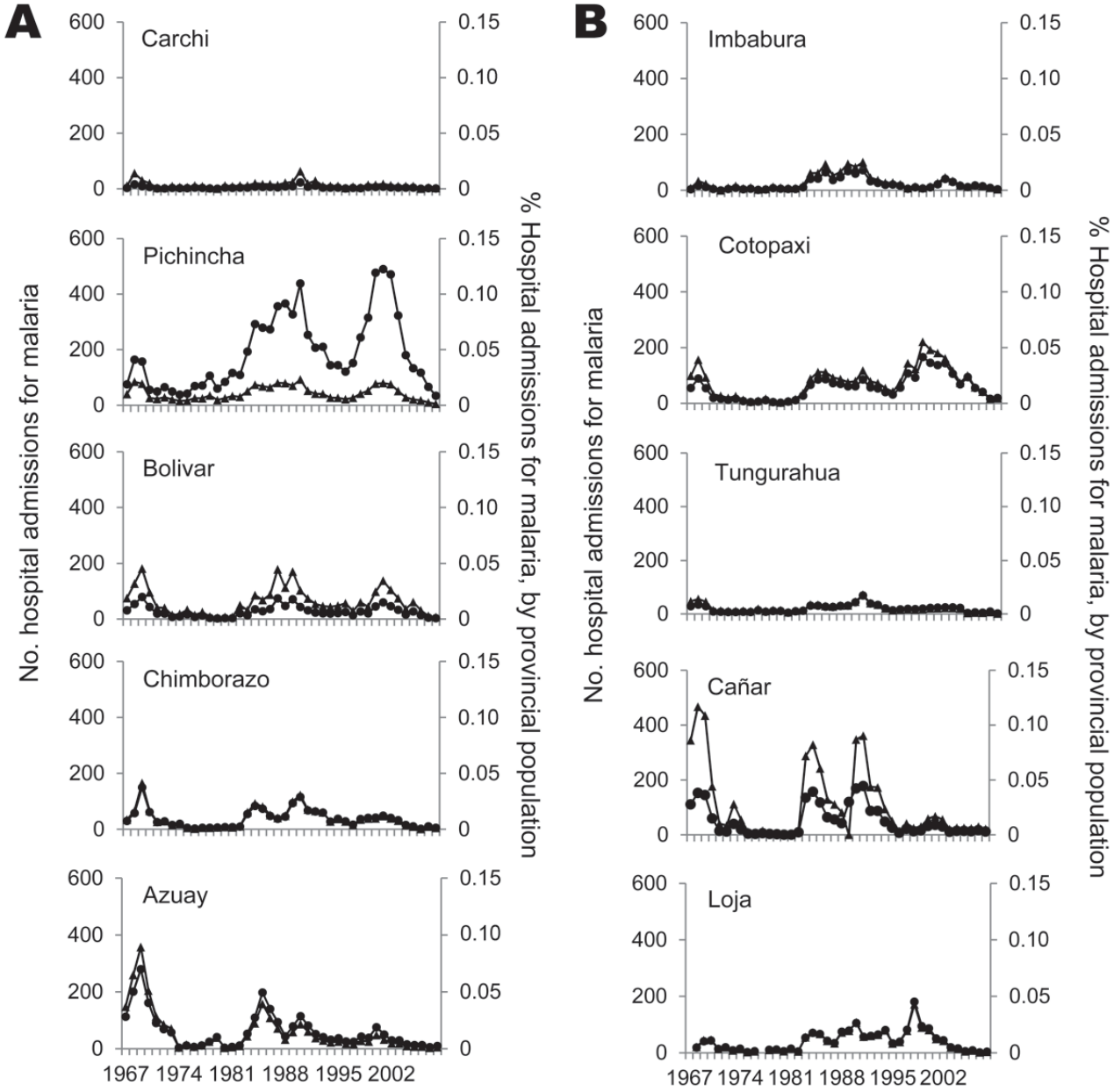


Figure 5. Number of hospital admissions for malaria (▲) per year for each province in the sierra region of Ecuador and percentage of admissions for malaria, by provincial population (●). Data were obtained from the Instituto de Nacional Estatisticas y Censos (*38,39*).

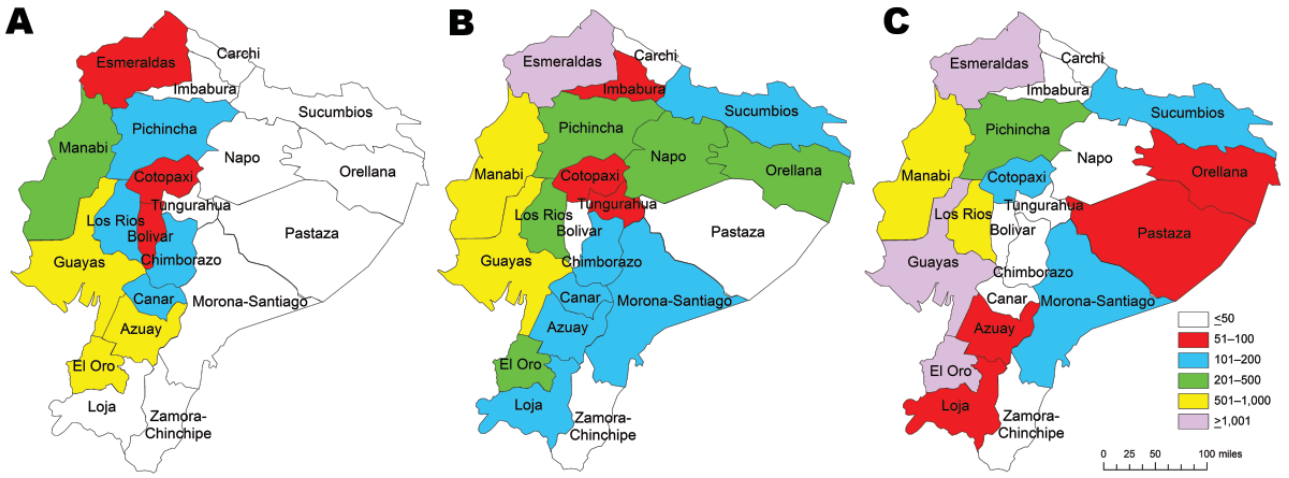


Figure 6. Number of hospital admissions for malaria in each province of Ecuador in peak malaria years of A) 1969, B) 1990, and C) 2000. Data were obtained from the Instituto de Nacional Estatisticas y Censos (*38*).

during 1900–1950, malaria spread into highland valleys along the railway linking Guayaquil and Quito (*10*,*17*,*18*). Although there have likely been a few highland epidemics since the 1940s, only 1 report in 1991 documented the presence of *An. pseudopunctipennis* mosquitoes in river-associated habitats of Guayllabamba (*28*). To effectively monitor establishment of highland malaria vectors, a focus on historically malaria-endemic highland valleys may be needed. Anopheline habitats in areas with steep topography are expected to differ from those in flat, low-altitude regions. Therefore, these differences will necessitate further study of local dynamics of mosquito ecology, meteorologic variables, and transmission cycles.

Acknowledgments

We thank Santiago Caizapanta, Julio Rivera, Jady Toscano, Edmundo Caizapanta, Clifford Keil, the staff at Servicio Nacional de Control de Enfermedades Transmitidas por Vectores Artrópodos in Guayaquil, Instituto Nacional de Estadísticas y Censos de Ecuador, G. García Idrovo, and residents of Alausí for assistance.

This study was supported by a Natural Sciences and Engineering Research Council of Canada discovery grant to F.F.H., an International Development Research Centre doctoral research award to L.L.P., and a Natural Sciences and Engineering Research Council of Canada postgraduate scholarship to L.L.P.

Dr Pinault just completed her doctorate at Brock University, Canada, studying the ecology of highland *Anopheles* spp. in the Andes in Ecuador. Her primary research interests include highland malaria, vector ecology, spatial ecology with geographic information systems, and human perspectives of vector-borne diseases.

Dr Hunter is a medical entomologist and professor in the Department of Biological Sciences, Brock University, Canada. Her primary research interests include behavioral and feeding ecology of biting flies, entomologic monitoring for public health, and molecular and chromosomal systematics of *Anopheles* spp. mosquitoes.

References

1. Moreno AR. Climate change and human health in Latin America: drivers, effects and policies. Reg Environ Change. 2006;6:157–64. http://dx.doi.org/10.1007/s10113-006-0015-z
2. Reiter P. Climate change and mosquito-borne disease. Environ Health Perspect. 2001;109:141–61.
3. Hay SI, Rogers DJ, Randolph SE, Stern DI, Cox J, Shanks GD, et al. Hot topic or hot air? Climate change and malaria resurgence in east African highlands. Trends Parasitol. 2002;18:530–4. http://dx.doi.org/10.1016/S1471-4922(02)02374-7
4. Rutar T, Baldomar Salgueiro EJ, Maguire JH. Introduced *Plasmodium vivax* malaria in a Bolivian community at an elevation of 2,300 meters. Am J Trop Med Hyg. 2004;70:15–9.
5. Pinault LL, Hunter FF. New highland distribution records of multiple *Anopheles* species in the Ecuadorian Andes. Malar J. 2011;10:236. http://dx.doi.org/10.1186/1475-2875-10-236
6. Pineo RF. Misery and death in the Pearl of the Pacific: Health care in Guayaquil, Ecuador, 1870–1925. Hisp Am Hist Rev. 1990;70:609–37. http://dx.doi.org/10.2307/2516575
7. Izquieta Pérez L. Public health in Ecuador. Bulletin of the Pan American Sanitary Bureau. 1941;20:1283–5.
8. Parks LF, Nuermberger GA. The sanitation of Guayaquil. Hisp Am Hist Rev. 1943;23:197–221. http://dx.doi.org/10.2307/2508015
9. Sanitary convention [in Spanish]. Grito del Pueblo. 1906 May 13, No. 4227.
10. Meitzner M. Malaria, bubonic plague, yellow fever, and ancylostomiasis in Ecuador [in Spanish]. Guayaquil (Ecuador): S.A. Sociedad Filantropica; 1938.
11. Astudillo C. Human parasitology [in Spanish]. Quito (Ecuador): Casa de la Cultura Ecuatoriana; 1971.

12. Bustamante ME. Public health administration in Latin America. Am J Public Health Nations Health. 1950;40:1067–71. http://dx.doi.org/10.2105/AJPH.40.9.1067
13. Espinoza López N, Triviño Yépez L, Alarcón y Alvarado J, Vélez Nieto L. Technical report: historical and current contribution of the national control of vector-borne disease service (SNEM) for the betterment of the health and the quality of life of Ecuadorians in relation to the illnesses transmitted by arthropod vectors, period 1956–2008 [in Spanish]. Guayaquil (Ecuador): SNEM Technical Commission, Ministry of Public Health, Government of Ecuador; 2009.
14. García Idrovo G. The most difficult railway in the world [in Spanish]. Quito (Ecuador): Ministry of Culture, Government of Ecuador; 2008.
15. Vásconez VR, Bravo Silva C. Our railway: past, present, future [in Spanish]. Quito (Ecuador); National State Railway Company; 1992.
16. From the capital, by telegraph [in Spanish]. Grito del Pueblo. 1906 Mar 17, No. 4170.
17. Levi Castillo R. The anophelines of the Republic of Ecuador [in Spanish]. Guayaquil (Ecuador): Artes Graficas Senlieider C.A. Ltda.; 1945.
18. Montalvan JA. Malaria in Ecuador [in Spanish]. Guayaquil (Ecuador): Director General of Sanitation, Instituto Nacional de Higiene Leopoldo Izquieta Perez; 1948.
19. Levi Castillo R. Studies of the anophelines of the region of Milagro [in Spanish]. Guayaquil (Ecuador): Revista de la Asociación Escuela de Ciencias Quimicas; 1944.
20. Levi Castillo R. Studies on the malaria in the Andean valleys of South America [in Spanish]. Revista Médica de Colombia. 1947;15:343–56.
21. Levi Castillo R. *Anopheles pseudopunctipennis* in the Los Chillos valley of Ecuador. J Econ Entomol. 1945;38:385–90.
22. Aguilar M. Space and malaria in Ecuador [in Spanish]. In: Aguilar M, Yepez RF, editors. La malaria es más que una picadura. Quito (Ecuador): Instituto Juan Cesar Garcia, Fundación Internacional de Ciencias Sociales y Salud; 1994. p. 1–26.
23. (Students of) Huerta B. Malaria [in Spanish]. Guayaquil (Ecuador): Impresora de El Tiempo; 1905.
24. Levi Castillo R. The pseudopunctipennis complex in Ecuador (Diptera: Culicidae) [in Spanish]. Guayaquil (Ecuador): Imprenta de la Universidad de Guayaquil; 1944.
25. Hanson H, Montalvan JA. Malaria in Balzapamba (epidemiological study) [in Spanish]. Quito (Ecuador): Pan American Sanitation Office; 1938.
26. Levi Castillo R. The vectors of malaria of the countries on the coast of the Pacific of South America and their control [in Spanish]. Revista Kuba de Medicina Tropical. 1949;5:101–4.
27. Sinclair JH, Wasson T. Explorations in eastern Ecuador. Geogr Rev. 1923;13:190–210. http://dx.doi.org/10.2307/208447
28. Gómez de la Torre Serrano JA, Gómez de la Torre Flores P, Cruz ME. Control of malaria at altitude [in Spanish]. In: Cruz ME, de Lourdes Chacon M, editors. New strategies against malaria. Presented at: Conferencia Andina Nuevas Estrategias Contra la Malaria; 1990 May 30–31. Quito (Ecuador): Centro de Documentatción del Sistema de Naciones Unidas Ecuador; 1991. p. 51–7.
29. Sarmiento FO. Anthropogenic change in the landscapes of highland Ecuador. Geogr Rev. 2002;92:213–34. http://dx.doi.org/10.2307/4140971
30. Summary of the meteorological observations made in the observatory and in the diverse stations of the Republic [in Spanish]. Quito (Ecuador): Astronomical and Meteorological Observatory of Quito, Ministry of Public Instruction, Government of Ecuador; 1929–1937; 1937.
31. Mackie TT, Hunter GW, Worth CB. Manual of tropical medicine [in Spanish]. 2nd ed. Mexico City: La Prensa Medica Mexicana; 1950.
32. Burt AL, Hitchcock CB, James PE, Jones CF, Minkel CW. Santo Domingo de los Colorados – a new pioneer zone in Ecuador. Econ Geogr. 1960;36:221–30. http://dx.doi.org/10.2307/141816
33. Barragán AR, Dangles O, Cárdenas RE, Onore G. The history of entomology in Ecuador. Ann Soc Entomol Fr. 2009;45:410–23.
34. Perez AR. Cascarilla and its economic importance in Ecuador [in Spanish]. Quito (Ecuador): Publicaciones del Instituto Superior de Pedagogia y Letras; 1944.
35. Dunham GC. The cooperative health program of the American Republics. Am J Public Health Nations Health. 1944;34:817–27. http://dx.doi.org/10.2105/AJPH.34.8.817
36. Russell PF. Malaria in the world today. Am J Public Health Nations Health. 1957;47:414–20. http://dx.doi.org/10.2105/AJPH.47.4_Pt_1.414
37. Stivers J. Emergency malaria situation in Ecuador. Washington: US Government Report, contract no. 510–0011–0-00–3131–00; 1983.
38. INEC (Instituto Nacional de Estatisticas y Censos). Hospital beds and ingressions (national statistical database), 1967–2008 [in Spanish]. Quito (Ecuador): Government of Ecuador; 2008.
39. INEC (Instituto Nacional de Estatisticas y Censos). Projection of the population of Ecuador (national statistical database), 1960–2000 [in Spanish]. Quito (Ecuador): Government of Ecuador; 2010.

Address for correspondence: Lauren L. Pinault, Department of Biological Sciences, Brock University, 500 Glenridge Ave, St. Catharines, Ontario L2T 3K2, Canada; email: lauren.pinault@gmail.com

Dengue and US Military Operations from the Spanish–American War through Today

Robert V. Gibbons, Matthew Streitz, Tatyana Babina, and Jessica R. Fried

Dengue is a major cause of illness among travelers and a threat to military troops operating in areas to which it is endemic. Before and during World War II, dengue frequently occurred in US military personnel in Asia and the South Pacific. From the 1960s into the 1990s, dengue often occurred in US troops in Vietnam, the Philippines, Somalia, and Haiti. We found attack rates as high as 80% and periods of convalescence up to 3-1/2 weeks beyond the acute illness. The increase in dengue throughout the world suggests that it will remain a problem for military personnel until an effective vaccine is licensed.

Dengue has proven itself a challenge to US military personnel. Even though case-fatality rates are low, dengue can rapidly incapacitate personnel. Dengue caused major illness among US service members stationed in the Philippines beginning after the Spanish–American War, and although not reported in the Iraq and Afghanistan conflicts, it has occurred during many others since that time.

To assess the effect of dengue on US military personnel stationed in dengue-endemic areas, we performed a literature search using "dengue" and "military" (109 titles), "army" (126), "navy" (22), "air force" (7), and "war" (29) and selected articles relevant to the US military. We searched personal files and reviewed military histories and books. References in these publications were reviewed for additional pertinent articles.

Author affiliations: Armed Forces Research Institute of Medical Science, Bangkok, Thailand (R.V. Gibbons); Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA (M. Streitz, T. Babina); and Mahidol-Oxford Tropical Medicine Research Unit, Bangkok (J.R. Fried)

DOI: http://dx.doi.org/10.3201/eid1804.110134

Before the Vietnam War, a diagnosis of dengue was usually based on clinical findings, sometimes supplemented by a complete blood count. The clinical diagnosis of dengue, especially in epidemiologically permissive settings of immunologically naive personnel assigned to tropical countries, is relatively accurate. Carefully described outbreaks of dengue in immunologically naive adults are almost pathognomonic. In 2 studies in the Philippines during 1924–1925 (*1*) and 1929–1930 (*2*), patients who had not traveled in dengue-endemic areas before or after the study were experimentally infected with the dengue virus, and clinical dengue developed . More than 40 years later, serologic testing confirmed that the patients in the first study had been infected with dengue virus serotype 1 and those in the second study with serotype 4 (*3*,*4*). In addition, a study from the Vietnam era serologically confirmed 77%–80% of clinically diagnosed dengue (*5*). Characteristics that identify a febrile outbreak as dengue include predominant leukopenia, maculopapular rash, retro-orbital headache, and a relatively long period of incapacitation after defervescence.

The references documented that since the Vietnam War, dengue has been diagnosed by hemagglutinin inhibition, plaque neutralization, complement fixation, and/or virus isolation. In most cases, assays (not sampling) were done after the illness to determine its etiology.

Before World War II

Cuba

After the Spanish–American War in 1898, US troops were stationed in Cuba, Puerto Rico, Panama, and the Philippines. In Cuba, troops had widespread and debilitating fevers from typhoid, malaria, and yellow fever, among other illnesses. The principal vector for dengue and

yellow fever, *Aedes aegypti* mosquitoes, was common in urban areas. Distinguishing dengue was a lower priority than distinguishing yellow fever and typhoid (*6*). The number of missed dengue diagnoses is unknown. A dengue epidemic in Cuba occurred in 1897, and some researchers have linked troop movements to subsequent outbreaks in Texas and Florida during the ensuing 3 years (*7*,*8*).

Among the second occupation force during the first decade of the 1900s, dengue reportedly occurred without causing any deaths. The most serious health threat throughout the new occupation was typhoid fever, which appeared in localized epidemics, occasionally causing deaths (*9*).

In 1903, with US encouragement, Panama proclaimed independence, and the Hay–Bunau-Varilla Treaty granted rights to the United States in a zone of ≈10 × 50 miles. In 1904, US Navy physicians reported 200 cases of dengue from the Isthmus of Panama (*10*). Exact numbers were not given, but reports noted that "[d]engue has already played an important part in increasing the ratio of sick days among the men stationed in our most recently acquired territory" (*11*).

Philippines

The Army Tropical Disease Board in the Philippines was created in 1898 to investigate a wide variety of health problems that threatened military and civilian populations. According to Brigadier General George H. Toney, dengue caused a "small constant non-effective rate" among the troops (*9*). In 1906, a dengue epidemic swept Fort William McKinley, located on a low site near Manila, and the study of dengue became a priority for members of the Board, including Percy Ashburn (Figure 1) and Charles Craig (Figure 2) (*9*,*12*).

The Philippine tour of duty was usually 2 years, and dengue-naive persons were arriving with each transport of troops. During 1902–1924, hospitalizations for dengue averaged 101 per 1,000 persons per year (range 12–213/1,000/year), and the average hospitalization lasted 7 days (*6*). Lieutenant Colonel J.F. Siler (Figure 3) recognized that the greatest risk was in the Manila urban environment; rates of disease were much lower in remote posts. Approximately 40% of newly arrived troops acquired dengue within 1 year; for 30% (12% of the total), illness recurred during their tour, and for 15% (<2% of the total and most of those staying beyond 2 years), dengue occurred a third time. Siler et al. proposed that these percentages underestimated disease incidence because most of the fevers of short duration (3–6 days) were of unknown cause and could have included dengue. Although rates of illness in general for troops in the Philippines had declined by half during 2 decades, rates for dengue did not appreciably change. Dengue was second only to venereal disease as the most common illness during this period (*1*).

Figure 1. Captain Percy Ashburn.

In 1928, Major James Simmons et al. found that annual hospitalizations for dengue per 1,000 troops per year were 6.84 for the entire Army (0.02 for the United States; 0.5 for Panama; and 177 for the Philippines) (*2*). Not only did >98% of cases occur in the Philippines, but also >96% were from Manila and surrounding areas. During 1925–1928, ≈4,000 work days were lost each year to hospitalization for dengue (*2*).

World War II

During 1942–1945, dengue was diagnosed in only 245 soldiers in Latin America (mostly from the Panama Canal Zone), compared with ≈80,000 who were hospitalized for dengue in the Pacific Theater, in addition to ≈8,000 in the China-Burma-India Theater (*13*). The epidemics engendered continued study of dengue, including Albert Sabin's research in pursuit of an effective dengue vaccine (Figure 4).

Australia

A dengue epidemic occurred in 1942 among US personnel stationed in Queensland and the Northern

Figure 2. First Lieutenant Charles Craig.

Territory; 80% of service members were affected during a 3-month period (*13*). A subsequent epidemic (463 cases) occurred during January–March 1943 (Table 1). Major Joseph Diasio et al., reporting from an analysis of 100 cases among US service members in Australia, found that the average hospitalization was 7.5 days. Informally, they observed in a small sample that patients needed another 7–10 days to return to full strength (*14*).

South Pacific

The Malaria and Epidemic Control Board of the South Pacific Area rated dengue second only to malaria as a tropical disease of military importance (*15*). This finding remains unchanged today (*19*). Dengue profoundly affected operations because of the weakness and fatigue that persisted for weeks after the acute phase. Dengue was reported to have caused nearly 1,600 hospitalizations during spring 1942 among Allied prisoners at the Changi Prisoner Camp on Singapore Island (*20*). The US military moved rapidly in the South Pacific to establish military bases without allowing time for precautions and prevention measures to avert the spread of dengue. The military focused on such imperative issues as food, ammunition, construction of defensive positions, and fighting; concern for local diseases, especially nonfatal diseases, was not a priority. The constant traffic of personnel and supplies between islands of the South Pacific contributed to the circulation of dengue by providing susceptible hosts and vector breeding sites.

Commander James Sapero and Lieutenant Commander Fred Butler reported "almost all troops" located in Tulagi (Solomon Islands) were affected by dengue shortly after ground action ceased in August 1942. They speculated that the evacuation of infected patients facilitated the spread of dengue in the South Pacific (Figure 5). Within 3 months in the Espiritu Santos area, dengue cases caused illness rates to increase from 12% to 40% (*21*); affected service members were absent from strenuous duty for at least 2 weeks (*22*). One publication reports an epidemic in the archipelago of New Hebrides (now Vanuatu) on the island of Espiritu Santo in 1943. The epidemic began in February, peaked in April and subsided in August; 25% of the base strength (≈5,000 personnel) was affected, with a maximum of 1,713 cases per 1,000 persons per year (*13*,*23*). The epidemic also affected New Caledonia but to a lesser extent (Table 1) (*13*).

Zeligs et al. reported that in July 1943, four members of an aviation unit flew from 1 unidentified island to

Figure 3. Lieutenant Commander J.F. Siler.

Figure 4. Major Albert Sabin.

another (*24*). Shortly after their arrival at the second island, dengue was diagnosed in all 4. At the same time, a dengue epidemic was identified on the first island. Traffic between the 2 islands could not be stopped because of the support required for combat operations, and the influx of personnel to the first island continued. To prevent the spread of disease, strict measures were enforced. Infected personnel were placed in an isolated camp, and the remaining servicemen were closely monitored for signs and symptoms (*24*). This transmission phenomenon was seen elsewhere. One author, reporting on an advanced base in Melanesia, wrote of dengue being brought by patients from neighboring islands, which resulted in 80,000 sick days and attack rates as high as 12% (*25*). In addition, in 1944, a total of 396 dengue-infected military personnel from the Gilbert Islands were evacuated to hospitals on Oahu, Hawaii (*16*).

Another author, writing of the epidemic in Marine and Navy personnel in the South Pacific, estimated that one third were affected and that a "large group were hospitalized." He noted, "The acute attack of dengue lasted for about 8 days, the convalescent period often ran into weeks before the patient could return to his previous type of duty" (*26*). One article noted that ≈2% of patients had pain so severe that they required morphine for relief (*27*).

Others reported 1,200 cases of dengue in March and April 1943 in Army troops on an unidentified island (*28*). Observers of this outbreak reported that temporary immunity existed for 5–10 months after an episode of dengue; after several attacks, more lasting immunity existed. The convalescent period was generally 2–3.5 weeks but even longer for older patients. The Thirteenth Air Force, operating in the South Pacific, reported that during March, 49 days were lost per 100 flying officers (*15*).

Severe outbreaks of dengue were reported on Saipan, an island in the Marianas. The first occurred in July 1944 in the Marshall Islands, when dengue was diagnosed in 744 persons, most of whom were on Saipan. The disease reportedly was much more clinically severe than it had been in 1943 (*16*). In August, 300 cases per 1,000 persons per year occurred and rapidly jumped to 3,500 per 1,000 per year by September 1944. With the arrival of DDT in September, the Army enacted a plan to control mosquitoes in the area. DDT and kerosene were sprayed from airplanes during September 13–22, 1944. Ten days of spraying seemed extremely effective; the attack rate decreased to 182 cases per 1,000 persons per year by October (*13*,*16*).

Table 1. Dengue in US service members during World War II*

Location	Dates	Attack rate, %	No. cases	Maximum no. cases/1,000/y	Reference
North Territory and Queensland, Australia	1942 Mar–May	80	ND	ND	(*13*)
Rockhampton/Brisbane, Australia	1943 Jan–Mar	ND	463	ND	(*14*)
Espiritu Santo, archipelago of New Hebrides (now Vanuatu)	1943 Feb–Aug	25	≈5,000	1,713	(*15*)
New Caledonia	1943 Jan–Aug	ND	ND	645	(*13*)
	1943 Jan–Aug	ND	ND	120	
Hawaii	1943	ND	56	ND	(*16*,*17*)
Gilbert Islands	1944	ND	396	26	(*16*)
New Guinea	1944 Jan–Dec	ND	24,079	198	(*13*)
	1945 Jan–Aug	ND	2,960	31	
Philippines†	1944 Nov–Dec	ND	2,012	49	(*13*)
	1945 Jan–Dec	ND	8926	32	
Saipan, Mariana Islands	1944 Jul–Sep	ND	~20,000	3,560	(*13*,*16*)
China-Burma-India	1943	ND	ND	25	(*13*)
	1944	ND	ND	31	
	1945	ND	ND	8	
Okinawa, Japan	1945 Apr–Aug	ND	≈865	275	(*18*)
Hankow, China	1945 Sep	83	40	ND	(*13*)

*ND, no data.
†Reported to have reached 68 cases/1,000 service members/year in the Sixth Army.

Figure 5. New Georgia Island medical clearing station, Solomon Islands, 1943.

Dengue cases among the staff of 2 major hospitals located on Saipan, the 148th General Hospital and the 176th Station Hospital, demonstrated the effectiveness of vector control through spraying. The former hospital arrived on August, 10, 1944, and the latter ≈6 weeks later. Spraying began on September 13, ≈1 week before the 176th Station Hospital opened. In the interim, the 148th General Hospital saw infection rates for staff as high as 47% (252 personnel), amounting to a rate of 3,500 cases per 1,000 persons per year. In contrast, the 176th Station Hospital experienced no dengue cases among its staff, probably because of improved vector control. Of 4,624 troops who arrived during September 17–30, a total of 41 (0.9%) cases occurred (232 cases/1,000 persons/year) (*16*,*23*).

Hawaii

After an absence of >30 years, dengue was reintroduced to Hawaii in July 1943 when commercial airline pilots carried the disease from the South Pacific to Honolulu. A dengue outbreak first appeared along Waikiki beach, resulting in the August 8 declaration of the area as off limits to the troops. Local authorities created a squad to go door to door inspecting premises and providing instructions and education to the public about preventing dengue (*17*,*23*).

Because of the strategic importance of the area and the role already played by dengue in combat operations, the Army designated soldiers to perform inspections along with the civilian squad. Travel was restricted among the Hawaiian Islands. Despite these measures, dengue cases in Waikiki increased. To prevent further spread, all premises in Waikiki were sprayed, and more soldiers were assigned to the inspection squad to help with mosquito elimination. Eventually, mosquito control was extended citywide, led by the US Public Health Service; most labor was provided by an Army medical service company (*13*). Additional areas were declared off limits to the troops (*17*). By June 1944, cases in 1,500 civilians and 56 military personnel had been reported (*16*,*17*).

Okinawa

The Army in Okinawa experienced a dengue outbreak during spring and summer 1945. Incidence peaked among members of an infantry unit at 275 cases per 1,000 persons per year in July. The authors noted 161 cases in a field hospital, 704 in a clearing station, and numerous others in various Army and Navy medical facilities. The average hospital stay was ≈7 days. None of the hospitalized patients required evacuation, and all returned to active duty (*18*).

New Guinea and Philippines

From the start of operations in New Guinea, dengue was a major cause of loss of troop strength. Statistics available for 1944–1945 indicate ≈27,000 cases; epidemics were reported in the Hollandia and Biak areas. By contrast, in the Philippines, dengue cases occurred only sporadically and without epidemic proportions, perhaps because of the extensive use of DDT in populated areas on Luzon from the beginning of the reoccupation (Figure 6) (*13*).

China-Burma-India Theatre

Most reported dengue cases in the China-Burma-India Theater occurred in Calcutta, reaching rates of 31 cases per 1,000 persons per year in 1944. In addition, the famed Merrill's Marauders reportedly were adversely affected by dengue. In September 1945, a dengue outbreak occurred in Hankow, China, which was reported to have affected 80% of the population, including Japanese personnel. Of the first 48 US troops to occupy the airport in Hankow, dengue developed in 40 within 5–10 days. The city area was deemed off limits, and a unit was ordered into the area for mosquito control (*13*).

Vietnam War

At the end of World War II, 2 dengue serotypes were discovered (*29*,*30*). During the decade leading up to the Vietnam War, 2 additional serotypes were identified, and dengue was found to cause a more severe illness, dengue hemorrhagic fever (*31*).

In 1964, an outbreak of dengue occurred in Ubol, Thailand, among US and Royal Australian Air Forces (Table 2). Of 294 men, dengue was confirmed for 16%–19% (*5*). A study conducted during 4 months in 1966 at the 93rd Evacuation Hospital in Long Binh, South Vietnam, evaluated 110 cases of fever of unknown origin (FUO, i.e., fever and a negative malaria smear in patients whose illness remained undiagnosed 24 hours after hospitalization). Of these, dengue was diagnosed in 31 (28%) and was the most

Figure 6. Airplane spraying of DDT over Manila, the Philippines, 1945.

prevalent disease causing FUOs. The researchers concluded that dengue was acquired within the urban setting of the base camp (*32*). Another study of FUOs (excluding malaria diagnosed during the first 72 hours after hospitalization) was conducted for 4 months during 1966–1967 at the Eighth Field Hospital in the semimountainous central coastlands of Vietnam. Ten (11%) of 94 cases were dengue (*33*). Nine patients came from more inhabited rather than rural regions. A third study of FUOs among 87 soldiers deployed to the rural Mekong River Delta in 1967 found that 3% of cases were caused by dengue (*34*).

During May 1965–April 1966, the average monthly incidence of dengue in US Army personnel in Vietnam was 3.5 cases per 1,000 troops (range 1.2–6.7/1,000) (*38*). As shown in FUO studies, dengue was underreported because of lack of laboratory capabilities. FUOs during the same period ranged from 9.1 to 101.0 cases per 1,000 persons per month (average 55.2/1,000/month); dengue constituted a substantial fraction. In 1967, the monthly incidence of dengue was 57–87 cases per 1,000 troops (*39*) (average 75/1,000) (*40*). A 1-year study from the 12th US Air Force Hospital at Cam Ranh Bay during 1967–1968 found that dengue caused 15 (5%) of 306 FUOs (*36*). In a 2-year study of servicemen residing separately from native populations and with 4 days of FUO in 6 Navy-Marine hospitals, 5 (0.6%) of 377 cases resulted from dengue (*37*). A summary of FUOs from Vietnam in 1969 found that 10% were caused by dengue (*35*). Unlike during World War II, dengue never reached major epidemic proportions among the troops in Vietnam. Nevertheless, a variety of studies attributed 3.4%–28% of "fever of undetermined origin" cases to dengue in service members who had had contact with the local population. Using these percentages with FUO numbers from the same period, we can calculate a monthly dengue incidence of 2–15 cases per 1,000 persons during 1965–1966 and 3–21 per 1,000 during 1967. Days lost because of FUOs averaged 225,000 per year during 1967–1970 (reference *41* in online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-0134-Techapp.pdf).

Although the more severe dengue hemorrhagic fever occurred among Vietnamese children, no cases were diagnosed in the troops. Most troops were unlikely to have been exposed to a second dengue virus infection, which predisposes them to more severe disease.

After the Vietnam War

Philippines

In 1984, Clark Air Base, north of Manila, had a population of ≈10,000 personnel. During June–September 1984, a total of 42 confirmed cases and 9 probable cases of dengue occurred. Of these, 35 occurred in military personnel and 25 (71%) persons were hospitalized. Hospitalization ranged from 3 to 11 days (average 5.9 days), and patients reported not being fit for duty for 3 to 18 days (average 14.6 days). One person was admitted to the intensive care unit and shock subsequently developed. By the end of September 1984, the vector populations were markedly reduced by an extensive education program and mosquito elimination strategies (*4*; *42* in online Technical Appendix).

Somalia

More than ≈30,000 US troops went to Somalia as part of Operation Restore Hope during 1992–3. Of 289 patients hospitalized with fever during that operation, 129 (45%)

Table 2. Dengue in US service members during the Vietnam War

Location or source of samples*	Dates	Dengue cases among fevers of unknown origin, %	Total no. fevers of unknown origin	Reference
Ubol, Thailand	1964 May–Aug	77–80	69*	(*5*)
Vietnam				
93rd Evacuation Hospital, Long Binh	1966 Apr–Aug	28	110	(*32*)
8th Field Hospital, Nha Trang	1967 Oct–Feb	11	94	(*33*)
Dong Tam, Mekong Delta	1967 Jun–Dec	3	87	(*34,35*)
I Corps	1967 Feb–Sep	3	295	(*35*)
12th US Air Force Hospital	1968 Jul–Jun	5	306	(*35,36*)
12th US Air Force Hospital	1969	10	1,256	(*35,36*)

*Attack rate in this study was 16%–19%.

did not have an immediately identified cause of illness. Of the 96 tested for dengue, 59 (61%) had positive results; dengue thus accounted for at least 20% of hospitalizations. Illnesses remained unspecified for 24%; many might have been dengue (*43* in online Technical Appendix).

An additional serologic study was performed on a military unit that had 26 (5%) members discharged from the hospital with unspecified febrile illness; dengue was confirmed for 17 (65%) (13 by virus isolation and 4 by IgM). A subsequent serosurvey showed that an additional 27 members of the unit had seroconverted to dengue virus; 16 had a febrile illness, 4 had nonfebrile illness, and 7 were asymptomatic. Thus, up to 7.5% (17 + 16 + 4 = 37 of 493) of the unit had dengue (*43* in online Technical Appendix). Some cases that remained unspecified were possibly dengue with waning IgM. In another study of patients consecutively hospitalized with fever, dengue viruses were isolated from 14 (17%) of 81, and serologic test results were positive for 15 (18%) of 84 (*44* in online Technical Appendix). In addition, dengue was confirmed in journalists and relief workers seeking care at US military field hospitals (*45* in online Technical Appendix). According to these studies of Operation Restore Hope, dengue accounted for 7%–21% of illness.

Haiti

In September 1994, ≈20,000 US military personnel deployed to Haiti as part of Operation Uphold Democracy. During the first 6 weeks, 30 (29%) of 103 patients hospitalized with febrile illness had confirmed dengue (22 virus isolation, 8 IgM); dengue was excluded for 40 (39%) cases, and cause was undetermined in 31 (30%). Patients came from urban and rural environments (*46* in online Technical Appendix). These numbers did not include outpatients. During the follow-up United Nations mission in Haiti, dengue was diagnosed in 79 (32%) of 249 soldiers and civilians who had fever and sought care at the 86th Combat Support Hospital. The actual numbers were probably much higher because only IgM testing was conducted (*47* in online Technical Appendix). In another report from the United Nations Mission in Haiti, dengue was confirmed in 233 (56%) of 414 suspected cases (*48* in online Technical Appendix).

The Present

Many US military operations involve small numbers of personnel in diverse locations. During October 2008–October 2010, dengue developed in at least 9 Special Forces soldiers. Recently, a report was published about a Special Forces soldier deployed in South America who became ill with dengue and required evacuation from a rural setting (*49* in online Technical Appendix); another report described a Marine who required hospitalization during deployment to the Philippines (*50* in online Technical Appendix). During 1999–2008, a total of 97 dengue cases (45 in the Army) (7 cases per million person-years) were reported among the active-duty personnel of the US Department of Defense (*51* in online Technical Appendix). A recent seroprevalence study of 500 samples from US Army Special Forces soldiers during 2006–2008 found antibodies against dengue in 11% (*52* in online Technical Appendix). No cases have been reported in the Iraq or Afghanistan conflicts.

The Future

Dengue has substantially weakened US military operations and reduced troop strength since the Spanish–American War. Recognizing these facts, the Military Infectious Disease Research Program and the Medical Research and Materiel Command have supported dengue vaccine research. A recent quantitative algorithm for prioritizing infectious disease threats to the US military rated dengue third behind malaria and bacterial diarrhea (*53* in online Technical Appendix). Historically, the military significance of dengue has probably been underestimated (*54*,*55* in online Technical Appendix). As US deployments around the globe continue, dengue prevention is needed for service members and other persons in dengue-endemic regions. Dengue vaccine development, despite many unique challenges, is moving forward and is the best hope for protection against dengue (*56* in online Technical Appendix).

COL Gibbons is chief of the Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok. He has held multiple clinical and research positions in the US Army, conducted dengue research at Walter Reed Army Institute of Research, and served as a CDC Epidemic Intelligence Services officer.

References

1. Siler JF, Hall MW, Hitchens AP. Dengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention. Philipp J Sci. 1926;29:1–302.
2. Simmons JS, St. John JH, Reynolds FHK. Experimental studies of dengue. Philipp J Sci. 1931;44:1–247.
3. Halstead SB. Etiologies of the experimental dengues of Siler and Simmons. Am J Trop Med Hyg. 1974;23:974–82.
4. Nishiura H, Halstead SB. Natural history of dengue virus (DENV)-1 and DENV-4 infections: reanalysis of classic studies. J Infect Dis. 2007;195:1007–13. http://dx.doi.org/10.1086/511825
5. Halstead SB, Udomsakdi S, Singharaj P, Nisalak A. Dengue chikungunya virus infection in man in Thailand, 1962–1964. 3. Clinical, epidemiologic, and virologic observations on disease in non-indigenous white persons. Am J Trop Med Hyg. 1969;18:984–96.
6. Gillett MC. Public health in Cuba. In: Clarke JJ, editor. Army historical series: the Army Medical Department 1865–1917. Washington (DC): Center of Military History, United States Army; 1995. p. 229–56.

7. Ehrenkranz NJ, Ventura AK, Cuadrado RR, Pond WL, Porter JE. Pandemic dengue in Caribbean countries and the southern United States—past, present, and potential problems. N Engl J Med. 1971;285:1460–9. http://dx.doi.org/10.1056/NEJM197112232852606
8. Levy MD. Dengue: observations on a recent epidemic. Medical Record. 1920;97:1040–2.
9. Gillett MC. Supporting the invasion forces. In: Clarke JJ, editor. Army historical series: the Army Medical Department 1865–1917. Washington (DC): Center of Military History, United States Army; 1995. p. 141–72.
10. Carpenter DN, Sutton RL. Dengue in the Isthmian Canal Zone. JAMA. 1905;XLIV:214–6.http://dx.doi.org/10.1001/jama.1905.92500300046001l
11. Sutton RL. Dengue in the Isthmian Canal Zone. JAMA. 1904;XLIII:1869–71.http://dx.doi.org/10.1001/jama.1904.92500250002k
12. Ashburn PM, Craig CF; US Army Board for the Study of Tropical Diseases. Experimental investigations regarding the etiology of dengue fever. 1907. J Infect Dis. 2004;189:1747–83.
13. McCoy OR. Preventive medicine in World War II: dengue. In: Coates JB, Hoff EC, editors. Medical Department, United States Army in World War II. Washington (DC): Surgeon General's Office; 1964. p. 29–62.
14. Diasio JS, Richardson FM. Clinical observations on dengue fever; report of 100 cases. Mil Surg. 1994;94:365–9.
15. Link MM, Coleman HA. Medical support of air combat in the Pacific. In: Medical support of the Army Air Forces in World War II. Washington (DC): United States Air Force: 1955. p. 794–810.
16. Mason VR. Internal medicine in World War II—activities of medical consultants: central Pacific area. In: Coates JB, Havens WP, editors. Medical Department, United States Army. Washington (DC): Surgeon General's Office; 1961. p. 625–92.
17. Gilbertson WE. Sanitary aspects of the control of the 1943–1944 epidemic of dengue fever in Honolulu. Am J Public Health Nations Health. 1945;35:261–70. http://dx.doi.org/10.2105/AJPH.35.3.261
18. Johnson JA, Marin WB, Breslow L. Dengue-like fever on Okinawa. Bull US Army Med Dep. 1946;5:306–11.
19. Memorandum for the Record, by COL MC Duane R. Hospenthal, MCHE-MDI, "Infectious Disease Threats to the US Military Prioritization Panel Results," 2010 Apr 23.
20. Smallman-Raynor MR, Cliff AD. War epidemics: an historical geography of infectious diseases in military conflict and civil strife, 1850–2000. New York: Oxford University Press; 2004.
21. Sapero JJ, Butler FA. Highlights on epidemic disease occurring in military forces. JAMA. 1945;127:502–6.http://dx.doi.org/10.1001/jama.1945.02860090008002
22. Carson DA. Observations on dengue. U S Nav Med Bull. 1944;42:1081–4.
23. Capps RB. Internal medicine in World War II: dengue. In: Coates JB, Havens WP, editors. Medical Department, United States Army in World War II. Washington (DC): Surgeon General's Office; 1963. p. 57–78.
24. Zeligs MA, Legant O, Webster EH. Epidemic dengue—its abortion in a combat area. U S Nav Med Bull. 1944;42:856–9.
25. Johnson LW. Sanitary problems of a tropical advanced base. Mil Surg. 1944;94:325–35.
26. Hyman AS. The heart of dengue; some observations made among Navy and Marine combat units in the South Pacific. War Medicine. 1943;4:497–501.
27. Cavanagh JR. Dengue; Observations on the disease as seen in the South Pacific area. War Medicine. 1943;4:549–55.
28. Kisner P, Lisansky ET. Analysis of an epidemic of dengue fever. Ann Intern Med. 1944;20:41–51.
29. Sabin AB. Research on dengue during World War II. Am J Trop Med Hyg. 1952;1:30–50.
30. Hotta S. Experimental studies on dengue. I. Isolation, identification and modification of the virus. J Infect Dis. 1952;90:1–9. http://dx.doi.org/10.1093/infdis/90.1.1
31. Hammon WM, Rudnick A, Sather GE. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. Science. 1960;131:1102–3. http://dx.doi.org/10.1126/science.131.3407.1102
32. Deller JJ Jr, Russell PK. An analysis of fevers of unknown origin in American soldiers in Vietnam. Ann Intern Med. 1967;66:1129–43.
33. Reiley CG, Russell PK. Observations of fevers of unknown origin in the Republic of Vietnam. Mil Med. 1969;134:36–42.
34. Colwell EJ, Boone SC, Brown JD, Legters LJ, Russell PK, Catino D. investigations on acute febrile illness in American servicemen in the Mekong Delta of Vietnam. Mil Med. 1969;134:1409–14.
35. Russell PK, Ognibene AJ, Barrett O, eds. Internal medicine in Vietnam. Volume II. General medicine and infectious diseases. Washington (DC): Surgeon General's Office, Medical Department of the US Army; 1982.
36. Deaton JG. Febrile illnesses in the tropics (Vietnam). Mil Med. 1969;134:1403–8.
37. Berman SJ, Irving GS, Kundin WD, Gunning JJ, Watten RH. Epidemiology of the acute fevers of unknown origin in South Vietnam: effect of laboratory support upon clinical diagnosis. Am J Trop Med Hyg. 1973;22:796–801.
38. Gilbert DN, Greenberg JH. Vietnam: preventive medicine orientation. Mil Med. 1967;132:769–90.
39. Gilbert DN, Moore WL Jr, Hedberg CL, Sanford JP. Potential medical problems in personnel returning from Vietnam. Ann Intern Med. 1968;68:662–78.
40. Greenberg JH. Public health problems relating to the Vietnam returnee. JAMA. 1969;207:697–702. http://dx.doi.org/10.1001/jama.1969.03150170023004

Address for correspondence: Robert V. Gibbons, Department of Virology, Armed Forces Research Institute of Medical Science (AFRIMS), USAMC-AFRIMS, APO AP 96546, USA; email: robert.gibbons@afrims.org

Bartonella spp. in Rats and Zoonoses, Los Angeles, California, USA

Vijay A.K.B. Gundi, Sarah A. Billeter, Michael P. Rood, and Michael Y. Kosoy

Bartonella spp. were detected in rats (*Rattus norvegicus*) trapped in downtown Los Angeles, California, USA. Of 200 rats tested, putative human pathogens, *B. rochalimae* and *B. tribocorum* were found in 37 (18.5%) and 115 (57.5%) rats, respectively. These bacteria among rodents in a densely populated urban area are a public health concern.

Bartonella spp. are vector-borne bacteria associated with an increasing array of emerging zoonotic infections in humans and animals (*1*). Some *Bartonella* spp. are widely distributed among small mammals in the United States and potentially cause human health concerns because these bacteria may be associated with human diseases (*2*). However, limited surveys have been conducted to identify infectious agents involved in zoonotic infections in rodents within urban areas of the United States (*3*). Norway rats (*Rattus norvegicus*) have been shown to harbor several *Bartonella* species, including *B. tribocorum*, *B. elizabethae*, *B. rattimassiliensis*, *B. phoceensis*, *B. queenslandensis*, and a strain closely related to *B. rochalimae* (*3*–*6*), of which *B. elizabethae*, *B. rochalimae*, and *B. tribocorum* were implicated in human diseases (*7*–*11*).

Our study had 3 purposes. The first purpose was to investigate prevalence of *Bartonella* spp. infections in rodent populations in downtown Los Angeles, California, USA. The second purpose was to evaluate genetic diversity of *Bartonella* spp. in blood of urban rats by analyzing variations of the citrate synthase (*gltA*) gene. The third purpose was to compare rates of detection of *Bartonella* spp. infections in rats between culture and molecular assays.

Author affiliations: Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (V.A.K.B. Gundi, S.A. Billeter, M.Y. Kosoy); and Los Angeles County Department of Public Health, Baldwin Park, California, USA (M.P. Rood)

DOI: http://dx.doi.org/10.3201/eid1804.110816

The Study

Rats were sampled during 2003–2007 at 16 sites in downtown Los Angeles in a rodent management/disease surveillance program. The rats were captured by using Tomahawk live traps (Tomahawk Live Trap Co., Hazelhurst, WI, USA) and anesthetized with CO_2. Blood samples were obtained by cardiac puncture, transferred to sterile cryovials, placed on dry ice, and preserved at –70°C until testing at the *Bartonella* Laboratory of the Centers for Disease Control and Prevention (Fort Collins, CO, USA).

Genomic DNA was extracted from animal blood according to the blood protocol of the QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA). Primers CS140f and CS443f (*12*) and CS781f and CS1137n (*13*), including newly designed primers CS120f-5′-TTTCACTTATGATCCTGGCTT-3′ and CS1210r-5′-GATCYTCAATCATTTCTTTCCA-3′, which amplify DNA fragments ranging from 380 to 1,091 bp encompassing a 327-bp–specific zone (between bp positions 801 and 1127) of the *gltA* gene (*14*), were used in different combinations.

Eight PCRs were performed for each sample. PCR products were purified by using the QIAquick PCR Purification Kit (QIAGEN, Germantown, MD, USA). The same primers were used for DNA sequencing. The obtained DNA sequences were trimmed to 327 bp between positions 801 and 1127 because most *Bartonella* spp. *gltA* sequences available in GenBank are limited to this size.

Details of procedures used for isolation of *Bartonella* spp. from mammalian blood have been described (*2*). Rat blood was diluted 1:4 in brain–heart infusion medium containing 5% fungizone and pipetted onto heart infusion agar plates containing 10% rabbit blood. Plates were incubated aerobically at 35°C in an atmosphere of 5% CO_2 for ≤4 weeks and monitored for bacterial growth at least 1× per week after initial plating. Colonies were subpassaged onto fresh agar plates until a pure culture, free from contamination, was obtained.

Analysis of DNA sequences and phylogenetic relationships was performed by using MEGA4 (www.megasoftware.net/) and the neighbor-joining method with the Kimura 2-parameter distance model. Stability of inferred phylogeny was assessed by using bootstrap analysis of 1,000 randomly generated trees. DNA sequences obtained in this study were deposited in GenBank under accession nos. JF429450–JF429625.

A total of 200 *R. norvegicus* rats were trapped in 16 sites in downtown Los Angeles. *Bartonella* DNA was detected in 135 (67.5%) of 200 rat blood samples. PCR-positive blood samples were subsequently cultured for viable *Bartonella* organisms. Fifty-nine (43.7%) of 135 blood samples were confirmed as bacteremic for bartonellae by culturing.

A total of 176 sequences were obtained either directly from blood samples (117 sequences) or from pure cultures

(59 sequences). Among 176 *gltA* sequences, 23 genotypes with ≥1 nt difference were found, and sequence similarity between genotypes ranged from 85.3% to 99.7%. These 23 genotypes were closely related to *B. tribocorum* (n = 16), *B. rochalimae* (n = 2), *B. queenslandensis* (n = 1), or 4 potentially novel genotypes. A total of 130 DNA sequences (JF429450–JF429579) obtained from 115 rats were grouped into 16 genotypes (1–16). These genotypes showed 98.2%–99.7% sequence similarity with each other and were genetically related to *B. tribocorum* IBS506^T (AJ005494) (97.9%–100% identity). All 16 genotypes clustered with *B. tribocorum* with a high bootstrap value, as shown in the phylogenetic tree (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/4/11-0816-FA1.htm).

Two genotypes (22 and 23) from 38 sequences (JF429588–JF429625) obtained from 37 rats had a similarity between 98.5% and 98.8% to *B. rochalimae* BMGH (DQ683195). These 2 genotypes joined with *B. rochalimae* with a high bootstrap branching pattern in phylogenetic analysis (online Appendix Figure). The *B. queenslandensis* group consisted of a single genotype (genotype 17) from 4 sequences (JF429580–JF429583) from 4 rats. This genotype demonstrated a sequence similarity to *B. queenslandensis* AUST/NH12^T (EU111800) of 99.0% for the *gltA* gene (online Appendix Figure).

Conclusions

In this study, 3 novel *Bartonella* genogroups identified in 4 *R. norvegicus* rats were not genetically related to any known *Bartonella* spp. and might represent novel *Bartonella* spp. Genotypes 18 (JF429586) and 19 (JF429587) formed a *Bartonella* genogroup with 95% similarity to *B. tribocorum* as the closest species. Likewise, genotypes 20 (JF429584) and 21 (JF429585) have 91.4% and 92.6% sequence identities, respectively, with *B. clarridgeiae,* which is their closest related species (online Appendix Figure).

Of 135 PCR-positive blood samples, 59 yielded cultures of 3 *Bartonella* spp.: *B. tribocorum* (n = 54), *B. rochalimae* (n = 4), and *B. queenslandensis* (n = 1). The number of CFU per 100 μL of blood varied: 800–1,400 CFU for *B. tribocorum*, 160–240 CFU for *B. rochalimae*, and 100 CFU for *B. queenslandensis*. Although 4 rats were positive by PCR for *B. queenslandensis*, this organism was cultured from only 1 of these animals.

Our study reports detection and identification of *Bartonella* spp. in urban rats in downtown Los Angeles, California. We demonstrated that *R. norvegicus* in downtown Los Angeles can serve as reservoirs of several *Bartonella* spp., such as *B. rochalimae*, *B. tribocorum*, *B. queenslandensis*, and possibly 3 additional novel species. Some genotypes identified in rats showed a high level of similarity (≤98.8%) with a *B. tribocorum* isolate obtained from a febrile patient in Thailand (GenBank accession no. GQ225706) (*11*). Another species (*B. rochalimae*) was isolated from a patient with splenomegaly who had traveled to South America (*14*). This bacterium has also been isolated from dogs, foxes, rats, shrews, gerbils, and raccoons, suggesting that multiple reservoirs may be involved in maintenance of this species. One *Bartonella* genotype found in 4 rats in this study was 99.0% similar to *B. queenslandensis* (GenBank accession no. EU111800), which was originally isolated from *R. fuscipes* rats in Australia (*15*).

Because most identified *Bartonella* species have been reported as human infectious agents elsewhere, the finding that they were circulating among rodents in a densely populated urban area is of serious public health concern. In this context, further studies should be conducted on a larger collection of rodents and clinical human samples to determine evolutionary, genetic, and pathogenic relationships.

Acknowledgment

We thank Robert Flores for assistance with rat trapping and processing.

This study was supported in part by an appointment to the Emerging Infectious Diseases Fellowship Program administered by the Association of Public Health Laboratories and funded by the Centers for Disease and Control and Prevention.

Dr Gundi is an Emerging Infectious Diseases Fellow at the *Bartonella* Laboratory, Division of Vector-Borne Diseases, Centers for Disease Control and Prevention, Fort Collins, Colorado. His research interests include tropical and emerging infectious diseases.

References

1. Breitschwerdt EB, Maggi RG, Chomel BB, Lappin MR. Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings. J Vet Emerg Crit Care (San Antonio). 2010;20:8–30. http://dx.doi.org/10.1111/j.1476-4431.2009.00496.x
2. Kosoy MY, Regnery RL, Tzianabos T, Marston EL, Jones DC, Green D, et al. Distribution, diversity, and host specificity of *Bartonella* in rodents from the southeastern United States. Am J Trop Med Hyg. 1997;57:578–88.
3. Ellis BA, Regnery RL, Beati L, Bacellar F, Rood M, Glass GG, et al. Rats of the genus *Rattus* are reservoir hosts for pathogenic *Bartonella* species: an Old World origin for a New World disease? J Infect Dis. 1999;180:220–4. http://dx.doi.org/10.1086/314824
4. Heller R, Riegel P, Hansmann Y, Delacour G, Bermond D, Dehio C, et al. *Bartonella tribocorum* sp. nov., a new *Bartonella* species isolated from the blood of wild rats. Int J Syst Bacteriol. 1998;48:1333–9. http://dx.doi.org/10.1099/00207713-48-4-1333
5. Gundi VA, Davoust B, Khamis A, Boni M, Raoult D, La Scola B. Isolation of *Bartonella rattimassiliensis* sp. nov. and *Bartonella phoceensis* sp. nov. from European *Rattus norvegicus.* J Clin Microbiol. 2004;42:3816–8. http://dx.doi.org/10.1128/JCM.42.8.3816-3818.2004

6. Lin JW, Chen CY, Chen WC, Chomel BB, Chang CC. Isolation of *Bartonella* species from rodents in Taiwan including a strain closely related to '*Bartonella rochalimae*' from *Rattus norvegicus*. J Med Microbiol. 2008;57:1496–501. http://dx.doi.org/10.1099/jmm.0.2008/004671-0
7. Daly JS, Worthington MG, Brenner DJ, Moss CW, Hollis DG, Weyant RS, et al. *Rochalimaea elizabethae* sp. nov. isolated from a patient with endocarditis. J Clin Microbiol. 1993;31:872–81.
8. Comer JA, Diaz T, Vlahov D, Monterroso E, Childs JE. Evidence of rodent-associated *Bartonella* and *Rickettsia* infections among intravenous drug users from central and east Harlem, New York City. Am J Trop Med Hyg. 2001;65:855–60.
9. Smith HM, Reporter R, Rood MP, Linscott AJ, Mascola LM, Hogrefe W, et al. Prevalence study of antibody to ratborne pathogens and other agents among patients using a free clinic in downtown Los Angeles. J Infect Dis. 2002;186:1673–6. http://dx.doi.org/10.1086/345377
10. Eremeeva ME, Gerns HL, Lydy SL, Goo JS, Ryan ET, Mathew SS, et al. Bacteremia, fever, and splenomegaly caused by a newly recognized *Bartonella* species. N Engl J Med. 2007;356:2381–7. http://dx.doi.org/10.1056/NEJMoa065987
11. Kosoy M, Bai Y, Sheff K, Morway C, Baggett H, Maloney SA, et al. Identification of *Bartonella* infections in febrile human patients from Thailand and their potential animal reservoirs. Am J Trop Med Hyg. 2010;82:1140–5. http://dx.doi.org/10.4269/ajtmh.2010.09-0778
12. Birtles RJ, Raoult D. Comparison of partial citrate synthase gene (*glt*A) sequences for phylogenetic analysis of *Bartonella* species. Int J Syst Bacteriol. 1996;46:891–7. http://dx.doi.org/10.1099/00207713-46-4-891
13. Norman AF, Regnery R, Jameson P, Greene C, Krause DC. Differentiation of *Bartonella*-like isolates at the species level by PCR-restriction fragment length polymorphism in the citrate synthase gene. J Clin Microbiol. 1995;33:1797–803.
14. La Scola B, Zeaiter Z, Khamis A, Raoult D. Gene-sequence-based criteria for species definition in bacteriology: the *Bartonella* paradigm. Trends Microbiol. 2003;11:318–21. http://dx.doi.org/10.1016/S0966-842X(03)00143-4
15. Gundi VA, Taylor C, Raoult D, La Scola B. *Bartonella rattaustraliani* sp. nov., *Bartonella queenslandensis* sp. nov. and *Bartonella coopersplainsensis* sp. nov., identified in Australian rats. Int J Syst Evol Microbiol. 2009;59:2956–61. http://dx.doi.org/10.1099/ijs.0.002865-0

Address for correspondence: Michael Y. Kosoy, Centers for Disease Control and Prevention, 3150 Rampart Rd, Fort Collins, CO 80521, USA; email: mck3@cdc.gov

Detection of *Plasmodium* spp. in Human Feces

Milan Jirků,[1] Kateřina Pomajbíková,[1] Klára J. Petrželková, Zuzana Hůzová, David Modrý, and Julius Lukeš

Comparison of diagnostic methods for *Plasmodium* spp. in humans from Uganda and the Central African Republic showed that parasites can be efficiently detected by PCR in fecal samples. These results, which rely solely on PCR-based examination of feces, validate numerous estimates of the prevalence of malaria in great apes.

In spite of a century of research, knowledge of *Plasmodium* spp. affecting the African great apes is limited. However, molecular tools have recently shown unexpectedly high diversity of these species (*1*–*5*). Available evidence supports the scenario in which humans acquired *P. falciparum* from western gorillas (*3*,*6*) and carried it in their blood throughout the world after migrating from Africa (*7*). However, recent discovery of a *P. falciparum*–related parasite in the African putty-nosed monkey calls into question this theory of the origin of this most malignant and widespread *Plasmodium* species (*8*). All data for identification of *Plasmodium* spp. were obtained only by PCR-based amplification of feces of free-living great apes (*9*). Because it is difficult to obtain their blood samples, which are used from for detection of *Plasmodium* spp. in other hosts, estimation of the prevalence of *Plasmodium* spp. in great apes is problematic.

Plasmodium spp. cause >200 million malaria cases in humans (*10*). The availability, sensitivity, and accuracy of diagnostic methods often rely on use of blood samples (*11*), making any attempts to inspect other material from humans for these pathogens unnecessary or impractical. Furthermore, a blood parasite would not be expected to be present in feces at detectable amounts (*12*). No attempts to identify *Plasmodium* spp. in feces of infected persons have been reported.

Author affiliations: Biology Centre, Institute of Parasitology, ASCR, České Budějovice, Czech Republic (M. Jirků, D. Modrý, J. Lukeš); Faculty of Science, University of South Bohemia, České Budějovice (M. Jirků, J. Lukeš); University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic (K. Pomajbíková, D. Modrý); CEITEC, Brno (D. Modrý); Institute of Vertebrate Biology, ASCR, Brno, and Zoo Liberec, Liberec, Czech Republic (K.J. Petrželková); and Health Institute, Prague, Czech Republic (Z. Hůzová)

DOI: http://dx.doi.org/10.3201/eid1804.110984

Studies of great apes (*3*,*4*,*9*) have focused on diversity and phylogeny of *Plasmodium* spp. However, fecal-based diagnostics open a plethora of questions regarding the prevalence, epidemiology, and clinical role of malaria in apes. To answer these questions, we urgently need to assess the reliability of fecal-based diagnostics. Motivated by prior feasibility of such an approach in closely related great apes, we examined whether malarial infection is detectable in feces of infected humans, and if so, in what fraction of infected persons does the parasite penetrate into feces?

The Study

We analyzed fecal samples from 16 patients given a diagnosis of malaria at Lwanga Hospital in Buikwe, Uganda, who were undergoing treatment during October–December 2010; twenty-eight employees of Dzanga-Sangha Protected Areas, Central African Republic, who when samples were obtained during November–December 2010, did not show any symptoms of malaria but were considered a high risk group; and 6 Europeans who had repeated cases of malaria during 2003–2010 (samples were provided in April 2011). Samples obtained from 2 Europeans who never visited regions to which malaria was endemic were used as negative controls. Blood and fecal samples were obtained from each person. Blood smears were also prepared from the 16 patients in Uganda. We adhered to the research protocol defined by Dzanga-Sangha Protected Areas. Permission to collect samples was obtained from all examined persons before samples were obtained.

Thick blood smears were prepared by spreading 2–3 drops of fresh blood on a slide. Slides were dried, stained for 20 min with a 1:9 dilution of Giemsa, washed with tap water for 3–5 min, dried, and inspected for *Plasmodium* stages by light microscopy (100 fields at a magnification of ×1,000). Parasitemia levels in positive samples were determined according to standard protocols (*13*). Feces and blood were fixed in 96% ethanol and stored at room temperature until processed by using the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). DNA from blood samples stored on filter paper in ethanol was isolated as follows. The ethanol was evaporated; the filter paper was then transferred into 200 μL of 5% Chelex 100 (Sigma, St. Louis, MO, USA), incubated at 56°C for 1 hour, boiled for 10 min, and stored at 4°C. Before use, samples were centrifuged for 1 min at 15,000 rpm.

We amplified part of the apocytochrome (*cyB*) gene of *Plasmodium* spp. by using a reported protocol (*9*) and primers DW2-F and DW4-R in a first-round PCR and primers CYTB1-F and CYTB2-R in a second-round nested PCR. The amplified 938-bp fragment was resolved by agarose gel electrophoresis (Figure). Samples were

[1]These authors contributed equally to this article.

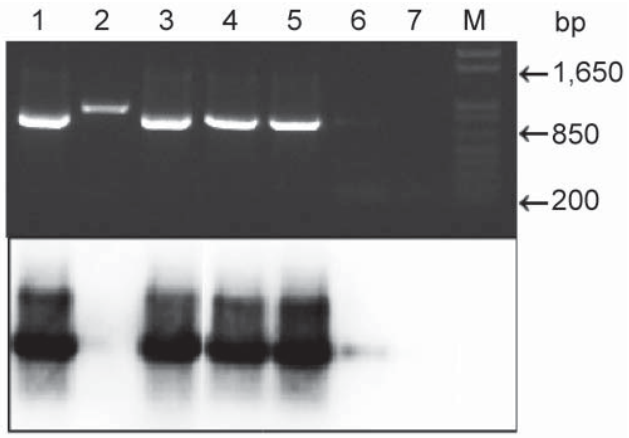


Figure. Top, agarose gel electrophoresis of nested PCR products of human fecal samples amplified with primers (pairs DW2-F + DW2-R and CYTB1-F + CYTB2-R) and stained with ethidium bromide. Bottom, autoradiograph of a Southern blot of the same gel. α-^{32}P-ATP–labeled acytochrome B gene of *Plasmodium falciparum* was used as a probe. Lanes 1–6, samples from humans with malaria (the infection sample in lane 6 is weak); lane 2, spurious amplicon; lane 7, sample from an uninfected person; lane M, 1-kb molecular mass (Invitrogen, Carlsbad, CA, USA).

subsequently blotted onto membranes and hybridized with the same PCR product labeled by random priming with ^{32}P-dATP (ICN, Costa Mesa, CA, USA). The membrane was hybridized at 65°C overnight and washed 3× in SSC (0.15 M NaCl, 0.015 M sodium citrate, 0.1% sodium dodecyl sulfate) at 65°C (20 min/wash), and analyzed by using a phosphoimager. The *cyB* gene of *P. falciparum* was used as a positive control.

We analyzed fecal and blood samples obtained from persons in Uganda and determined their suitability for identification of *Plasmodium* spp. Parasites in various stages were detected in 13 blood smears (Table); all were identified as *P. falciparum*. PCR amplified a band of expected size from blood of 15 persons, and a weak band was consistently obtained from 6 samples. Amplification of *Plasmodium cyB* from feces of these patients was equally efficient; only 2 negative samples were identified (Table). A good correlation was observed between intensity of PCR products obtained from blood and feces, indicating that low parasitemia levels result in poor amplification regardless of the biological material used.

PCR products for 2 fecal samples were then subjected to Southern blotting, which in 1 of 2 negative samples detected the cyB amplicon, which was not visible on an agarose gel (Figure). This method also confirmed that 1 band of slightly different mobility was a spurious amplicon (Figure). Therefore, with 1 exception, samples from 15 persons with *Plasmodium*–spp. positive blood samples contained *Plasmodium* DNA in their feces. Ten amplicons were sequenced and confirmed as being from *P. falciparum*.

We compared the capacity of blood and feces from persons clinically asymptomatic for malaria from the Central African Republic to amplify *cyB*. Blood and fecal samples from 5 of 28 persons showed bands of expected size, indicating asymptomatic or chronic malaria. Samples with positive and negative PCR results were also subjected to Southern blotting, which confirmed these results. In this dataset, the ability of blood and feces to accurately diagnose malaria was equal. All blood and fecal samples obtained from the 8 Europeans showed negative PCR results.

Conclusions

We have shown that similar to apes, infected humans shed a detectable amount of *P. falciparum* in their feces, which correlates with results obtained by PCR. Southern blotting slightly enhanced the sensitivity of the PCR,

Table. Comparative analysis of diagnostic methods for *Plasmodium* spp. with different biologic material from humans, Africa*

Sample origin, no.	Blood smear parasitemia (%)	Blood PCR	Fecal PCR	Fecal Southern blot
Buikwe, Uganda				
T2767/11	+ (0.31)	+	+	+
T2768/11	+ (0.10)	+	+	+
T2770/11	+ (0.11)	+ (w)	+	+
T2774/11	+ (0.5)	+	+	+
T2775/11	+ (0.90)	+	+	+
T2776/11	+ (0.16)	+	+	+
T2777/11	+ (0.24)	+	+	+
T2779/11	+ (0.40)	+ (w)	+	+
T2781/11	+ (0.65)	+	+	+
T2785/11	+ (2.10)	+	+	+
T2786/11	+ (0.18)	+	+	+
T2772/11	+ (1.2)	+	–	–
T2778/11	+ (0.36)	–	+	+
T2778/11	–	+ (w)	+	+
T2782/11	–	+ (w)	+	+
T2780/11	–	+ (w)	–	+
Bayanga, Central African Republic	NA	+ (6)†	+ (6)†	+ (6)†

*+, positive; w, weak band; –, negative; NA, not available.
†Samples from 6 persons.

but visual inspection of gel-resolved PCR products from feces was nearly equally sufficient (Table and Figure). We conclude that feces are as suitable as blood for malaria diagnostics for humans.

Although informative in terms of parasite diversity, prevalence of *Plasmodium* spp. amplified from feces of gorillas and chimpanzees was not determined in blood samples. A cautionary note regarding this issue and other issues has been reported (*14*). Our results show that in humans, *P. falciparum* efficiently penetrates the feces at levels detectable by PCR. Use of humans as proxies in our study validates previous estimates of malaria infection rates determined from feces of great apes (*3,4,7,9,12*). The diagnostic method we describe is suitable in situations in which feces are easier to obtain than blood and for use with small children.

Acknowledgments

We thank R. Ssentongo, K. Karásková, M. Chmelařová, B. Kalousová, P. Smejkalová, and K. A. Shutt for assistance with collecting and transporting material; the government of the Central African Republic and the World Wildlife Fund for granting permission to conduct our research in the Central African Republic; the Ministre de l'Education Nationale, de l'Alphabetisation, de l'Enseignement Superieur, et de la Recherche for providing research permits; and Primate Habituation Project and A. Todd for providing logistical support in the field.

This study was conducted with support from the Czech Science Foundation (grant #206/09/0927) awarded to K.J.P. and D.M. and a Praemium Academiae award to J.L.

Dr Jirků is a research scientist at the Institute of Parasitology in České Budějovice, Czech Republic. His research interests include kinetoplastid and apicomplexan parasites.

References

1. Duval L, Nerrienet E, Rousset D, Mba SA, Houze S, Fourment M, et al. Chimpanzee malaria parasites related to *Plasmodium ovale* in Africa. PLoS ONE. 2009;4:e5520. http://dx.doi.org/10.1371/journal.pone.0005520
2. Krief S, Escalante AA, Pacheco MA, Mugisha L, André C, Halbwax M, et al. On the diversity of malaria parasites in African apes and the origin of *Plasmodium falciparum* from bonobos. PLoS Pathog. 2010;6:e1000765. http://dx.doi.org/10.1371/journal.ppat.1000765
3. Liu W, Li Y, Learn GH, Rudicell RS, Robertson JD, Keele BF, et al. Origin of the human malaria parasite *Plasmodium falciparum* in gorillas. Nature. 2010;467:420–5. http://dx.doi.org/10.1038/nature09442
4. Rayner JC, Liu W, Peeters M, Sharp PM, Hahn BH. A plethora of *Plasmodium* species in wild apes: a source of human infection? Trends Parasitol. 2011;27:222–9. http://dx.doi.org/10.1016/j.pt.2011.01.006
5. Ollomo B, Durand P, Prugnolle F, Douzery E, Arnathau C, Nkoghe D, et al. A new malaria agent in African hominids. PLoS Pathog. 2009;5:e1000446. http://dx.doi.org/10.1371/journal.ppat.1000446
6. Duval L, Fourment M, Nerrienet E, Rousset D, Sadeuh SA, Goodman SM, et al. African apes as a reservoir of *Plasmodium falciparum* and the origin and diversification of *Laverania* subgenus. Proc Natl Acad Sci U S A. 2010;107:10561–6. http://dx.doi.org/10.1073/pnas.1005435107
7. Tanabe K, Mita T, Jombart T, Eriksson A, Horibe S, Palacpac N, et al. *Plasmodium falciparum* accompanied the human expansion out of Africa. Curr Biol. 2010;20:1283–9. http://dx.doi.org/10.1016/j.cub.2010.05.053
8. Prugnolle F, Ollomo B, Durand P, Yalcindag E, Arnathau C, Elguero E, et al. African monkeys are infected by *Plasmodium falciparum* nonhuman primate–specific strains. Proc Natl Acad Sci U S A. 2011;108:11948–53. http://dx.doi.org/10.1073/pnas.1109368108
9. Prugnolle F, Durand P, Neel C, Ollomo B, Ayala FJ, Arnathau C, et al. African great apes are natural hosts of multiple related malaria species, including *Plasmodium falciparum.* Proc Natl Acad Sci U S A. 2010;107:1458–63. http://dx.doi.org/10.1073/pnas.0914440107
10. Hay SI, Guerra CA, Gething PW, Patil AP, Tatem AJ, Noor AM, et al. A world malaria map: *Plasmodium falciparum* endemicity in 2007. PLoS Med. 2009;6:e1000048. http://dx.doi.org/10.1371/journal.pmed.1000048
11. Chotivanich K, Silamut K, Day NP. Laboratory diagnosis of malaria infection: a short review of the methods. New Zealand Journal of Medical Laboratory Science. 2007;61:4–7.
12. Prugnolle F, Durand P, Ollomo B, Duval L, Ariey F, Arnathau C, et al. A fresh look at the origin of *Plasmodium falciparum*, the most malignant malaria agent. PLoS Pathog. 2011;7:e1001283. http://dx.doi.org/10.1371/journal.ppat.1001283
13. Garcia LS, Bullock-Lacullo SL, Fristche TR, Grady KK, Healy GR, Palmer J, et al. Laboratory diagnosis of blood-borne parasitic diseases. Approved guideline M15-A. 2000; 20(12) [cited 2012 Jan 5]. http://www.clsi.org/source/orders/free/m15-a.pdf
14. Valkiūnas G, Ashford RW, Bensch S, Killick-Kendrick R, Perkins S. A cautionary note concerning *Plasmodium* in apes. Trends Parasitol. 2011;27:231–2. http://dx.doi.org/10.1016/j.pt.2011.02.008

Address for correspondence: Julius Lukeš, Biology Centre, Branišovská 31, 37005 České Budějovice, Czech Republic; email: jula@paru.cas.cz

Increase in Extraintestinal Infections Caused by *Salmonella enterica* Subspecies II–IV

Sharon L. Abbott, Frank C.Y. Ni, and J. Michael Janda

To garner information regarding site of infection and age and sex of persons infected with *Salmonella enterica* subspecies II–IV, we retrospectively analyzed data on *Salmonella* spp. infections in California, USA, 1985–2009. These subspecies were found to cause significantly more frequent invasive disease (e.g., bacteremia) than did *Salmonella* subspecies I strains.

The genus *Salmonella* has 2 species, *bongori* and *enterica*; the latter species is divided into 6 subspecies: *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI). *S. bongori* and *Salmonella* subsp. VI are rarely isolated from humans (*1*). However, for the more commonly isolated subspecies (II, IIIa, IIIb, IV), little information is available on their distribution in extraintestinal infections or on the demographic characteristics of the patients from whom they are recovered. Therefore, we retrospectively analyzed salmonellae data (>75,000 isolates) collected by a large state laboratory over 25 years.

The Study

From 1985 through 2009, the Microbial Diseases Laboratory of the California Department of Public Health serotyped 1,342 isolates of *Salmonella* from individual patients; the isolates belonged to subspecies II–IV and encompassed 126 different serotypes. Serotypes of subspecies II were the most rare, with a total of 60 (4%) isolates; subspecies IIIa, IIIb, and IV had isolate totals of 463 (35%), 443 (33%), and 376 (28%), respectively. Overall, patients were more likely to have had subspecies II–IV isolated from feces (n = 947) than from an extraintestinal site (n = 395; p<0.001). Only patients from whom subspecies IIIa was isolated were equally likely to have had an extraintestinal infection as to have had diarrhea only (Table 1). Sources of extraintestinal infections included cerebrospinal fluid, blood, urine, cervix, bile, wounds and abscesses, and the respiratory tract. Blood and urine were the most common extraintestinal sites, comprising 42% (n = 167) and 33% (n = 129) of the isolates. Although blood was the most common site for subspecies IIIa and IV (p<0.001), the most common site for subspecies II and IIIb was urine (p<0.001). The only 2 subspecies isolated from cerebrospinal fluid were subspecies IIIa serotypes.

When information on the patient's sex was included, male patients were only slightly more likely (707/1,342, 53%) than were female patients (617/1,342, 46%) to have had an infection caused by non–subspecies I salmonellae. The numbers of male and female patients were also comparable, regardless of whether the site of salmonellae infection was fecal or extraintestinal (Table 2). Only when patient's sex was considered according to individual subspecies did the number of infections associated with male patients differ significantly from the number associated with female patients. Male patients were more likely than female patients to have fecal (63% vs. 36%; p>0.001) and extraintestinal (66% vs. 33%; p<0.001) infections caused by subspecies IIIa, whereas female patients were more likely to have extraintestinal infections caused by subspecies IIIb (68% vs. 31%; p<0.001).

Few remarkable associations were observed between patient age, site of infection, and specific subspecies (Table 2). In general, patients in the 11- to 60-year-old age group were more likely to have disease caused by subspecies IIIa or IIIb, whereas those ≤1 year of age were more likely to have infections caused by subspecies II or IV. Notably, however, 82% of all extraintestinal infections occurred in persons 11–60 (51%) or ≥61 years of age (31%) (p<0.001).

Conclusions

Although the infections in the patients in this review were primarily diarrheal, the relative percentage of extraintestinal infections (29%) as well as the ratio (0.42) to diarrheal cases is intriguing. In 2009, only 15% (898/5,888, ratio 0.20) of nontyphoidal subspecies I salmonellae were isolated from extraintestinal sites. Countries other than the United States have reported markedly lower numbers (2%–3%) of nontyphoidal subspecies I extraintestinal infections than subspecies II–IV infections (*2*,*3*). Weiss et al. (*4*), at the US Centers for Disease Control and Prevention (CDC), described a similar high frequency of extraintestinal infections (24%, ratio 0.32) for the Arizona group (now subspecies IIIa and IIIb) between 1967 and 1976. In their review, they ascribed an enhanced virulence for humans to certain serotypes and noted the prevalence of those serotypes within the Arizona group.

Author affiliations: California Department of Public Health, Richmond, California, USA (S.L. Abbott, F.C.Y. Ni, J.M. Janda); and Los Angeles County Public Health Laboratory, Downey, California, USA (J.M. Janda).

DOI: http://dx.doi.org/10.3201/eid1804.111386

Table 1. Location of infections caused by salmonellae other than subspecies I among 1,342 patients, California, USA, 1985–2009

Site	% Subspecies				% Total, n = 1,342
	II, n = 60	IIIa, n = 463	IIIb, n = 443	IV, n = 376	
Feces*	85	33	59	79	71
Extraintestinal*	15	67	41	21	29
Blood†	0	51	19	57	42
Urine†	80	21	60	18	33
Wound/abscess†	0	7	4	5	6
Respiratory tract†	0	5	10	2	6
Other‡	20	15	6	18	13
Cerebrospinal fluid	0	5	0	0	0.5

*Percentage of total of each subspecies relative to intestinal or extraintestinal sites.
†Percentage of total within each subspecies.
‡Includes genital tract, eye, tissue, gallbladder, and ascites fluid.

Within subgroup I, a limited number of certain serotypes are recognized as having a higher propensity for causing extraintestinal disease on the basis of distinct virulence-associated characteristics. These serotypes include Typhi, Dublin, Cholerasuis, and Paratyphi A and C. In contrast, little information is available regarding comparable pathogenicity traits in *Salmonella* subspecies II–IV, with the exception of the Arizona group.

Data available did not enable us to determine how these patients acquired their infections. However, exotic pets are well recognized as a source of infection caused by unusual subspecies of salmonellae (*5*–*7*). Similarly, the most current salmonellae data available from CDC show that 65% of all subspecies II–IV strains were obtained from reptiles (*1*).

The distribution of subspecies II–IV infections by patient sex in this study differs somewhat from that of subspecies I, according to CDC records, which show that subspecies I infections, in general, are slightly more common in female patients (*1*). In our data, overall, male patients were more likely than female patients to be infected with subspecies II–IV salmonellae. This situation was particularly true for subspecies IIIa. Subspecies II and IIIb extraintestinal infections were an exception to this trend; cases were more prevalent in female patients than in male patients, and in both groups, subtypes were predominantly isolated from urine rather than blood.

Most reports in the literature emphasize the prevalence of exotic pet–associated subspecies II–IV infections in children <1 year of age (*8*–*12*). Our data showed an equivalent number of cases in children ≤1 year of age and in persons 11–60 years of age (from isolates obtained from fecal samples), but the preponderance of extraintestinal infections occurred in the older patient groups. These patients, as owners or handlers of exotic pets, may be exposed to a greater inoculum, whereas children ≤1 year primarily acquire infections secondarily from fomites or surfaces such as sinks used previously to bathe reptiles or by transmission from handler to child (*5*,*12*).

Little information is available regarding this small, but obviously substantially pathogenic, group of organisms. Our report and those of others (*4*) clearly show that *Salmonella* subspecies II–IV serotypes are capable of causing serious infections, including septicemia and wounds or abscesses. Unfortunately, despite recommendations to the US public, beginning in the mid-1990s, regarding the potential risk for acquisition of salmonellae infections from exotic pets, the number of infections in the United States caused by subspecies associated with these sources does not appear to be abating (*9*,*12*). In California, for subspecies IIIa isolates

Table 2. Distribution of infections caused by salmonellae other than subspecies I, by sex and age, among 1,342 patients, California, USA, 1985–2009

Subspecies	No. (%) patients by sex		No. (%) patients by age group, y			
	M	F	≤1	2–10	11–60	≥61
II						
Feces	25 (45)	29 (53)	31 (56)	11 (20)	7 (13)	0
Extraintestinal	1 (20)	4 (80)	1 (20)	0	4 (80)	0
IIIa						
Feces	146 (63)	84 (36)	30 (13)	108 (47)	32 (14)	8 (3)
Extraintestinal	154 (66)	77 (33)	4 (2)	126 (54)	79 (34)	15 (6)
IIIb						
Feces	160 (48)	161 (49)	48 (15)	155 (47)	44 (13)	18 (5)
Extraintestinal	35 (31)	77 (68)	9 (8)	56 (50)	36 (32)	6 (5)
IV						
Feces	168 (50)	159 (48)	173 (52)	46 (14)	67 (20)	15 (5)
Extraintestinal	18 (41)	26 (59)	12 (27)	9 (21)	15 (34)	7 (16)
Total*	707 (53)	617 (46)	349 (26)	157 (12)	538 (40)	213 (16)
Feces	499 (53)	433 (46)	321 (34)	135 (14)	337 (35)	91 (10)
Extraintestinal	208 (53)	184 (46)	28 (7)	22 (6)	201 (51)	122 (31)

*Numbers do not total 1,342 because data were not available for some patients.

alone, which are predominantly associated with reptiles, the number of infections doubled from 1993 to 1997 and from 2005 to 2009 (68 vs. 147, respectively). We hope this report will stimulate further epidemiologic investigations into these infections and that this information can then be used to generate a more effective strategy that public health agencies and the exotic pet industry can implement to reduce the extent of disease caused by these organisms.

Dr Abbott is a postdoctoral fellow training coordinator in the Microbial Diseases Laboratory, Richmond, California. Her research interests are focused on the members of the family *Enterobacteriaceae,* particularly with reference to foodborne disease.

References

1. Centers for Disease Prevention and Control. Public Health Laboratory Information System surveillance data: annual *Salmonella* summaries 1995–2006 [cited 2011 Sep 9]. http://www.cdc.gov/ncidod/dbmd/phlisdata/salmonella.htm
2. Sechter I, Katzenelson E, Reisfeld A. *Salmonella* serovars (others than Typhi or Paratyphi) from extraintestinal sources. Israel. 1984–9. Epidemiol Infect. 1991;106:485–8. http://dx.doi.org/10.1017/S0950268800067534
3. Wilkins EGL, Roberts C. Extraintestinal salmonellosis. Epidemiol Infect. 1988;100:361–8. http://dx.doi.org/10.1017/S095026880006711X
4. Weiss SH, Blaser MJ, Paleologo FP, Black RE, McWhorter AC, Asbury MA, et al. Occurrence and distribution of serotypes of the Arizona subgroup of *Salmonella* strains in the United States from 1967 to 1976. J Clin Microbiol. 1986;23:1056–64.
5. Centers for Disease Control and Prevention. Reptile-associated salmonellosis—selected states, 1998–2002. MMWR Morb Mortal Wkly Rep. 2003;52:1206–9.
6. Litwin CM. Pet-transmitted infections: diagnosis by microbiologic and immunologic methods. Pediatr Infect Dis J. 2003;22:768–77. http://dx.doi.org/10.1097/01.inf.0000083827.20027.64
7. Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, et al. for the Emerging Infections Program FoodNet Working Group. Reptiles, amphibians, and human *Salmonella* infection: a population-based, case–control study. Clin Infect Dis. 2004;38(suppl 3):S253–61. http://dx.doi.org/10.1086/381594
8. Editorial team; Bertrand S, Rimhanen-Finne R, Weill FX, Rabsch W, Thornton L, et al. *Salmonella* infections associated with reptiles: the current situation in Europe. Euro Surveill. 2008;13:pii:18902.
9. Centers for Disease Control and Prevention. Reptile-associated salmonellosis—selected states, 1996–1998. MMWR Morb Mortal Wkly Rep. 1999;48:1009–13. Erratum in MMWR Morb Mortal Wkly Rep 1999;48:1051.
10. de Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. Emerg Infect Dis. 2005;11:398–403.
11. Wells EV, Boulton M, Hall W, Bidol SA. Reptile-associated salmonellosis in preschool-aged children in Michigan, January 2001–June 2003. Clin Infect Dis. 2004;39:687–91. http://dx.doi.org/10.1086/423002
12. Mermin J, Hoar B, Angulo J. Iguanas and *Salmonella* Marina infection in children: a reflection of the increasing incidence of reptile-associated salmonellosis in the United States. Pediatrics. 1997;99:399–402. http://dx.doi.org/10.1542/peds.99.3.399

Address for correspondence: Sharon L. Abbott, Microbial Diseases Laboratory, E-164, 850 Marina Bay Parkway, Richmond, CA 94804, USA; email: sharon.abbott@cdph.ca.gov

Subclinical Infections with Crimean-Congo Hemorrhagic Fever Virus, Turkey

Hürrem Bodur, Esragül Akinci, Sibel Ascioglu, Pinar Öngürü, and Yavuz Uyar

To investigate Crimean-Congo hemorrhagic fever virus in Turkey, we conducted a seroepidemiologic survey during January–April 2009. Seroprevalence of infection was 10% in a sample from an outbreak region and increased with patient age, indicating that the virus had been previously present in Turkey. We also estimated that 88% of infections were subclinical.

Crimean-Congo hemorrhagic fever virus (CCHFV) infection was first recognized in Turkey in 2002. Since that time, the number of diagnosed cases has increased to the magnitude of an outbreak with major public health consequences (*1*,*2*). Although the clinical spectrum may change from asymptomatic infection to severe hemorrhagic disease, most studies to date have included only symptomatic cases (*1*–*6*). Therefore, the true incidence of infection with the virus, the full spectrum of severity of disease, and its epidemiologic features are unknown. The purpose of our study was to investigate the seroprevalence of CCHFV infection in a sufficiently large sample representative of the region affected during this outbreak in Turkey and to describe the main epidemiologic features, including the proportion of subclinical cases.

The Study

During January–April 2009, we conducted a survey that included obtaining venous blood samples from participants. The study sample was selected by using a random geographic cluster sampling stratified by age and sex from rural residential areas of Turkey where 99.5% of CCHF cases were reported (Figure 1). Only adults >18 years of age who were living in the study area for ≥1 year were eligible for the study. The study was approved by the Central Ethics Committee, and informed consent was obtained from all participants. A study questionnaire included questions on demographics, socioeconomic status, behavior characteristics, medical history, known risk factors for CCHFV infection, and participants' awareness of the outbreak and infection prevention methods.

Serum samples were tested for IgG against CCHFV at the Virology Reference Laboratory of the Refik Saydam National Public Health Agency, (Ankara, Turkey) by using a commercial ELISA kit (Vector-Best, Novosibrisk, Russia). Although the sensitivity and specificity of the kit were not specified by the manufacturer, studies that used this method have reported a sensitivity of 87%–98.3% and a specificity of 99%–100% (*7*,*8*). We defined subclincial cases as those in persons who were seropositive although they were not given a diagnosis or had not had severe symptoms compatible with CCHF at any time.

In addition, we compared information in our database with that in the database of reported cases at the Ministry of Health, Turkey. We used the χ^2 test, *t* test, and Mann-Whitney U test for univariate statistical comparisons, as appropriate. Multivariable logistic regression was used to assess independent risk factors for seropositivity. We used the direct standardization method to adjust age-specific seropositivity rates for our study population with the age composition of the entire outbreak region. This adjustment enabled us to calculate expected numbers of infected (seropositive) persons in the outbreak region (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1374-Techapp.pdf). Clinically diagnosed cases were compared with expected numbers of infections, and an observed:expected ratio was calculated.

The survey included 3,671 adults of whom 3,557 (97%) provided blood samples for serologic analysis. The mean ± SD age of the study population was 44.3 ± 16.2 years, the female:male ratio was 1.04 (51%:49%), and the most common occupation was farming (52.4%). Only 18.2% had a history of tick bite.

Of 3,557 serum samples tested, 356 (10%) were positive for IgG against CCHFV. Mean ± SD age was 43.4 ± 16.2 years for seronegative persons and 52 ± 17.1 years for seropositive persons ($p<0.001$). Categorizing persons by

Figure 1. Provinces in Turkey where study was conducted and to which Crimean-Congo hemorrhagic fever virus is endemic (green), January–April 2009. Gray indicates other provinces and dots indicate major cities.

Author affiliations: Ankara Numune Education and Research Hospital, Ankara, Turkey (H. Bodur, E. Akinci, P. Öngürü); Hacettepe University Medical School, Ankara (S. Ascioglu); and Refik Saydam National Public Health Agency, Ankara (Y. Uyar)

DOI: http://dx.doi.org/10.3201/eid1804.111374

age in 10-year intervals showed that seropositivity increased with age (p<0.001) (Figure 2). Univariate analysis showed that seropositive persons had less education (p<0.001), were more likely to be involved in farming, (p<0.001), and had a higher frequency of tick bites (p<0.001) than seronegative persons (Table 1). Animal husbandry as an occupation and a history of hunting were not more frequent among seropositive persons than among seronegative persons. A high proportion of seropositive persons (73.8%) and seronegative persons (71.3%), claimed that they had sufficient information about the infection and how to protect themselves (p = 0.329). Multivariable analysis results showed that an age >60 years, less schooling, farming as an occupation, and a history of tick bites were independent risk factors for seropositivity (Table 2).

None of the study population had been given a diagnosis of CCHF or had been hospitalized for an acute febrile illness or severe bleeding compatible with CCHF. During 2002–2009, a total of 1,806 adults were given a diagnosis of CCHF in the outbreak region where we conducted our study (Ministry of Health, Turkey). Direct standardization of age-specific seroprevalence rates for the study population with ages of persons in the entire region showed that 15,156 infections would be expected during 2002–2008 (observed:expected ratio 0.12; 95% CI 0.114–0.125). This finding shows that only 12% of the infections were diagnosed and 88% were subclinical.

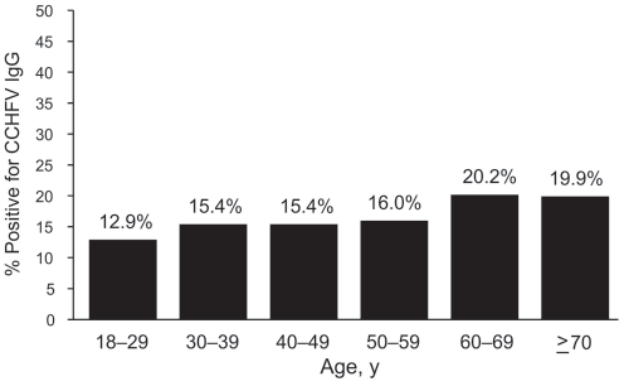


Figure 2. Distribution of Crimean-Congo hemorrhagic fever virus (CCHFV)–positive persons, by age group, Turkey, January–April 2009. p<0.001, by trend test.

Conclusions

We found that the seroprevalence of CCHF in the study region was 10%. Hoogstraal et al. reported that the expected seroprevalence of CCHF was ≈10% during epidemics (*9*). In a small survey in Turkey, IgG against CCHFV was detected in 12.8% of the population in high-risk areas (*10*). Our study showed that the distribution of seropositivity increased with age (Figure 2). This finding was unexpected and showed that CCHF was present in the region long before it was recognized. However, we could not assess whether incidence or severity were amplified in recent years, which led to its detection in 2002. Seroprevalence surveys with representative samples at regular intervals may be the only way for determining if incidence of infection has increased.

Another useful result from this study was the ability to predict that ≈90% of CCHFV infections were subclinical

Table 1. Characteristics of study population tested for CCHFV, Turkey, January–April 2009*

Characteristic	Seronegative, n = 3,201	Seropositive, n = 356	p value
Sex			
F	1,634 (51.0)	168 (47.2)	0.167
M	1,567 (49.0)	188 (52.8)	
Age, y, mean ± SD	43.38 ± 15.81	51.97 ± 17.14	<0.001
18–29	765 (23.9)	46 (12.9)	<0.001
30–39	721 (22.5)	55 (15.4)	
40–49	616 (19.2)	55 (15.4)	
50–59	511 (16.0)	57 (16.0)	
60–69	352 (11.0)	72 (20.2)	
≥70	236 (7.4)	71 (19.9)	
Education			
None	607 (19.0)	105 (29.7)	<0.001
Elementary school	1,873 (58.8)	214 (60.6)	
High school or university	707 (22.2)	34 (9.6)	
Occupation			
Unemployed	618 (19.5)	66 (18.9)	<0.001
Farming	1,186 (37.5)	174 (49.9)	
Animal husbandry	553 (17.5)	58 (16.6)	
Other†	809 (25.5)	51 (14.6)	
Persons living in same residence, mean ± SD	5.01 ± 2.54	5.28 ± 2.97	0.177
History of hunting	548 (17.6)	54 (15.5)	0.325
History of tick bite	540 (17.0)	105 (29.7)	<0.001
Sufficiently informed about CCHFV	2,269 (71.3)	261 (73.7)	0.329

*Values are no. (%) unless otherwise indicated. CCHFV, Crimean-Congo hemorrhagic fever virus.
†Retired, civil servant, factory, worker, or housewife.

Table 2. Multivariable logistic regression of risk factors for infection with CCHFV, Turkey, January–April 2009*

Characteristic	Odds ratio (95% CI)
Age, y	
18–29	1
30–39	0.965 (0.630–1.480)
40–49	1.034 (0.669–1.599)
50–59	1.297 (0.832–2.023)
60–69	2.687 (1.723–4.191)
≥70	4.176 (2.638–6.611)
Education	
None	1
Elementary school	0.977 (0.736–1.297)
High school or university	0.580 (0.357–0.942)
Occupation	
Unemployed and other†	1
Farming	1.688 (1.301–2.190)
Animal husbandry	1.299 (0.922–1.832)
History of tick bite	2.292 (1.768–2.971)

*CCHFV, Crimean-Congo hemorrhagic fever virus.
†Retired, civil servant, factory worker, or housewife.

(online Technical Appendix). This finding has clinical and epidemiologic implications. First, it shows that the spectrum of severity is highly skewed toward milder disease, although CCHF is believed to be a severe infection similar to other hemorrhagic fevers such as those caused by Ebola or Marburg viruses. Epidemiologically, information about subclinical cases is necessary for estimating the level of herd immunity in the population and predicting the characteristics of the outbreak. Finally, we do not know precisely why CCHF develops into a serious or fatal disease in some patients but is only a mild or asymptomatic in others (*11*). Some studies have shown that factors such as immune response of the host, viral load, or lack of some receptors may affect the clinical form of infection (*12*,*13*). Some authors have suggested that geographic variation in pathogenicity of the virus (*14*) may also be a factor in severity, although the supporting data are lacking (*15*). Therefore, timely detection and comparison of different clinical groups will be helpful in understanding the pathogenicity of the virus or host responses and developing effective treatments for infection.

This study was supported by the Scientific and Technological Research Council of Turkey.

Dr Bodur is chief of the Infectious Diseases and Clinical Microbiology Department at Ankara Numune Education and Research Hospital. His primary research interests are Crimean-Congo hemorrhagic fever, brucellosis, chronic hepatitis, and nosocomial infections.

References

1. Bakir M, Ugurlu M, Dokuzoguz B, Bodur H, Tasyaran MA, Vahaboglu H, et al. Crimean-Congo haemorrhagic fever outbreak in middle Anatolia: a multicenter study of clinical features and outcome measures. J Med Microbiol. 2005;54:385–9. http://dx.doi.org/10.1099/jmm.0.45865-0
2. Yilmaz GR, Buzgan T, Irmak H, Safran A, Uzun R, Cevik MA, et al. The epidemiology of Crimean-Congo hemorrhagic fever in Turkey, 2002–2007. Int J Infect Dis. 2009;13:380–6. http://dx.doi.org/10.1016/j.ijid.2008.07.021
3. Elaldi N, Bodur H, Ascioglu S, Celikbas A, Ozkurt Z, Vahaboglu H, et al. Efficacy of oral ribavirin treatment in Crimean-Congo haemorrhagic fever: a quasi-experimental study from Turkey. J Infect. 2009;58:238–44. http://dx.doi.org/10.1016/j.jinf.2009.01.014
4. Ozkurt Z, Kiki I, Erol S, Erdem F, Yilmaz N, Parlak M, et al. Crimean-Congo hemorrhagic fever in eastern Turkey: clinical features, risk factors and efficacy of ribavirin therapy. J Infect. 2006;52:207–15. http://dx.doi.org/10.1016/j.jinf.2005.05.003
5. Jabbari A, Besharat S, Abbasi A, Moradi A, Kalavi K. Crimean-Congo hemorrhagic fever: case series from a medical center in Golestan Province, northeast of Iran (2004). Indian J Med Sci. 2006;60:327–9. http://dx.doi.org/10.4103/0019-5359.26609
6. Alavi-Naini R, Moghtaderi A, Koohpayeh HR, Sharifi-Mood B, Naderi M, Metanat M, et al. Crimean-Congo hemorrhagic fever in southeast of Iran. M. J Infect. 2006;52:378–82. http://dx.doi.org/10.1016/j.jinf.2005.07.015
7. Saijo M, Qing T, Niikura M, Maeda A, Ikegami T, Prehaud C, et al. Recombinant nucleoprotein-based enzyme-linked immunosorbent assay for detection of immunoglobulin G antibodies to Crimean-Congo hemorrhagic fever virus. J Clin Microbiol. 2002;40:1587–91. http://dx.doi.org/10.1128/JCM.40.5.1587-1591.2002
8. Emmerich P, Avsic-Zupanc T, Chinikar S, Saksida A, Thomé-Bolduan C, Langroudi AG, et al. Early serodiagnosis of acute human Crimean-Congo hemorrhagic fever virus infections by novel capture assays. J Clin Virol. 2010;48:294–5. http://dx.doi.org/10.1016/j.jcv.2010.05.002
9. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol. 1979;15:307–417.
10. Gunes T, Engin A, Poyraz O, Elaldi N, Kaya S, Dokmetas I, et al. Crimean-Congo hemorrhagic fever virus in high-risk population, Turkey. Emerg Infect Dis. 2009;15:461–4. http://dx.doi.org/10.3201/eid1503.080687
11. Cevik MA, Erbay A, Bodur H, Gulderen E, Bastug˘ A, Kubar A, et al. Clinical and laboratory features of Crimean-Congo hemorrhagic fever: predictors of fatality. Int J Infect Dis. 2008;12:374–9. http://dx.doi.org/10.1016/j.ijid.2007.09.010
12. Cevik MA, Erbay A, Bodur H, Eren SS, Akinci E, Sener K, et al. Viral load as a predictor of outcome in Crimean-Congo hemorrhagic fever. Clin Infect Dis. 2007;45:e96–100. http://dx.doi.org/10.1086/521244
13. Saksida A, Duh D, Wraber B, Dedushaj I, Ahmeti S, Avsic-Zupanc T. Interacting roles of immune mechanisms and viral load in the pathogenesis of Crimean-Congo hemorrhagic fever. Clin Vaccine Immunol. 2010;17:1086–93. http://dx.doi.org/10.1128/CVI.00530-09
14. Vorou R, Pierroutsakos IN, Maltezou HC. Crimean-Congo haemorrhagic fever. Curr Opin Infect Dis. 2007;20:495–500. http://dx.doi.org/10.1097/QCO.0b013e3282a56a0a
15. Burt FJ, Swanepoel R. Molecular epidemiology of African and Asian Crimean-Congo haemorrhagic fever isolates. Epidemiol Infect. 2005;133:659–66. http://dx.doi.org/10.1017/S0950268805003730

Address for correspondence: Hürrem Bodur, Infectious Diseases and Clinical Microbiology Department, Ankara Numune Education and Research Hospital, 06100 Ankara, Turkey; email: hurrembodur@gmail.com

Crimean-Congo Hemorrhagic Fever, Kazakhstan, 2009–2010

Barbara Knust,[1] Zhumagul B. Medetov,[1] Kakimzhan B. Kyraubayev, Yekaterina Bumburidi, Bobbie Rae Erickson, Adam MacNeil, Stuart T. Nichol, Baurzhan S. Bayserkin, and Kenes S. Ospanov

We evaluated Crimean-Congo hemorrhagic fever (CCHF) surveillance data from southern Kazakhstan during 2009–2010 and found both spatial and temporal association between reported tick bites and CCHF cases. Public health measures should center on preventing tick bites, increasing awareness of CCHF signs and symptoms, and adopting hospital infection control practices.

Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne pathogen of the *Bunyaviridae* family (*1*). The primary modes of transmission to humans are tick bites, handling of ticks, exposure to blood or tissues of viremic livestock, and direct contact with blood and body fluids of infected persons. After a 3–7-day incubation period, sudden onset of fever, myalgia, headache, and gastrointestinal symptoms develop. Hemorrhagic signs can include petechiae; cutaneous hematomas; or bleeding from the nose, gastrointestinal tract, or urogenital tract (*2*). Among hospitalized patients, case-fatality rates range from 5% to 30% (*3*,*4*).

Ticks of the genus *Hyalomma* are the primary vectors for CCHV, and the virus is endemic throughout Africa, the Middle East, eastern Europe, and central Asia. *Hyalomma* spp. ticks are 2- or 3-host parasites, and adults feed mainly on large mammals, such as livestock. Although viremia and antibodies develop in infected livestock, no disease appears to be associated with CCHFV infection (*5*).

Crimean-Congo hemorrhagic fever (CCHF) is endemic to Kazakhstan (*6*,*7*). Most CCHF cases have been reported from Southern Kazakhstan Oblast. In 2009 and 2010, reported CCHF cases increased in Southern Kazakhstan Oblast, prompting the Kazakhstan Ministry of Health to expand surveillance and disease control activities. As a part of surveillance, a tick bite reporting system was initiated. Our objectives were to summarize CCHF surveillance data and evaluate the association between reported tick bites and CCHF in Kazakhstan.

The Study

CCHF is a reportable disease in Kazakhstan. A suspected case was defined as fever and ≥1 hemorrhagic sign or thrombocytopenia. A probable case was a suspected case in a person with a known risk factor for CCHF, such as a tick bite, handling of livestock, or exposure to blood or body fluids of a CCHFV-infected patient. A confirmed case was defined as laboratory evidence of infection by IgM, IgG, or antigen-capture ELISA (VECTOR-BEST, Novosibirsk, Russia), or quantitative real-time PCR (*8*). We reviewed lists of persons with confirmed and suspected CCHF cases in Southern Kazakhstan Oblast during 2009 and 2010 and compiled summary statistics. Data regarding date of disease onset and residential location were assessed.

Data on humans and animals were collected for diagnostic and surveillance purposes and were analyzed anonymously. Permission was sought from livestock owners before tick collection. No animal sampling was done as a part of this study.

Residents of Southern Kazakhstan Oblast were instructed to go to their local health care provider if they noted a tick bite. The health care provider registered them and instructed them to monitor their temperature at home for 14 days and return if fever developed. We obtained weekly summaries of tick bites and fevers reported in Southern Kazakhstan Oblast during spring and summer 2009 and 2010. In 2010, tick bites were additionally reported by rayon (local municipality). Tick bite data were compared with CCHF cases reported by date and location. We analyzed summary statistics by using standard software (SAS Institute, Inc. Cary, NC, USA) and considered $p<0.05$ significant. Maps were made by using geographic information systems software (Arc-GIS, ESRI, Redlands, CA, USA).

During 1999–2010, a total of 98 probable and confirmed CCHF cases were reported; 22 resulted in death. Fewer than 10 CCHF cases were reported per year, except for 1999 (19 cases), 2009 (22 cases), and 2010 (17 cases). Epidemiologic and clinical data were reviewed for 22 probable and confirmed cases in 2009 and 17 confirmed CCHF cases in 2010, all in residents of Southern Kazakhstan Oblast. An additional 34 suspected cases were identified in 2010, but sufficient data were not available for descriptive analysis. Ages of patients with probable and confirmed cases in 2009 and confirmed cases in 2010 (total

Author affiliations: Centers for Disease Control and Prevention, Atlanta, Georgia, USA (B. Knust, Y. Bumburidi, B.R. Erickson, A. MacNeil, S.T. Nichol); and Kazakhstan Ministry of Health, Almaty, Kazakhstan (Z.B. Medetov, K.B. Kyraubayev, B.S. Bayserkin, K.S. Ospanov).

DOI: http://dx.doi.org/10.3201/eid1804.111503

[1]These authors contributed equally to this article.

of 39 patients) ranged from 0 to 72 years; 17 (44%) were 21–40 years of age. Nosocomial transmission occurred in 5 patients in 2009 and 1 patient in 2010, accounting for 15% of the cases during 2009–2010. Livestock exposures were reported for 15 (38%), and tick exposures for 13 (33%), of the CCHF case-patients. No persons with confirmed CCHF who reported a tick bite were initially recorded in the tick bite registry. CCHF was laboratory confirmed for 14 (64%) reported cases in 2009 and for 17 (100%) in 2010. Eleven (28%) case-patients died.

Tick bite surveillance was conducted during April 17–October 22, 2009 (Figure 1, panel A) and March 3–October 28, 2010 (Figure 1, panel B). A total of 1,660 tick bites were registered in 2009; fever developed in 182 (9.7%) patients during the monitoring period (Figure 1, panel C). A total of 13,908 tick bites were registered in 2010; fever developed in 573 (4%) persons (Figure 1, panel D). In both years, peaks in reported tick bites temporally coincided with peak numbers of CCHF cases in Southern Kazakhstan Oblast; most bites and cases occurred during July–August 2009 and April–May 2010. Reported tick bites were significantly associated with number of CCHF cases per week (2009: $r = 0.48$, $p = 0.01$; 2010: $r = 0.64$, $p<0.0001$). No patients within the tick bite registry were registered as having confirmed CCHF in 2009 or 2010; however, diagnostic testing was not performed for persons who reported only a fever after a registered tick bite.

For 2010, we examined the geographic distribution of reported tick bites and CCHF cases in the 15 rayons in Southern Kazakhstan Oblast (Figure 2). The tick bite density (no. tick bites registered/ 1,000 persons) varied considerably among rayons. Mean tick bite density among rayons with ≥1 CCHF case in 2010 (6.6 bites/1,000 persons, range 3.1–15.7) was greater than that in rayons with no CCHF cases (2.5 bites/1,000 persons, range 1.7–3.8). A nonparametric Wilcoxon rank-sum exact test found a significant difference in tick bite density scores between rayons with and without reported CCHF cases (2-sided, $p = 0.01$).

Conclusions

Tick bites have long been recognized as a means of CCHFV transmission to humans (*9*–*12*), and the novel tick bite registry system provided an opportunity to examine the association between the population-level incidence of tick bites and CCHF. We demonstrated spatial and temporal correlation between reported tick bites and CCHF cases; distinct peaks in tick activity and disease were observed in both years and in regions with higher risk for CCHF in 2010. Such a registry is useful for timely deployment of tick control measures and preventive educational efforts. Exposures to ticks and livestock were commonly reported by persons with CCHF; at-risk populations should be educated about the disease and protective measures to reduce tick bites or exposure to blood and tissues of infected livestock.

Although clinical data available in this investigation were limited, we observed that disease severity of recent CCHF cases in Southern Kazakhstan Oblast are similar to those described previously in Kazakhstan and in other

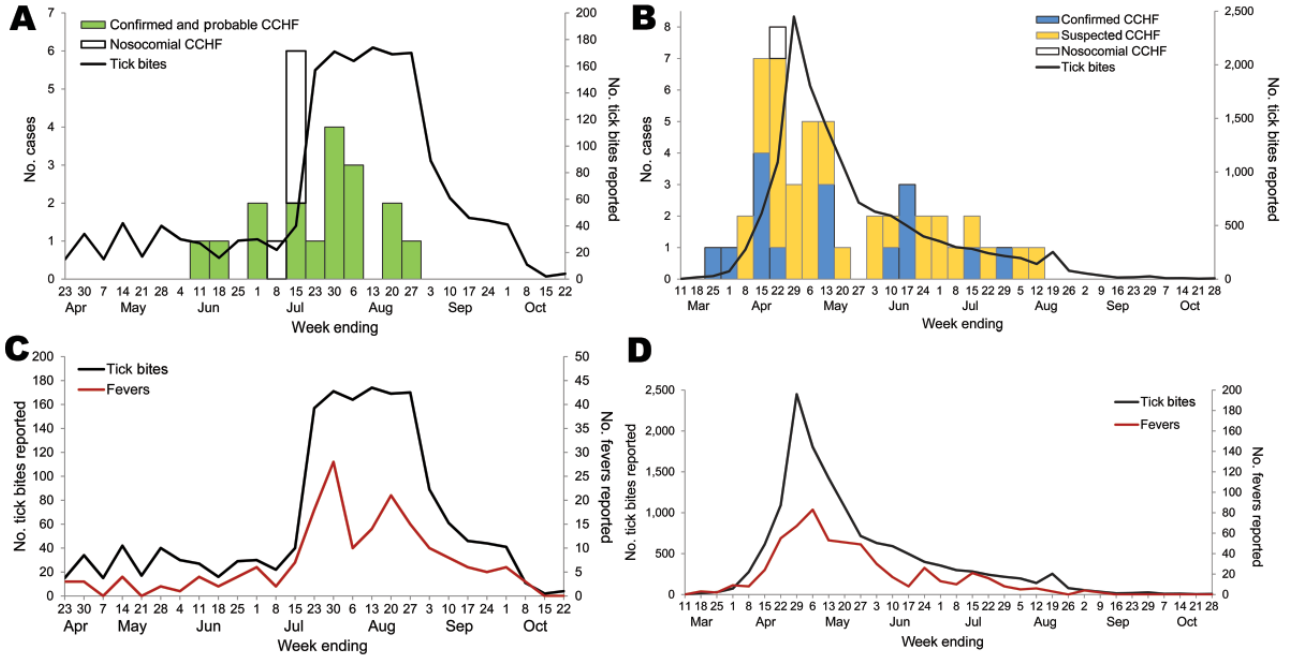


Figure 1. Reported Crimean-Congo hemorrhagic fever (CCHF) cases and reported tick bites in Southern Kazakhstan Oblast, Kazakhstan, April 23–October 22, 2009 (A) and March 11–October 28, 2010 (B), and reported tick bites and fevers in persons who registered a tick bite in the previous 14 days by week, April 23–October 22, 2009 (C), and March 11–October 28, 2010 (D).

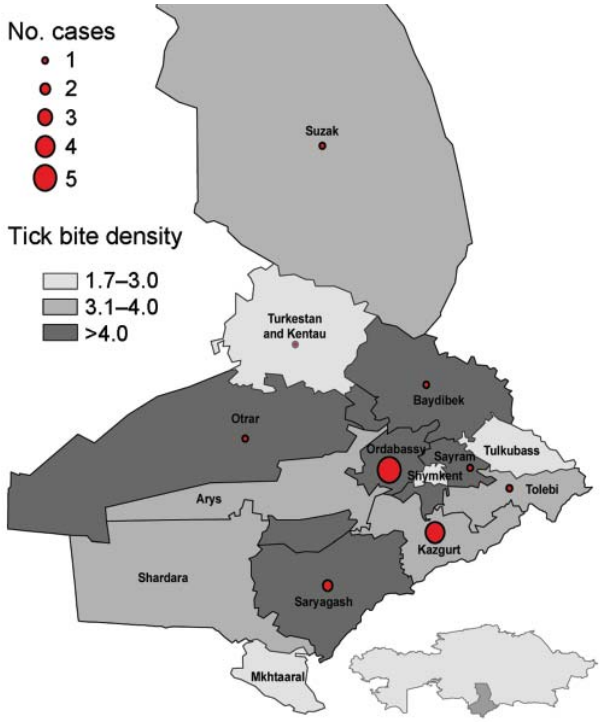


Figure 2. Crimean-Congo hemorrhagic fever cases and tick bite density per 1,000 persons, by rayon, Southern Kazakhstan Oblast, Kazakhstan, 2010.

regions to which CCHF is endemic (*6*,*13*,*14*). The recent occurrence of nosocomial transmissions in Southern Kazakhstan Oblast underscores the need for barrier nursing techniques. Education to raise awareness among physicians of the clinical signs and symptoms, infection control measures, and treatment strategies for CCHF remains critical (*15*).

Our analysis of CCHF surveillance data in Kazakhstan found a high number of reported tick bites during the spring and summer and spatial and temporal association between tick bites and CCHF cases. Public health measures should center on preventing tick bites, increasing clinician awareness of CCHF signs and symptoms, and adopting infection control practices in hospitals.

Funding for this study was provided by the governments of Kazakhstan and the United States.

Dr Knust is an epidemiologist in the Viral Special Pathogens Branch at the Centers for Disease Control and Prevention. Her research interests include epidemiology and ecology of zoonotic disease viruses.

References

1. Schmaljohn CS, Nichol ST. *Bunyaviridae*. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, editors. Fields virology, 5th ed. Philadelphia: Lippincott Williams and Wilkins; 2007. p. 1741–89.
2. Ergönül O. Crimean-Congo haemorrhagic fever. Lancet Infect Dis. 2006;6:203–14. http://dx.doi.org/10.1016/S1473-3099(06)70435-2
3. Swanepoel R, Shepherd AJ, Leman PA, Shepherd SP, McGillivray GM, Erasmus MJ, et al. Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. Am J Trop Med Hyg. 1987;36:120–32.
4. Yilmaz GR, Buzgan T, Irmak H, Safran A, Uzun R, Cevik MA, et al. The epidemiology of Crimean-Congo hemorrhagic fever in Turkey, 2002–2007. Int J Infect Dis. 2009;13:380–6. http://dx.doi.org/10.1016/j.ijid.2008.07.021
5. Hoogstraal H. The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe, and Africa. J Med Entomol. 1979;15:307–417.
6. Butenko A. Crimean-Congo hemorrhagic fever in Russia and other countries of the former Soviet Union. In: Ergonul O, Whitehouse CA, editors. Crimean-Congo hemorrhagic fever: a global perspective. Dordrecht (the Netherlands): Springer; 2007. p. 99–115.
7. Casals J, Henderson BE, Hoogstraal H, Johnson KM, Shelokov A. A review of Soviet viral hemorrhagic fevers, 1969. J Infect Dis. 1970;122:437–53. http://dx.doi.org/10.1093/infdis/122.5.437
8. Garrison AR, Alakbarova S, Kulesh DA, Shezmukhamedova D, Khodjaev S, Endy TP, et al. Development of a TaqMan minor groove binding protein assay for the detection and quantification of Crimean-Congo hemorrhagic fever virus. Am J Trop Med Hyg. 2007;77:514–20.
9. Swanepoel R, Gill DE, Shepherd AJ, Leman PA, Mynhardt JH, Harvey S. The clinical pathology of Crimean-Congo hemorrhagic fever. Rev Infect Dis. 1989;11(Suppl 4):S794–800. http://dx.doi.org/10.1093/clinids/11.Supplement_4.S794
10. Goldfarb LG, Chumakov MP, Myskin AA, Kondratenko VF, Reznikova OY. An epidemiological model of Crimean hemorrhagic fever. Am J Trop Med Hyg. 1980;29:260–4.
11. Chapman LE, Wilson ML, Hall DB, LeGuenno B, Dykstra EA, Ba K, et al. Risk factors for Crimean-Congo hemorrhagic fever in rural northern Senegal. J Infect Dis. 1991;164:686–92. http://dx.doi.org/10.1093/infdis/164.4.686
12. Fisher-Hoch SP, McCormick JB, Swanepoel R, Middelkoop AV, Harvey S, Kustner HGV. Risk of human infections with Crimean-Congo hemorrhagic fever virus in a South African rural community. Am J Trop Med Hyg. 1992;47:337–45.
13. Chinikar S, Goya M, Shirzadi M, Ghiasi S, Mirahmadi R, Haeri A, et al. Surveillance and laboratory detection system of Crimean-Congo haemorrhagic fever in Iran. Transbound Emerg Dis. 2008;55:200–4. http://dx.doi.org/10.1111/j.1865-1682.2008.01028.x
14. Dobritsa PG. Epidemiology and prophylaxis of hemorrhagic fever in Chimkent Oblast of southern Kazakhstan. In: Chumakov MP, editor. Moscow: Sborn. Trud. Inst. Polio, Virus, Encephal. Acad. Med. Nauk USSR (Medicine Moscow): 1965. p. 262–70.
15. Tarantola A, Ergonul O, Tattevin P. Estimates and prevention of Crimean-Congo hemorrhagic fever risks for health-care workers. In: Ergonul O, Whitehouse CA, editors. Crimean-Congo hemorrhagic fever. Dordrecht (the Netherlands): Springer 2007. p. 281–94.

Address for correspondence: Stuart T. Nichol, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop G14, Atlanta, GA 30333, USA; email: stn1@cdc.gov

Vector Blood Meals and Chagas Disease Transmission Potential, United States

Lori Stevens, Patricia L. Dorn, Julia Hobson, Nicholas M. de la Rua, David E. Lucero, John H. Klotz, Justin O. Schmidt, and Stephen A. Klotz

A high proportion of triatomine insects, vectors for *Trypanosoma cruzi* trypanosomes, collected in Arizona and California and examined using a novel assay had fed on humans. Other triatomine insects were positive for *T. cruzi* parasite infection, which indicates that the potential exists for vector transmission of Chagas disease in the United States.

Chagas disease is a vector-borne disease caused by *Trypanosoma cruzi* trypanosomes. Although these parasites are rarely transmitted by insects in the United States, there is concern that vector transmission may increase (*1*). Chagas disease, endemic to most of Latin America, can be transmitted to mammals by >130 species of blood-feeding insect vectors (subfamily Triatominae). In the United States, the most common result of a triatomine bite is allergic reaction, including anaphylaxis, elicited in sensitized persons (*2*). Despite only 7 cases of vector transmission to humans reported in the United States (*3*,*4*), *T. cruzi* trypanosomes are present in >20 wildlife species. In Latin America, 8–10 million persons are infected with these parasites (*5*), and an estimated 300,000 of the ≈13 million persons from disease-endemic areas now living in the United States carry the parasite (*6*). Although vector transmission of *T. cruzi* trypanosomes is a minimal risk, 9 of the 11 triatomine species in the United States are potential vectors (*1*,*7*), and parasite transmission could increase because of climate change (*1*).

Author affiliations: University of Vermont, Burlington, Vermont, USA (L. Stevens, J. Hobson, N.M. de la Rua, D.E. Lucero); Loyola University, New Orleans, Louisiana, USA (P. L. Dorn); Southwestern Biological Institute, Tucson, Arizona, USA (J.O. Schmidt); University of California, Riverside, California, USA (J.H. Klotz); and University of Arizona, Tucson (S.A. Klotz)

DOI: http://dx.doi.org/10.3201/eid1804.111396

One critical aspect of transmission is parasite reservoirs; hence, the epidemiologic importance of identifying animal sources of the vectors' blood meals, the likelihood of these vectors also feeding on humans, and their incidence of *T. cruzi* infection. Blood meals consumed by insect vectors have been detected by using several molecular techniques (*8*), but assays are challenging because of degradation of the blood in the vector's gut, PCR inhibition, the often small size of a blood meal, and the difficulty of distinguishing multiple blood meals. We analyzed *T. cruzi* vectors collected in California and Arizona by using a novel technique— cloning following PCR amplification using universal vertebrate primers—to determine the source of blood meals and indicate the likelihood of parasite transmission to humans.

The Study

Insects were collected by using light traps at Redington Road, Tucson, Arizona, and Escondido, California, in 2007, and within the Arizona-Sonora Desert Museum, Tucson, in 2009 (Table 1). We examined blood meals (*9*) and *T. cruzi* parasite infection (*10*) of 13 insects from 2 species of kissing bugs, *Triatoma rubida* and *T. protracta*. A mouse-fed *T. recurva* bug served as a control (*11*). Using universal vertebrate primers for *cytB* and *12S* (*12*,*13*), we identified as many blood meals as possible (Table 1). We cloned and sequenced the PCR products to isolate multiple blood meals within a single insect (Table 2). Blood meal sources were inferred by using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Pearson χ^2 tests compared the likelihood of feeding on humans between vector species and compared the *cytB* and *12S* assays for differences in the number of blood meal taxa and blood meal haplotypes (i.e., unique DNA sequences) per insect (JMP Ver9; SAS, Cary, NC, USA).

Five of the 13 bugs (38%) had positive test results for human blood (Table 1); *T. rubida* bugs were significantly more likely than *T. protracta* bugs to have fed on humans (χ^2 9.24; p<0.01). *T. rubida* bugs had also fed on dogs and *T. protracta* bugs on woodrats (*Neotoma* spp.), chickens, dogs, and pigs. *T. cruzi* infection was found in 5/9 *T. protracta* and 0/4 *T. rubdia* bugs. No insect that had fed on humans was infected with *T. cruzi* trypanosomes.

The *cytB* and *12S* assays differed in the specificity of primers for vertebrate DNA and number of blood meals per insect (Figure). More than 70% of DNA cloned in the *cytB* assay was from the insect (64/87 clones); ≈10% of clones did not produce interpretable sequences. In contrast, the *12S* assay did not clone insect DNA, and all sequences were interpretable. The average number of blood meals per insect was not statistically different (*cytB* 0.56 taxa/insect, *12S* 1.75 taxa/insect; χ^2 8.31; p<0.10); however, the average number of haplotypes/insect was significantly higher for *12S* (*cytB* 0.78, *12S* 2.75; χ^2 9.09; p<0.02).

Table 1. Blood meal sources of *Trypanosoma cruzi* insect vectors collected in Arizona and California, USA, 2007 and 2009, as determined by using *cytB* and *12S* rDNA assays, and haplotypes identified*

Assay and *Triatoma* spp.	*T. cruzi*	Location†	No. vertebrate blood meal sources			Haplotypes (no.) of vertebrate blood meal sources amplified in clones						No. non–blood meal clones	
			Clones	Taxa	Haplo	Human	Rat	Chick	Dog	Pig	Mouse‡	Vector§	ND
cytB													
T. rubida	–	R	8	1	1	A						6	1
T. rubida	–	R	10	1	2	B, C						7	1
T. protracta	–	E	8	1	1	D						5	2
T. protracta	–	E	11	1	1			A				9	1
T. protracta	+	E	9	0	0							7	1
T. protracta	+	E	8	1	2		A, B					6	
T. protracta	–	E	9	0	0							9	
T. protracta	–	E	8	0	0							7	1
T. protracta	+	E	8	0	0							8	
T. recurva‡	–		8								A (7), B		
12S rRNA													
T. protracta	+	M	8	1	2				A (7), B				
T. protracta	+	M	8	2	4				A (4)	A (2), B, C			
T. rubida	–	M	6	2	3	A (4), B			A				
T. rubida	–	M	7	2	2	A			A (6)				

*Vector species, *T. cruzi* infection status, collection location, number of clones sequenced, number and identity of taxa, and number of haplotypes represented in the clone sequences are indicated. Blank cells indicate clones were not found. For the *cytB* assay, the number of clones that were *Triatoma* spp. vector DNA or had uninterpretable sequences are indicated. The mouse-fed control (*cytB* assay) had 2 mouse haplotypes. Haplo, haplotypes; rat, woodrat; chick, chicken; ND, not determined because of low quality sequence data; –, negative; +, positive.
†Insects were collected by using light traps at Redington Road, Tucson, Arizona (R), and Escondido, CA (E), in 2007, and within the Arizona-Sonora Desert Museum, Tucson, AZ (M), in 2009. The light traps were in "wilderness" (museum) and "sylvatic" (Redington Road and Escondido) habitats and not in human habitations.
‡Control.
§*Triatoma* spp.

The *cytB* assay detected more haplotypes from each blood meal taxon, indicating the bugs fed on unique individuals; 4 human and 2 woodrat sequences were all different from each other. In contrast, for *12S*, 17/18 dog sequences were identical, as were 2/3 human and 3/4 pig sequences.

The mouse-fed control (*11*) was the only insect for which no vector DNA was cloned in the *cytB* assay. All 8 clones from the control were mouse; 7 were identical. Although unexpected, heteroplasmic mitochondrial DNA has been reported for inbred mice (*14*).

Conclusions

We found that 38% bugs of 2 species of *T. cruzi* vectors endemic to the United States, *T. rubida* and *T. protracta*, fed on humans. Infection with the Chagas parasite, *T. cruzi,* was high (55% for *T. protracta* bugs), but no insect was positive for human blood meals and the parasite. Both vectors are common in the foothills of Tucson, Arizona (*2*); although the *T. rubida* bugs in this study were uninfected, another study found that 67% of adult bugs collected around Tucson were infected with *T. cruzi* parasites (*15*).

The Arizona-Sonora Desert Museum in Tucson exhibits desert animals. All 4 insects collected from near the museum had fed on canids (dog/coyote/wolf; *Canis* spp.); 1 had fed on pigs and 2 on humans. Although canid samples are not distinguishable by *12S*, there are no dogs at the museum, so the insects probably fed on coyotes or wolves. Although javelina (*Tayassu tajacu*), a species similar to pigs, are at the museum, sequences were 99% identical to pig (*Sus scrofa*) and only a 90% match with javelina. The source of the human blood meals is not

Table 2. Assays used to determine the source of blood meals and *Trypanosoma cruzi* trypanosome infection in insects collected in Arizona and California, USA*

Assay and reference	Primers, 5′ → 3′	PCR cycling†	Amplicon size
cytB (*12*)	cca tcc aac atc tca gca tga tga a ccc ctc aga atg att att tgt cct ca	95°C, 40 s; 44°C, 40 s; 72°C, 40 s	358 bp
12S (*13*)	ccc aaa ctg gga tta gat acc c gtt tgc tga aga tgg cgg ta	95°C, 30 s; 57°C, 15 s; 72°C, 30 s	215 bp
TCZ‡ (*11*)	cga gct ctt gcc cac acg ggt gct cct cca agc agc gga tag ttc agg	94°C, 20 s; 57°C, 10 s; 72°C, 30 s	188 bp

*Insects were collected by using light traps in Tucson, Arizona, and Escondido, CA, in 2007, and within the Arizona-Sonora Desert Museum, Tucson, in 2009. For the blood meal assays, cloned PCR products (pGEM-T, Promega, Madison, WI), USA were sequenced by using the BigDye v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and analyzed by using an ABI PRISM 3730*xl* (Beckman Coulter, Fullerton, CA, USA).
†For all assays: initial denaturation of 95°C for 5 m; 35 cycles of PCR and final extension of 72°C for 10 m.
‡A negative control (lacking *T. cruzi* DNA template) was included with every assay. Samples that failed to amplify were spiked with 1 μL of *T. cruzi* parasites boiled in 1× PCR buffer and retested to ensure that the lack of product was not caused by PCR inhibition.

clear. No one lives at the museum, but there is camping in the area.

Around Escondido, we found *T. protracta* bugs fed on humans, woodrats, and domestic chickens, according to the *cytB* assay. This assay amplified only vector DNA from 4/7 insects, which could mean it had been a long time since the last blood meal and thus the DNA was highly degraded. We detected only 1 blood meal source in the other 3 insects from California but found 2 woodrat sequences in a single insect. Both *T. rubida* bugs collected in California had fed only on humans; 2 unique human sequences from 2 clones from 1 insect suggest it had fed on 2 humans.

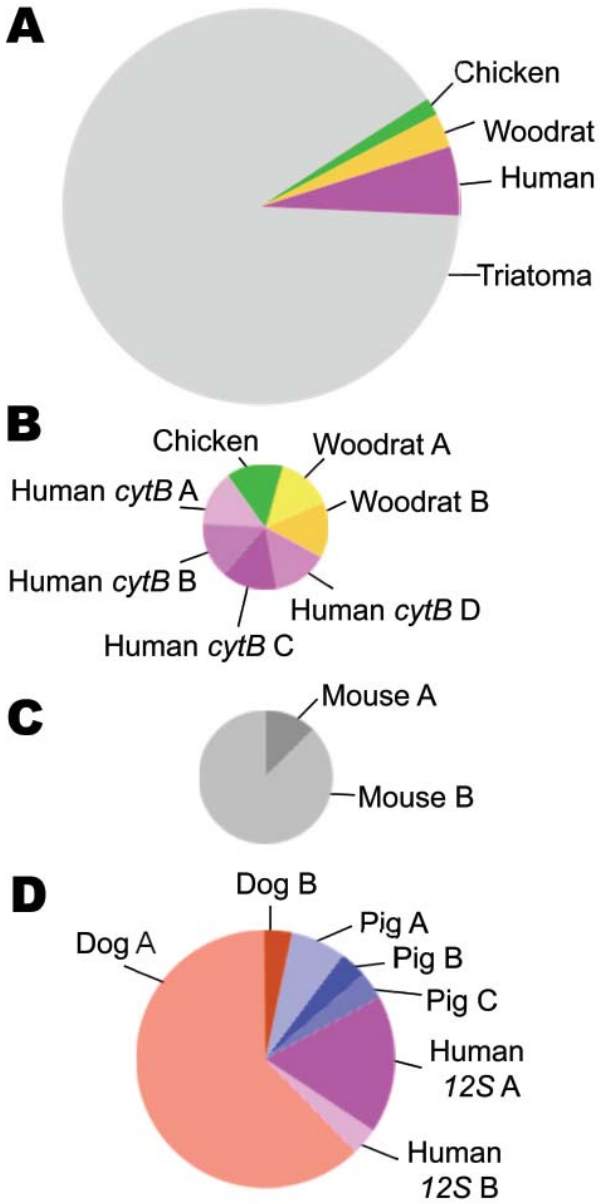


Figure. Types of blood meals found by using *cytB* and *12S* assays in insect vector species that carry *Trypanosoma cruzi*, the pathogen that causes Chagas disease, Arizona and California, USA, 2007 and 2009. Circle size is proportional to the sample size for that comparison. A) Vertebrate taxa and vector DNA (n = 71 sequences), showing that the *cytB* assay amplified vector DNA more often than blood meal DNA. B) Four vertebrate taxa among the blood meals detected by the *cytB* assay (n = 7 sequences). Unique haplotypes (DNA sequences or alleles) of human and woodrat are indicated by letters. C) Two mouse haplotypes detected in the mouse-fed control insect (n = 8 sequences). D) Types of blood meal based on the *12S* assay (n = 29 sequences).

Compared with *cytB,* the *12S* assay had better amplification and sequence quality and no recovery of insect vector DNA. Attempts to improve the *cytB* assay (e.g., higher annealing temperature) were unsuccessful, leading us to try the *12S* assay, which had a smaller amplicon size and higher primer specificity for vertebrate DNA (*13*). Only mouse DNA was detected from the control bug that had fed on mouse in the laboratory (*2*), demonstrating the *cytB* assay worked well for fresh blood meals; however, for degraded DNA, *12S* is a better assay.

Overall, *cytB* is more variable than *12S*, producing more haplotypes, and thus can detect feeding on multiple individuals of the same taxon. Because identifying the source of blood meals depends on the availability of similar sequences in GenBank, another advantage of *cytB* is that GenBank contains 3–4× as many vertebrate sequences for comparison.

In conclusion, although allergic reaction from triatomine bites is well known (*12*), the high incidence of human blood meals in these bugs in our study suggests that the potential for human transmission of *T. cruzi* parasites might be greater than previously thought. Our assays using vertebrate primers and cloning PCR products may be especially useful for detecting unpredicted blood meal sources and multiple blood meals.

Part of this material is based on work supported by the National Science Foundation under core faculty funding from grant no. NSF EPS-0701410 (to L.S.) and National Institutes of Health grant 1R15 A1079672-01A1 (to P.L.D.). All procedures using mice were first approved by the Southwestern Biological Institute Animal Care and Use Committee.

Dr Stevens is professor of biology at the University of Vermont. Her research interests include feeding sources and population genetics of triatomine vectors in North, Central, and South America.

References

1. Click Lambert R, Kolivras KN, Resler LM, Brewster CC, Paulson SL. The potential for emergence of Chagas disease in the United States. Geospat Health. 2008;2:227–39.
2. Klotz JH, Dorn PL, Logan JL, Stevens L, Pinnas JL, Schmidt JO, et al. "Kissing bugs": potential disease vectors and cause of anaphylaxis. Clin Infect Dis. 2010;50:1629–34. http://dx.doi.org/10.1086/652769

3. Kjos SA, Snowden KF, Olson JK. Biogeography and *Trypanosoma cruzi* infection prevalence of Chagas disease vectors in Texas, USA. Vector Borne Zoonotic Dis. 2009;9:41–50. http://dx.doi.org/10.1089/vbz.2008.0026
4. Dorn PL, Perniciaro L, Yabsley MJ, Roellig DM, Balsamo G, Diaz J. Autochthonous transmission of *Trypanosoma cruzi*, Louisiana. Emerg Infect Dis. 2007;13:605–7. http://dx.doi.org/10.3201/eid1304.061002
5. Hotez PJ, Bottazzi ME, Franco-Paredes C, Ault SK, Periago MR. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. PLoS Negl Trop Dis. 2008;2:e300. http://dx.doi.org/10.1371/journal.pntd.0000300
6. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. Clin Infect Dis. 2009;49:e52–4. http://dx.doi.org/10.1086/605091
7. Stevens L, Dorn PL, Schmidt JO, Klotz JH, Lucero D, Klotz SA. Kissing bugs. The vectors of Chagas. Adv Parasitol. 2011;75:169–92. http://dx.doi.org/10.1016/B978-0-12-385863-4.00008-3
8. Kent RJ. Molecular methods for arthropod blood meal identification and applications to ecological and vector-borne disease studies. Mol Ecol Resour. 2009;9:4–18. http://dx.doi.org/10.1111/j.1755-0998.2008.02469.x
9. Pizarro JC, Stevens L. A new method for forensic DNA analysis of the blood meal in Chagas disease vectors demonstrated using *Triatoma infestans* from Chuquisaca, Bolivia. PLoS ONE. 2008;3:e3585. http://dx.doi.org/10.1371/journal.pone.0003585
10. Pizarro JC, Lucero DE, Stevens L. PCR reveals significantly higher rates of *Trypanosoma cruzi* infection than microscopy in the Chagas vector, *Triatoma infestans*: high rates found in Chuquisaca, Bolivia. BMC Infect Dis. 2007;7:66–73. http://dx.doi.org/10.1186/1471-2334-7-66
11. Klotz SA, Dorn PL, Klotz JH, Pinnas JL, Weirauch C, Kurtz JR, et al. Feeding behavior of triatomines from the southwestern United States: an update on potential risk for transmission of Chagas disease. Acta Trop. 2009;111:114–8. http://dx.doi.org/10.1016/j.actatropica.2009.03.003
12. Kocher TD, Thomas WK, Meyer A, Edwards SV, Paabo S, Villablanca FX, et al. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proc Natl Acad Sci U S A. 1989;86:6196–200. http://dx.doi.org/10.1073/pnas.86.16.6196
13. Kitano T, Umetsu K, Tian W, Osawa M. Two universal primer sets for species identification among vertebrates. Int J Legal Med. 2007;121:423–7. http://dx.doi.org/10.1007/s00414-006-0113-y
14. Sachadyn P, Zhang XM, Clark LD, Naviaux RK, Heber-Katz E. Naturally occurring mitochondrial DNA heteroplasmy in the MRL mouse. Mitochondrion. 2008;8:358–66. http://dx.doi.org/10.1016/j.mito.2008.07.007
15. Reisenman CE, Gregory T, Guerenstein PG, Hildebrand JG. Feeding and defecation behavior of *Triatoma rubida* (Uhler, 1894) (Hemiptera: Reduviidae) under laboratory conditions, and its potential role as a vector of Chagas disease in Arizona, USA. Am J Trop Med Hyg. 2011;85:648–56. http://dx.doi.org/10.4269/ajtmh.2011.11-0137

Address for correspondence: Lori Stevens, Department of Biology, University of Vermont, 321 Marsh Life Science Bldg, 109 Carrigan Drive, Burlington, VT 05405, USA; email: lori.stevens@uvm.edu

Genomic Analysis of *emm59* Group A *Streptococcus* Invasive Strains, United States

Nahuel Fittipaldi, Randall J. Olsen, Stephen B. Beres, Chris Van Beneden, and James M. Musser

Genomic analysis of type *emm59* group A *Streptococcus* invasive strains isolated in the United States discovered higher than anticipated genetic heterogeneity among strains and identified a heretofore unrecognized monoclonal cluster of invasive infections in the San Francisco Bay area. Heightened monitoring for a potential shift in the epidemic behavior of *emm59* group A *Streptococcus* is warranted.

Group A *Streptococcus* (GAS) causes human diseases ranging in severity from uncomplicated pharyngitis to life-threatening necrotizing fasciitis (*1*). GAS strains have traditionally been classified based on a serologic reaction against the M protein, a polymorphic cell surface adhesin and anti-phagocytic factor; GAS strains are currently typed by sequencing the 5′ hypervariable region of the *emm* gene, encoding M protein (*2*–*4*). Studies of large numbers of GAS isolates causing invasive infections and pharyngitis worldwide have shown that type *emm59* GAS strains rarely cause disease (*5*,*6*). However, an increase in the frequency and severity of invasive infections caused by type *emm59* strains (>500 cases since 2006) has been reported recently in Canada (*7*). One of the most striking features of the *emm59* epidemic in Canada was its rapid spread; invasive *emm59* disease was reported in most Canadian provinces and territories in a matter of only a few years (*7*). By using whole-genome sequencing and animal models of invasive disease, we recently discovered that virtually all type *emm59* GAS invasive cases in Canada were caused by a single, recently emerged, hypervirulent *emm59* clone (*8*).

Whole-genome sequence analysis also revealed distinct spatiotemporal patterns of subclone diversification of the epidemic clone in Canada (*8*). Furthermore, we discovered that several geographically clustered cases of type *emm59* GAS invasive infections in south-central Montana in 2010 were caused by a distinct subclone of the *emm59* epidemic clone that disseminated from Canada (*8*). This finding led us to evaluate the hypothesis that the epidemic *emm59* clone disseminated further and caused invasive infections in other regions of the United States.

Author affiliations: The Methodist Hospital Research Institute, Houston, Texas, USA (N. Fittipaldi, R.J. Olsen, S.B. Beres, J.M. Musser); and Centers for Disease Control and Prevention, Atlanta, Georgia, USA (C. Van Beneden)

DOI: http://dx.doi.org/10.3201/eid1804.111803

The Study

In this study, we sequenced the genomes of all available invasive *emm59* GAS strains (n = 40) collected during 2000–2009 by the Active Bacterial Core surveillance (ABCs), a core component of the Centers for Disease Control and Prevention Emerging Infections Programs network. ABCs, an active, laboratory- and population-based surveillance system operating in 10 geographically disparate sites across the United States, represents a population of ≈32 million persons under surveillance for invasive GAS infections (www.cdc.gov/abcs/methodology/surv-pop.html).

Genome sequencing was performed by using a Genome Analyzer II (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. Polymorphism discovery and phylogenetic analysis were performed as described (*8*). The reference genome sequence for polymorphism discovery was that of the *emm59* Canadian strain MGAS15252 (GenBank accession no. CP003116). On average, the 40 *emm59* strains in the ABCs sample differed from the reference strain by 157 single-nucleotide polymorphisms (SNPs) and 15 insertions or deletions. We recently reported that, consistent with our hypothesis, we identified 5 strains that were genetically closely related to the epidemic clone in Canada: 1 strain from Oregon, 2 from California, and 2 from Minnesota (*8*). Here, we report that the core genomes (i.e., the ≈1,670-kbp portion of the genome that lacks mobile genetic elements and whose gene content is conserved among all sequenced GAS serotypes) of these 5 strains differed from the core genome of reference strain MGAS15252 by ≤16 SNPs (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/4/11-1803-FA1.htm).

Although our hypothesis regarding further dissemination of this recently emerged clone into the United States was correct, most *emm59* GAS strains collected by the ABCs program were genetically distinct from the clone in Canada. We therefore investigated in more detail the population of *emm59* GAS organisms responsible for invasive disease in the United States. We used the whole-genome SNP data to identify 2 major phylogenetic lineages of *emm59* organisms (Figure, panel A). All strains recovered during 2000 and 2001 (originating from Minnesota, Maryland, and Georgia) form 1 lineage (Figure, panel B). On average, the core genomes of these strains differed from that of reference strain MGAS15252 by 141 SNPs. The second lineage consists of 22 strains isolated in California, Connecticut, New Mexico, New York, and Tennessee

during 2003–2008 (Figure, panel A). On average, the core genomes of these 22 type *emm59* organisms differed from that of reference strain MGAS15252 by 169 SNPs. Seven strains from California, isolated during 2006–2008, were separated from the reference strain by increasing numbers of SNPs (ranging from 36 to 105 SNPs for the most closely to the most distantly related of the 7 strains, respectively) (Figure, panels A, B).

Closer examination of the second branch of the phylogenetic tree identified a conspicuous group formed by 14 closely related strains isolated from patients in the San Francisco Bay area, California, USA, during 2005–2008 (Figure, panel B). These 14 type *emm59* GAS organisms differed from one another, on average, by only 10 SNPs. The phylogenetic and epidemiologic data suggest that these 14 strains constitute a distinct clone that caused a geographic cluster of invasive infections.

The *emm59* strains causing the epidemic in Canada were isolated in high percentages from patients with bacteremia and soft tissue infections (*7*). In the only other well-documented *emm59* outbreak, which occurred in Scotland (*9*), most of the patients had skin lesions and wound infections (*9*). These data suggest that *emm59* GAS strains have a predilection for abscess formation and soft tissue infection. The most common clinical syndromes associated with infections caused by *emm59* GAS strains in the ABCs collection were similar to those associated with infections caused by all other *emm* types (*10*) and included bacteremia (40%), cellulitis (28%), pneumonia (13%), septic arthritis (5.0%), necrotizing fasciitis (7.5%), and abscess (5.3%). The initial report of type *emm59* GAS strains indicated this *emm* type to be associated with pyoderma and acute glomerulonephritis (*11*). However, the ABCs program tracks only invasive infections, so it would probably not detect most cases of GAS pyoderma and glomerulonephritis.

In Canada, an epidemiologic association with alcohol abuse, homelessness, hepatitis C virus infection, and illicit drug use was identified, suggesting that the *emm59* GAS epidemic was more common among a specific susceptible population consisting primarily of middle-aged persons with underlying medical conditions or histories of substance abuse (*7*). In our study of type *emm59* GAS invasive infection in the United States, we found that 30% of infected persons used illicit drugs and 17.5% abused alcohol; these percentages were higher than those for persons infected with all other *emm* types (*10*). However, substance abuse was higher only among US case-patients with infections caused by strains belonging to the epidemic clone from Canada and among case-patients from the San Francisco Bay area. This finding likely reflects circulation of the *emm59* strain among subpopulations with similar behaviors. Of note, substance abuse has been shown to be a major

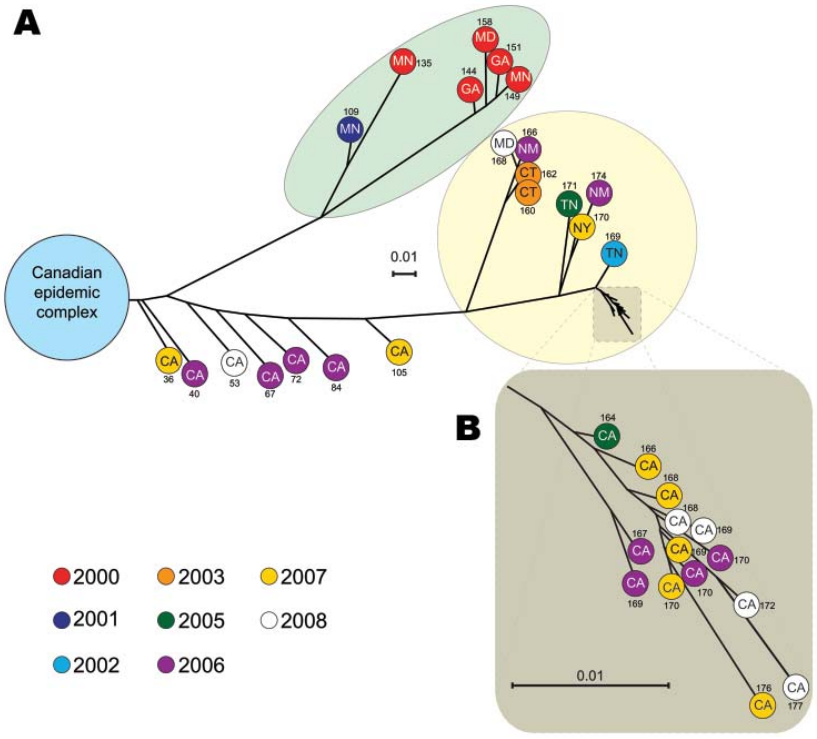


Figure. Inferred genetic relationships among 40 *emm59* group A *Streptococcus* (GAS) strains isolated in the United States during 2000–2009, based on 635 concatenated single-nucleotide polymorphism (SNP) loci identified by whole-genome sequencing. A) Phylogenetic tree showing that most of the strains are genetically distinct from an *emm59* GAS clone responsible for >500 cases of invasive disease in Canada. Light green highlight indicates older (US) strains (isolated during 2000–2001) that form 1 clearly differentiated branch of the phylogenetic tree. Yellow highlighting indicates a second lineage isolated in the United States (CA, CT, NM, NY, and TN) during 2003–2008. Circles represent individual isolates. The number of SNPs separating each isolate from strain MGAS15252 from Canada is indicated. Five strains (2 from MN, 2 from CA, and 1 from OR; not represented individually in the phylogenetic tree) are members of the epidemic complex from Canada. B) Magnification of the phylogenetic tree showing a conspicuous discrete clonal complex formed by 14 strains isolated in the San Francisco Bay area during 2005–2008. Genome-wide, these 14 strains are separated by an average of 10 SNPs. CA, California; CT, CT, Connecticut; GA, Georgia; MD, Maryland; MN, Minnesota; NM, New Mexico; NY, New York; TN, Tennessee. A matrix displaying the total number of core genome SNPs separating each individual strain from any other is available in the online Appendix Figure (wwwnc.cdc.gov/EID/article/18/4/11-1803-FA1.htm). Scale bars indicate nucleoide subsitutions per site.

risk factor for invasive GAS disease in the San Francisco Bay area (*12*).

Conclusions

Next-generation DNA sequencing technologies are highly successful for infectious disease epidemiology (*13*). The *emm59* GAS organisms causing invasive infections in the United States were closely related and indistinguishable by multilocus sequence typing. The strains could be differentiated from one another only by the use of high-throughput genome sequencing. The level of genetic diversity we identified among *emm59* GAS strains collected by the ABCs program in the United States was considerably greater than that among strains from the epidemic in Canada, where a monoclonal population was found to be responsible for virtually all of the >500 invasive cases reported (*8*). Of note, the high resolving power of whole-genome sequencing also enabled us to discover a cluster of genetically related invasive *emm59* GAS strains in the San Francisco Bay area.

Until recently, cases of invasive infection caused by *emm59* GAS have been uncommon in North America. However, the recent epidemic in Canada (*7*,*8*) and the data presented here for the United States suggest a potential shift in the epidemic behavior of *emm59* GAS strains that warrants heightened monitoring awareness by public health authorities.

Acknowledgments

We acknowledge the *Streptococcus* Laboratory and *emm* database (www.cdc.gov/ncidod/biotech/strep/strepblast.htm), Centers for Disease Control and Prevention, for typing and for ABCs isolates. We also thank the following Emerging Infections Programs sites and staff: California Emerging Infections Program; Minnesota Department of Health; and the Emerging Infections Programs in Connecticut, Georgia, Maryland, New Mexico, New York, Oregon, and Tennessee. We are grateful to C.C. Cantu for technical assistance and K. Stockbauer for critical reading of the manuscript.

This work was funded by the Methodist Hospital System. N.F. is funded in part by the Canadian Institutes of Health Research.

Dr Fittipaldi is a postdoctoral fellow in the Department of Pathology and Genomic Medicine of the Methodist Hospital Research Institute, Houston, Texas. His primary research interest is the molecular basis of GAS pathogen–host interactions.

References

1. Olsen RJ, Shelburne SA, Musser JM. Molecular mechanisms underlying group A streptococcal pathogenesis. Cell Microbiol. 2009;11:1–12. http://dx.doi.org/10.1111/j.1462-5822.2008.01225.x
2. Lancefield RC. The antigenic complex of *Streptococcus haemolyticus*: I. Demonstration of a type-specific substance in extracts of *Streptococcus haemolyticus.* J Exp Med. 1928;47:91–103. http://dx.doi.org/10.1084/jem.47.1.91
3. Scott JR, Pulliam WM, Hollingshead SK, Fischetti VA. Relationship of M protein genes in group A streptococci. Proc Natl Acad Sci U S A. 1985;82:1822–6. http://dx.doi.org/10.1073/pnas.82.6.1822
4. Manjula BN, Acharya AS, Fairwell T, Fischetti VA. Antigenic domains of the streptococcal Pep M5 protein. Localization of epitopes crossreactive with type 6 M protein and identification of a hypervariable region of the M molecule. J Exp Med. 1986;163:129–38. http://dx.doi.org/10.1084/jem.163.1.129
5. Luca-Harari B, Darenberg J, Neal S, Siljander T, Strakova L, Tanna A, et al. Clinical and microbiological characteristics of severe *Streptococcus pyogenes* disease in Europe. J Clin Microbiol. 2009;47:1155–65. http://dx.doi.org/10.1128/JCM.02155-08
6. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global *emm* type distribution of group A streptococci: systematic review and implications for vaccine development. Lancet Infect Dis. 2009;9:611–6. http://dx.doi.org/10.1016/S1473-3099(09)70178-1
7. Tyrrell GJ, Lovgren M, St Jean T, Hoang L, Patrick DM, Horsman G, et al. Epidemic of group A *Streptococcus* M/*emm59* causing invasive disease in Canada. Clin Infect Dis. 2010;51:1290–7. http://dx.doi.org/10.1086/657068
8. Fittipaldi N, Beres SB, Olsen RJ, Kapur V, Shea PR, Watkins ME, et al. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A *Streptococcus.* Am J Pathol. 2012. Epub ahead of print. PubMed http://dx.doi.org/10.1016/j.ajpath.2011.12.037
9. Phillips G, Efstratiou A, Tanna A, Beall B, Ferguson J, Roworth M. An outbreak of skin sepsis in abattoir workers caused by an 'unusual' strain of *Streptococcus pyogenes.* J Med Microbiol. 2000;49:371–4.
10. O'Loughlin RE, Roberson A, Cieslak PR, Lynfield R, Gershman K, Craig A, et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000–2004. Clin Infect Dis. 2007;45:853–62. http://dx.doi.org/10.1086/521264
11. Dillon HC, Dillon MS. New streptococcal serotypes causing pyoderma and acute glomerulonephritis types 59, 60, and 61. Infect Immun. 1974;9:1070–8.
12. Passaro DJ, Smith DS, Hett EC, Reingold AL, Daily P, Van Beneden CA, et al. Invasive group A streptococcal infections in the San Francisco Bay area, 1989–99. Epidemiol Infect. 2002;129:471–8. http://dx.doi.org/10.1017/S0950268802007823
13. Pallen MJ, Loman NJ, Penn CW. High-throughput sequencing and clinical microbiology: progress, opportunities and challenges. Curr Opin Microbiol. 2010;13:625–31. http://dx.doi.org/10.1016/j.mib.2010.08.003

Address for correspondence: James M. Musser, Department of Pathology and Genomic Medicine, The Methodist Hospital System, 6565 Fannin St, B490, Houston, TX 77030, USA; email: jmmusser@tmhs.org

Characterization of *Mycobacterium orygis* as *M. tuberculosis* Complex Subspecies

Jakko van Ingen, Zeaur Rahim, Arnout Mulder, Martin J. Boeree, Roxane Simeone, Roland Brosch, and Dick van Soolingen

The oryx bacilli are *Mycobacterium tuberculosis* complex organisms for which phylogenetic position and host range are unsettled. We characterized 22 isolates by molecular methods and propose elevation to subspecies status as *M. orygis*. *M. orygis* is a causative agent of tuberculosis in animals and humans from Africa and South Asia.

Traditionally, the *Mycobacterium tuberculosis* complex comprises tubercle bacilli of 8 distinct subgroups: *M. tuberculosis*, *M. africanum*, *M. canettii*, *M. bovis*, *M. caprae*, *M. pinnipedii*, *M. microti*, and *M. mungi* (*1*–*4*). Two other distinct branches of the *M. tuberculosis* complex phylogenetic tree exist, the dassie and oryx bacilli, causative agents of tuberculosis in the animal species after which they are named. Neither has been validly described as separate taxa, nor have they been associated with disease in humans (*1*–*4*).

Oryx bacilli have been isolated from members of the *Bovidae* family, i.e., oryxes, gazelles (*3*), deer, antelope, and waterbucks (*5*), although their exact host range remains unsettled. No human disease caused by the oryx bacilli has been reported. These bacilli most likely constitute a separate phylogenetic lineage; however, their exact position has not been established with valid phylogenetic markers, such as large genomic deletions or single nucleotide polymorphisms (SNPs). To settle the phylogenetic position and host range of the oryx bacilli, we collected all oryx bacillus isolates from our laboratory database to establish their sources and subjected the isolates to extended phylogenetic analysis.

Author affiliations: Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands (J. van Ingen, M.J. Boeree, D. van Soolingen); International Center for Diarrheal Disease Research, Bangladesh, Dhaka, Bangladesh (Z. Rahim); National Institute for Public Health and the Environment, Bilthoven, the Netherlands (A. Mulder, D. van Soolingen); and Institut Pasteur, Paris, France (R. Simeone, R. Brosch)

DOI: http://dx.doi.org/10.3201/eid1804.110888

The Study

We selected 22 isolates on the basis of >90% similarity of the IS*6110* restriction fragment-length polymorphism (RFLP) pattern to that of established and previously published oryx bacillus strains; 11 isolates originated from animals, and 11 originated from 10 human patients (Figure 1) (*1*–*3*). All isolates yielded smooth to greasy domed nonchromogenic colonies in culture (online Technical Appendix Figure, wwwnc.cdc.gov/EID/pdfs/11-0888-Techapp.pdf).

For phylogenetic analysis, we performed SNP and region of difference (RD) analysis (*2*,*6*). RD and SNP typing showed a consistent pattern among the isolates, with presence of regions RD1, RD2, RD4, RD5a (*Rv2348*), RD6, and RD13–RD16 and absence of regions RD3, RD5b (*plcA*), and RD7–RD12 (online Technical Appendix Table 1). The deleted region for RD12 ($RD12^{oryx}$) was larger than that for *M. bovis* and *M. caprae*. Analysis of the flanking regions indicated an IS*6110* insertion at the *M. tuberculosis* H37Rv coordinates of 3479670 and 3491252 with deletion of the intermediate area covering the open reading frames of the *Rv3111* to *Rv3125c* genes (online Technical Appendix Table 2). Isolates also showed the RDoryx_1, RDoryx_4, and RDoryx_wag22 deletions and the $mmpL6^{551}$AAG mutation (online Technical Appendix Table 1). Results agreed with those from previous studies (*1*,*6*).

Using pncA-1F 5′-GGC CGC GAT GAC ACC TCT-3′, pncA1-R 5′-GCC GCA GCC AAT TCA GCA GT-3′, pncA-2F 5′-CGA AGC GGC GGA CTA CCA TCA CG-3′, and pncA-2R 5′-CCC CAC CTG CGG CTG CGA ACC-3′ primers, we partially sequenced *Rv2042c*, *Rv2044c*, and the full *pncA* gene. The *pncA* sequences of the isolates from animals and humans were identical to those of *M. tuberculosis* H37Rv; in codon 38 of the *Rv2042c* gene, directly upstream from *pncA*, a GTC to GGC (Ser→Ala) mutation was noted in all 22 isolates; the partial *Rv2042c* sequence is stored in GenBank (accession no. JF417976). To assess the specificity of the $Rv2042^{38}$ GGC mutation, we screened 2 isolates of all *M. tuberculosis* complex (sub) species and 2 isolates of all *M. tuberculosis* groupings, on the basis of >60% IS*6110* similarity, for this mutation; we did not find it in any of the strains tested (data not shown).

We performed spoligotyping and 24-locus mycobacterial interspersed repetitive units–variable-number tandem repeat (MIRU-VNTR) typing, as described (*7*,*8*). Spoligotyping mostly showed the sequence type (ST) 587 pattern in the spolDB4 database and labeled *M. africanum* (*9*); minor variations in spoligotype were observed (Figure 1, panel A). All isolates had unique IS*6110* RFLP patterns, although with >75% similarity; patterns were characterized by high (i.e., 17–20) numbers of IS*6110* copies (data not shown). VNTR typing showed closely related patterns (online Appendix Table, wwwnc.cdc.gov/EID/

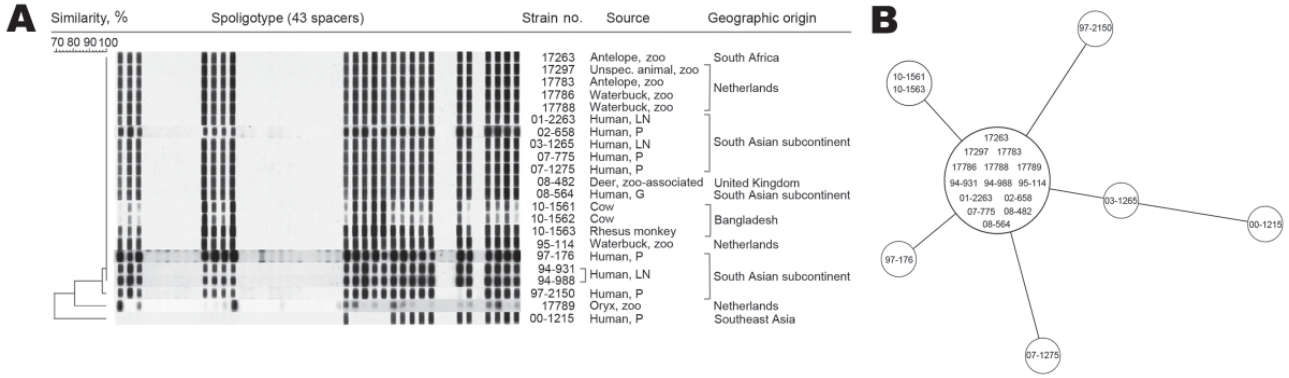


Figure 1. Spoligotyping and 24-locus variable number tandem repeat (VNTR) typing results for *Mycobacterium orygis*. A) Spoligotyping patterns for the oryx bacillus isolates in this study; ST587 is the most common pattern (labeled SB0422 at www.mbovis.org), with minor deviations. B) Minimum spanning tree based on 24-locus VNTR typing results for the oryx bacillus isolates in this study. One type dominates, with few strains representing minor variations. The dominant clone includes isolates from humans and animals. P, pulmonary; LN, lymph node; G, gastric juice. Both panels were created by using BioNumerics version 6.1 software (Applied Maths, Sint-Martens-Latem, Belgium); similarity coefficients were calculated by using Dice (spoligotyping) and Pearson (VNTR) methods; cluster analysis was done by the UPGMA (unweighted pair group method with arithmetic mean). Isolate 10–1562 (cow, Bangladesh) could not be included in (B) because of insufficient DNA.

article/18/4/11-0888-TA1.htm). A minimum spanning tree showed the clonality of the *M. orygis* isolates (Figure 1, panel B). The GenoType MTBC assay (Hain Lifesciences, Nehren, Germany) identified all isolates as *M. africanum*.

Baseline clinical data of humans were extracted from the anonymized National Tuberculosis Register. Ethical approval was waived for this retrospective laboratory-based study. Nine of the 10 human patients were of South Asian origin; the other was of Southeast Asian origin (Figure 1); patients' average age was 41 years (range: 0–69 years). Clinically, 6 patients had pulmonary tuberculosis, 3 had lymphadenitis, and 1 child had tuberculosis diagnosed by gastric fluid culture. All isolates were susceptible to all first-line antituberculosis drugs, including pyrazinamide, and hence the standard treatment regimen was started for all patients. Patients received treatment for an average of 9 months; no details about individual regimens were available. No bacteriologically proven relapses were noted. No information was available about contact-tracing studies.

Conclusions

The oryx bacillus is a phylogenetically distinct lineage of the clonal *M. tuberculosis* complex and thus deserves a separate subspecies status; we propose the name *M. orygis* (Latin: oryx, genitive: *orygis*, of the oryx) to convey that this subspecies was first characterized after its isolation from an oryx (Figure 2).

The most common spoligotype (ST587) is present in the spolDB4 database and labeled *M. africanum* (*9*). The *M. orygis* bacteria share the *gyrB*^{1450} (G→T) mutation with *M. africanum*, *M. microti*, and *M. pinnipedii* (*1*). Hence, the GenoType MTBC assay identifies *M. orygis* as *M. africanum*. Thus, *M. orygis* isolates may have previously been misidentified as *M. africanum* (*9*,*10*).

The animal-adapted *M. tuberculosis* complex lineage is thought to have evolved in Africa when an *M. africanum*–like clone diverged from *M. tuberculosis*, as shown by the loss of the RD9 locus. Consecutive loss of DNA during the adaptation to novel hosts led to the distinct subspecies with its distinct host range that we know today (*1*,*4*,*5*,*11*). This matches geographically with the habitats of *Oryx* species, gazelles, and waterbucks.

For *M. orygis*, the host range remains unknown but may include oryxes, waterbucks, and gazelles in eastern Africa and the Arabian Peninsula; cows and rhesus monkeys in South Asia; and humans. The evolutionary explanation for the diversity in geographic distribution and hosts of *M. orygis* remains elusive. This diversity contrasts starkly with the conserved VNTR and spoligotype patterns.

The presence of *M. orygis* in diseased cows and a monkey in Bangladesh, unique RFLP patterns, and lack of onward transmission suggest animal-to-human transmission. As for *M. bovis*; humans may be accidental, dead-end hosts.

M. orygis, unlike *M. microti*, the dassie bacillus, and *M. mungi*, shows an intact RD1 region. This region encodes part of the virulence-related ESX-1 secretion system of tubercle bacilli (*12*).

Molecular characteristics define the isolates previously labeled as oryx bacilli as a distinct subspecies in the *M. tuberculosis* complex for which we propose the name *M. orygis*. The *Rv2042*^{38} GGC mutation is a novel, useful genetic marker to identify *M. orygis*, which is otherwise characterized by the presence of genomic regions RD1,

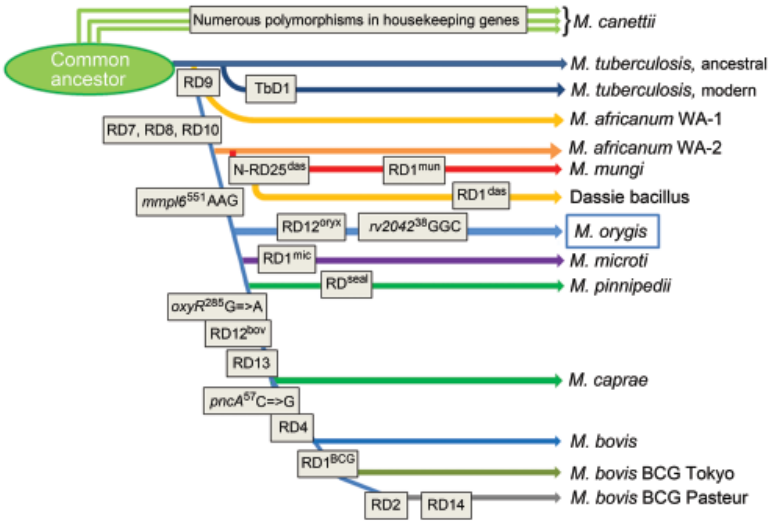


Figure 2. Updated phylogeny of the *Mycobacterium tuberculosis* complex based on the findings of Brosch et al. (*2*). Combined findings place *Mycobacterium orygis* at a distinct phylogenetic position between the *M. africanum*/dassie bacillus/*M. mungi* cluster and *M. microti*.

RD2, RD4, RD5a (*Rv2348*), RD6, RD13–RD16, and the *mmpL6*^{551}AAG polymorphism, with absence of regions RD3, RD5b (*plcA*), RD7–RD12, RDoryx_1, RDoryx_4, and RDoryx_wag22. The deletion of RD12 is subspecies specific. Isolates yield the ST587 or closely related spoligotypes, 17–20 copies of IS*6110*, and a distinct 24-locus VNTR pattern with minor variations. *M. orygis* is a causative agent of tuberculosis in oryxes, gazelles, and waterbucks of African origin; cows and rhesus monkeys of South Asian origin; and humans.

R.B. and R.S. were supported by the European Community's Seventh Framework Program (FP7/2007-2013) under grant agreement no. 201762.

Dr van Ingen is a resident in clinical microbiology at the Radboud University Nijmegen Medical Center. His primary research interests are the phylogeny and taxonomy of the genus *Mycobacterium* and treatment of tuberculosis and nontuberculous mycobacterial disease.

References

1. Huard RC, Fabre M, de Haas P, Oliveira Lazzarini LC, van Soolingen D, Cousins D, et al. Novel genetic polymorphisms that further delineate the phylogeny of the *Mycobacterium tuberculosis* complex. J Bacteriol. 2006;188:4271–87. http://dx.doi.org/10.1128/JB.01783-05
2. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, et al. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proc Natl Acad Sci U S A. 2002;99:3684–9. http://dx.doi.org/10.1073/pnas.052548299
3. van Soolingen D, de Haas PEW, Haagsma J, Eger T, Hermans PWM, Ritacco V, et al. Use of various genetic markers in differentiation of *Mycobacterium bovis* strains from animals and humans and for studying epidemiology of bovine tuberculosis. J Clin Microbiol. 1994;32:2425–33.
4. Alexander KA, Laver PN, Michel AL, Williams M, van Helden PD, Warren RM, et al. Novel *Mycobacterium tuberculosis* complex pathogen, *M. mungi*. Emerg Infect Dis. 2010;16:1296–9. http://dx.doi.org/10.3201/eid1608.100314
5. Smith NH, Kremer K, Inwald J, Dale J, Driscoll JR, Gordon SV, et al. Ecotypes of the *Mycobacterium tuberculosis* complex. J Theor Biol. 2006;239:220–5. http://dx.doi.org/10.1016/j.jtbi.2005.08.036
6. Mostowy S, Inwald J, Gordon S, Martin C, Warren R, Kremer K, et al. Revisiting the evolution of *Mycobacterium bovis*. J Bacteriol. 2005;187:6386–95. http://dx.doi.org/10.1128/JB.187.18.6386-6395.2005
7. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J Clin Microbiol. 1997;35:907–14.
8. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit–variable-number repeat typing of *Mycobacterium tuberculosis*. J Clin Microbiol. 2006;44:4498–510. http://dx.doi.org/10.1128/JCM.01392-06
9. Brudey K, Driscoll JR, Rigouts L, Prodinger WM, Gori A, Al-Hajoj SA, et al. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol. 2006;6:23. http://dx.doi.org/10.1186/1471-2180-6-23
10. Rahim Z, Möllers M, te Koppele-Vije A, de Beer J, Zaman K, Matin MA, et al. Characterization of *Mycobacterium africanum* subtype I among cows in a dairy farm in Bangladesh using spoligotyping. Southeast Asian J Trop Med Public Health. 2007;38:706–13.
11. Smith NH, Hewinson RG, Kremer K, Brosch R, Gordon SV. Myths and misconceptions: the origin and evolution of *Mycobacterium tuberculosis*. Nat Rev Microbiol. 2009;7:537–44. http://dx.doi.org/10.1038/nrmicro2165
12. Simeone R, Bottai D, Brosch R. ESX/type VII secretion systems and their role in host-pathogen interaction. Curr Opin Microbiol. 2009;12:4–10. http://dx.doi.org/10.1016/j.mib.2008.11.003

Address for correspondence: Jakko van Ingen, Radboud University Nijmegen Medical Center, Department of Clinical Microbiology (574), PO Box 9101, 6500HB Nijmegen, the Netherlands; email: j.vaningen@mmb.umcn.nl

Cosavirus Infection in Persons with and without Gastroenteritis, Brazil

Andreas Stöcker,[1]
Breno Frederico de Carvalho Dominguez Souza,[1]
Tereza Cristina Medrado Ribeiro,[1]
Eduardo Martins Netto, Luciana Oliveira Araujo,
Jefferson Ivan Corrêa, Patrícia Silva Almeida,
Angela Peixoto de Mattos,
Hugo da Costa Ribeiro Jr.,
Diana Brasil Pedral-Sampaio, Christian Drosten,
and Jan Felix Drexler

To determine possible cosavirus association with clinical disease, we used real-time reverse transcription PCR to test children and HIV-positive adults in Brazil with and without gastroenteritis. Thirteen (3.6%) of 359 children with gastroenteritis tested positive, as did 69 (33.8%) of 204 controls. Low prevalence, frequent viral co-infections, and low fecal cosavirus RNA concentrations argue against human pathogenicity.

The family *Picornaviridae* comprises 12 genera and includes several leading pathogens affecting human and animal health, e.g., foot-and-mouth-disease virus and polioviruses. With the advent of metagenomics, 3 novel human picornaviruses have been described since 2008: klassevirus, salivirus, and cosavirus (*1*–*3*).

Besides being detected in raw sewage (*4*), cosaviruses have been detected in human fecal specimens and were tentatively associated with gastroenteritis in 2 patients from Australia and Scotland (*3*,*5*). Cosavirus pathogenicity in humans has remained unknown, however, because detection rates in patients and healthy controls were similar in the only available cohort studies of patients with acute flaccid paralysis in Southeast Asia (*3*) and with gastroenteritis in China (*6*). We investigated cosavirus prevalence in 464 children and HIV-infected adults with gastroenteritis and 253 controls without gastroenteritis in Brazil.

Author affiliations: University of Bonn Medical Centre, Bonn, Germany (A. Stöcker, C. Drosten, J.F. Drexler); and Federal University of Bahia, Salvador, Brazil (A. Stocker, B.F.C.D. Souza, T.C.M. Ribeiro, E.M. Netto, L.O. Araujo, J.I. Corrêa, P.S. Almeida, A.P. de Mattos, H.C. Ribeiro Jr, D.B. Pedral Sampaio)

DOI: http://dx.doi.org/10.3201/eid1804.111415

The Study

Fecal specimens from 6 clinical cohorts in Salvador, northeastern Brazil, were analyzed (Table 1). Adult cohorts comprised HIV-infected patients with gastroenteritis (105 persons) and without (49 persons) gastroenteritis. Child cohorts included 359 children with gastroenteritis and 204 healthy children from child-care centers**.** Nasal swab specimens were obtained from controls attending child-care centers and from children with gastroenteritis and concomitant respiratory symptoms. All specimens were stored at −30° to −80°C until further processing.

Viral RNA was purified from ≈200 mg fecal specimen and 140 μL nasal swab specimen suspended in phosphate-buffered saline by using the Viral RNA Mini kit (QIAGEN, São Paulo, Brazil) as described (*7*). A nested reverse transcription PCR (*3*) detected cosavirus. After nucleotide sequencing of all PCR-positive specimens, we developed a specific real-time RT-PCR for quantifying viruses from Brazil (Table 2). Assay optimization and quantification relied on photometrically quantified cRNA in vitro transcripts, as described (*8*). After assay optimization, sensitivity was 6.8 copies per reaction.

In the adult cohorts, 1 of 105 HIV-infected persons with gastroenteritis had positive results for cosavirus. In the control group of 49 HIV-infected adults without gastroenteritis, none had positive cosavirus results (χ^2 0.5; $p = 0.49$).

Considerably higher prevalence was detected among child cohorts. Of children with gastroenteritis, 13 (3.6%) of 359 patients were cosavirus positive. The proportion of cosavirus-positive controls without gastroenteritis, sampled in 2008, was significantly higher: 65 (49.2%) of 132 controls were cosavirus positive (χ^2 149.1; $p<0.0001$). On resampling in 2011, 4 (6.5%) of 62 controls were cosavirus positive. Although the difference was not statistically significant (χ^2 1.1; $p = 0.3$), this result was almost double that for patients. In another child-care center, none of 10 healthy children were cosavirus positive.

The higher prevalence detected in controls in 2008 could indicate a seasonal infection pattern because specimens were collected in a single weekend in a child-care center. The lower cosavirus-positive results in the same child-care center 3 years after the initial sampling support this idea. However, in sick children, cosavirus-positive specimens were obtained at similarly low rates throughout the year (Figure 1), which might argue against seasonal variation as a generic property of cosavirus infection.

To evaluate whether cosavirus causes disease in ill children, we analyzed co-infections with common viral pathogens causing diarrhea (astrovirus, norovirus, rotavirus, adenovirus) (Figure 2, panel A). In 10 (76.9%) of

[1]These authors contributed equally to this article.

Table 1. Clinical cohorts tested for cosavirus, Salvador, Brazil*

Cohort no.	Cohort description†	Sampling site‡	Sampling time	Participant age, mo, mean (SD)	No. participants	No. (%) RT-PCR positive§	Virus concentration, $\log_{10}$ RNA copies/g feces, mean (SD)¶
1	HIV-infected adults with gastroenteritis	Infectious Diseases HIV	2007 Mar–2010 Mar	487.6 (114.4)	105	1 (1.0)	4.43
2	HIV-infected adults without gastroenteritis	Outpatient Department	2007 Mar–2010 Mar	533.8 (115.9)	49	0	–
3	Children with gastroenteritis	Department of Pediatrics Metabolic Unit#	2006 Feb–2007 Sep	19.0 (15.6)	359	13 (3.6)	3.40 (0.93)
4	Control children without gastroenteritis	Community child-care center**	2008 Dec	29.6 (13.1)	132	65 (49.2)	2.97 (0.97)
5	Control children without gastroenteritis	Community child-care center**	2011 Nov–2011 Dec	14.3 (5.5)	62	4 (6.5)	3.41 (0.49)
6	Control children without gastroenteritis	University child-care center**	2011 Oct–2011 Nov	18.6 (4.2)	10	0	–
Total					717	83 (11.6)	

*RT-PCR, reverse transcription PCR; –, no virus obtained.
†Gastroenteritis was defined as acute diarrhea with >3 watery stools in the previous 24 h and within 13 d before hospital admission.
‡All hospital units were located within the Hospital Professor Edgard Santos, Federal University of Bahia. All sites were located in Salvador, Bahia, in northeastern Brazil.
§Samples only considered if positive in nested RT-PCR as in (*3*) and in strain-specific real-time RT-PCR.
¶Measured by strain-specific RT-PCR.
#Reports of diarrhea in the preceding 2 wk served as an exclusion factor.
**Written consent was obtained from adult family members.

13 cosavirus-positive patients, ≥1 of these pathogens could be detected.

Acute infection with an enteric virus is usually associated with high viral RNA shedding. Cosavirus concentrations were low in outpatient and control children (≈10^3 copies/g) on 2 sampling occasions (Table 1) and did not differ significantly (analysis of variance F 2.0; p = 0.14; Figure 2, panel B).

Low cosavirus concentrations in the enteric tract could result from swallowing viruses originating in the respiratory tract. Therefore, we tested for cosavirus in 96 nasal swab specimens from gastroenteritis patients, including 3 nasal swab specimens from persons with cosavirus-positive fecal specimens and 65 nasal swab specimens from cosavirus-positive controls sampled in 2008. None of the nasal specimens from controls with positive fecal specimens and only 1 nasal specimen from a patient unrelated to the 3 aforementioned persons was weakly positive (10^3 RNA copies/mL), which refutes this hypothesis.

To analyze whether a preceding point-source infection caused high cosavirus prevalence in the controls without gastroenteritis sampled in 2008, we determined the genomic sequence of the 5′ untranslated region PCR amplicons and phylogenetically analyzed the sequence (GenBank accession no. JN228118–JN228188). Cosaviruses from these controls were distributed across the phylogenetic tree (online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-1415-Techapp.pdf). Maximum nucleotide distance within these cosaviruses was up to 22.5% in the analyzed 398-nt fragment, making a recent point-source infection unlikely.

Conclusions

Human cosavirus infections were reported previously from a limited number of persons and geographic areas

Table 2. Oligonucleotides used for detection and quantification of cosaviruses*

Oligonucleotide identity	Sequence, 5′ → 3′	Genomic target region, RT-PCR type	Use	Reference
DKV-N5U-F1	CGTGCTTTACACGGTTTTTGA (+)	5′-UTR, nested RT-PCR 1st round	Cosavirus detection†	(*3*)
DKV-N5U-R2	GGTACCTTCAGGACATCTTTGG (–)			
DKV-N5U-F2	ACGGTTTTTGAACCCCACAC (+)	5′-UTR, nested RT-PCR 2nd round		
DKV-N5U-R3	GTCCTTTCGGACAGGGCTTT (–)			
HCosV-rtF735-1	TTGTAGYGATGCTGTRTGTGTGTG (+)	5′-UTR, real time RT-PCR	Brazilian cosavirus quantification†	This study
HCosV-rtP783	FAM-AGCCTCACAGGCCRRAAGCCCTGTC-DDQ1 (+, Probe)			
HCosV-rtR827-1	CCAYTGTGTGGGTCCTTTCG (–)			

*RT-PCR, reverse transcription PCR; UTR, untranslated region; FAM, fluorescein; R, G/A; DDQ1, deep dark quencher 1; Y, C/T.
†RT-PCR reactions were carried out using the QIAGEN One-step RT-PCR kit as described by the manufacturer (QIAGEN, São Paulo, Brazil), 300 nmol/L of each primer, 200 nmol/L of the probe (real time RT-PCR assay), 1 μg bovine serum albumin, and 5 μL RNA extract. Second-round reactions used the Platinum Taq DNA Polymerase Kit as described by the manufacturer (Invitrogen, São Paulo, Brazil) with 2.5 mol/L MgCl and 1 μL of first-round PCR product. Real time RT-PCR amplification involved 55°C for 15 min, 95°C for 15 min, and 45 cycles of 95°C for 15 s and 58°C for 30 s (fluorescence measured).Nested RT-PCR involved 30 min at 50°C; 15 min at 95°C; 10 cycles of 20 s at 94°C, 30 s starting at 60°C with a decrease of 1°C per cycle, and 50 s at 72°C; and 40 cycles of 20 s at 95°C, 30 s at 54°C, and 50 s at 72°C; and a final elongation step of 5 min at 72°C. Second-round reactions used 3 min at 94°C and thermal cycling as for the first round.

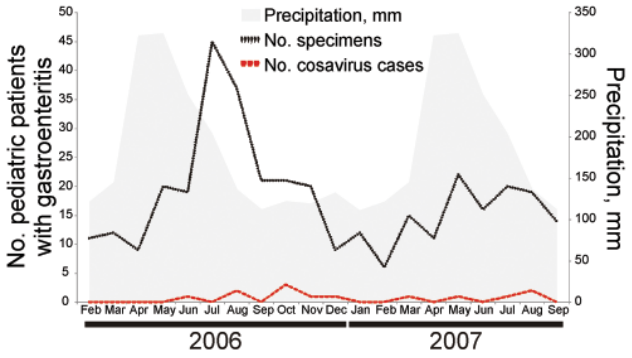


Figure 1. Detection pattern of cosavirus in children with gastroenteritis throughout different seasons during 2006–2007, Brazil. Temperature was not plotted because it varied little from mean 25.2°C through the year (range 23.6–26.7°C). Precipitation data were obtained from the German Weather Service and represent means throughout 1961–1990.

(*3*–*6*). In Brazil, the 3.6% detection rate in children with gastroenteritis was comparable to the 1.8% rate in a cohort study of gastroenteritis patients in China (*6*). Although the 6.5% detection rate in 1 control cohort in Brazil was compatible with the 1.7% rate in 60 healthy controls in China, the combined 33.8% prevalence detected in controls from 3 different samplings in Brazil was much higher. Nonetheless, the prevalence was comparable to the 43.9% detected in 41 healthy Southeast Asian children in the only other cohort study (*3*). Detecting cosavirus in 1 of 154 adults in Brazil was compatible with finding a single cosavirus-positive patient among 1,000 adults with gastroenteritis in Scotland, confirming that cosaviruses are rare and probably neither pathogenic nor commensal in adults (*3*).

The higher prevalence of cosavirus found in controls than in patients, the frequent co-infections with established pathogens, and the unusually low RNA virus concentrations give evidence against cosavirus involvement in human gastroenteritis. Viruses that replicate in the human gut generally reach concentrations 1,000- to 100,000-fold higher than those of cosavirus. This finding is exemplified by genetically related picornaviruses (Aichi viruses, parechoviruses, and cardioviruses) and established enteric pathogens (e.g., noroviruses and rotaviruses) (*8*–*12*). Notably, the aforementioned study on cardioviruses included the same specimens from Brazil, which indicates that poor sample quality was not a factor.

These low concentrations would be compatible with absence of replication in the enteric tract and passive virus ingestion, e.g., from nutritional sources, drinking water, or the respiratory tract. However, nutritional patterns of the tropical countries in which cosavirus have been detected certainly differ. Furthermore, in Brazil, adults are unlikely to have a completely different diet from infants and children. Moreover, the unprecedented detection of cosavirus in a respiratory tract specimen makes ingestion of viruses from nutritional sources alone unlikely, although a link to fluid droplets from drinking water in the respiratory tract is hypothetically possible.

Another explanation for low cosavirus RNA levels in fecal samples is that a cosavirus infection occurred early in the person's life and produced partial mucosal immunity and limited subsequent cosavirus replication in the gut. This is exemplified for viruses transmitted by the fecal–oral

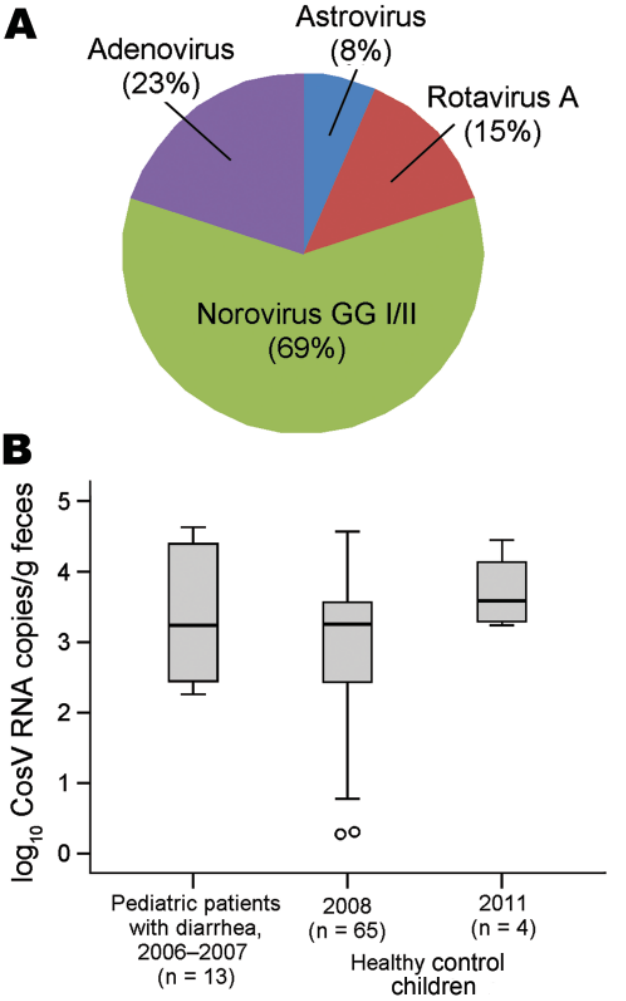


Figure 2. Co-infections and fecal cosavirus (CosV) RNA concentrations. A) Co-infections with established viral causes of diarrhea in children with gastroenteritis who were positive for CosV. Viral RNA and DNA were detected by real-time PCR (methods available upon request) in the same eluates used for CosV detection. B) Boxplot generated with SPSS V19 (SPSS, Munich, Germany) of $\log_{10}$ CosV RNA concentrations per gram of feces in children with gastroenteritis and healthy control children from a child-care center in 2008 and 2011. Boxes show the medians and interquartile ranges (box length). The whiskers represent an extension of the 25th or 75th percentiles by 1.5× the interquartile range. Datum points beyond the whisker range are considered as outliers and marked as circles. GGI/II, genogroups I and II.

route by up to 100-fold higher fecal shedding of vaccine rotavirus and poliovirus among seronegative persons than among seropositive or previously vaccinated persons (*13*,*14*). However, this explanation would be incompatible with the high prevalence of cosavirus in many control children, who were generally older than patients.

Prolonged low concentrations of picornavirus shedding has been demonstrated, e.g., by detectable hepatitis A virus RNA up to 3 months after acute infection (*15*). Nonetheless, this circumstance is unlikely to explain the low cosavirus concentrations, given the overall high number of persons with positive results.

Although our study extends the known geographic occurrence of cosavirus, whether it is a human pathogen at all remains to be determined. Future studies would be enhanced by serologic analyses and investigations focusing on nutrition and drinking water in tropical countries.

Acknowledgments

We thank Tobias Bleicker, Sebastian Brünink, Monika Eschbach-Bludau, Célia Pedroso, Carlos Brites, Vanusa dos Santos Estrela, Maria Goreth Barberino, Ana-Rute Santos Oliveira, and Milena Carvalho Bastos for outstanding support and Alexander N. Lukashev for helpful suggestions. We are also grateful to the child-care center staff, children, and parents involved in this study.

The study was funded by the Foundation for Research Support of the State of Bahia (Fundação de Amparo à Pesquisa do Estado da Bahia), project codes APR 125/2006/ethics committee protocol 120/2005 and SUS0004/2007/ethics committee protocol 06/2007, and the European Union FP7 project European Management Platform for Emerging and Re-emerging Infectious Disease Entities (grant agreement no. 223498).

Dr Stöcker is a physician affiliated with the Federal University of Bahia, Salvador, Brazil, and the University of Bonn, Germany. His primary research interest is the implementation of molecular diagnostics in resource-limited settings.

References

1. Li L, Victoria J, Kapoor A, Blinkova O, Wang C, Babrzadeh F, et al. A novel picornavirus associated with gastroenteritis. J Virol. 2009;83:12002–6. http://dx.doi.org/10.1128/JVI.01241-09
2. Greninger AL, Runckel C, Chiu CY, Haggerty T, Parsonnet J, Ganem D, et al. The complete genome of klassevirus—a novel picornavirus in pediatric stool. Virol J. 2009;6:82. http://dx.doi.org/10.1186/1743-422X-6-82
3. Kapoor A, Victoria J, Simmonds P, Slikas E, Chieochansin T, Naeem A, et al. A highly prevalent and genetically diversified *Picornaviridae* genus in South Asian children. Proc Natl Acad Sci U S A. 2008;105:20482–7. http://dx.doi.org/10.1073/pnas.0807979105
4. Blinkova O, Rosario K, Li L, Kapoor A, Slikas B, Bernardin F, et al. Frequent detection of highly diverse variants of cardiovirus, cosavirus, bocavirus, and circovirus in sewage samples collected in the United States. J Clin Microbiol. 2009;47:3507–13. http://dx.doi.org/10.1128/JCM.01062-09
5. Holtz LR, Finkbeiner SR, Kirkwood CD, Wang D. Identification of a novel picornavirus related to cosaviruses in a child with acute diarrhea. Virol J. 2008;5:159. http://dx.doi.org/10.1186/1743-422X-5-159
6. Dai XQ, Hua XG, Shan TL, Delwart E, Zhao W. Human cosavirus infections in children in China. J Clin Virol. 2010;48:228–9. http://dx.doi.org/10.1016/j.jcv.2010.03.024
7. Drexler JF, Baumgarte S, Luna LK, Stöcker A, Almeida PS, Ribeiro TC, et al. Genomic features and evolutionary constraints in Saffold-like cardioviruses. J Gen Virol. 2010;91:1418–27. http://dx.doi.org/10.1099/vir.0.018887-0
8. Baumgarte S, de Souza Luna LK, Grywna K, Panning M, Drexler JF, Karsten C, et al. Prevalence, types, and RNA concentrations of human parechoviruses, including a sixth parechovirus type, in stool samples from patients with acute enteritis. J Clin Microbiol. 2008;46:242–8. http://dx.doi.org/10.1128/JCM.01468-07
9. Bishop RF. Natural history of human rotavirus infection. Arch Virol Suppl. 1996;12:119–28.
10. Drexler JF, Luna LK, Stocker A, Almeida PS, Ribeiro TC, Petersen N, et al. Circulation of 3 lineages of a novel Saffold cardiovirus in humans. Emerg Infect Dis. 2008;14:1398–405. http://dx.doi.org/10.3201/eid1409.080570
11. Drexler JF, Baumgarte S, de Souza Luna LK, Eschbach-Bludau M, Lukashev AN, Drosten C. Aichi virus shedding in high concentrations in patients with acute diarrhea. Emerg Infect Dis. 2011;17:1544–8.
12. Henke-Gendo C, Harste G, Juergens-Saathoff B, Mattner F, Deppe H, Heim A. New real-time PCR detects prolonged norovirus excretion in highly immunosuppressed patients and children. J Clin Microbiol. 2009;47:2855–62. http://dx.doi.org/10.1128/JCM.00448-09
13. Anderson EJ. Rotavirus vaccines: viral shedding and risk of transmission. Lancet Infect Dis. 2008;8:642–9. http://dx.doi.org/10.1016/S1473-3099(08)70231-7
14. Laassri M, Lottenbach K, Belshe R, Wolff M, Rennels M, Plotkin S, et al. Effect of different vaccination schedules on excretion of oral poliovirus vaccine strains. J Infect Dis. 2005;192:2092–8. http://dx.doi.org/10.1086/498172
15. Yotsuyanagi H, Koike K, Yasuda K, Moriya K, Shintani Y, Fujie H, et al. Prolonged fecal excretion of hepatitis A virus in adult patients with hepatitis A as determined by polymerase chain reaction. Hepatology. 1996;24:10–3. http://dx.doi.org/10.1002/hep.510240103

Address for correspondence: Jan Felix Drexler, Institute of Virology, University of Bonn Medical Centre 53127, Bonn, Germany; email: drexler@virology-bonn.de

Drug Susceptibility of *Mycobacterium tuberculosis* Beijing Genotype and Association with MDR TB

Jurriaan E.M. de Steenwinkel, Marian T. ten Kate, Gerjo J. de Knegt, Kristin Kremer, Rob E. Aarnoutse, Martin J. Boeree, Henri A. Verbrugh, Dick van Soolingen, and Irma A.J.M. Bakker-Woudenberg

To determine differences in the ability of *Mycobacterium tuberculosis* strains to withstand antituberculosis drug treatment, we compared the activity of antituberculosis drugs against susceptible Beijing and East-African/Indian genotype *M. tuberculosis* strains. Beijing genotype strains showed high rates of mutation within a wide range of drug concentrations, possibly explaining this genotype's association with multidrug-resistant tuberculosis.

The emergence of *Mycobacterium tuberculosis* resistance to antituberculosis (anti-TB) drugs is a major public health challenge that is threatening World Health Organization targets set for the elimination of TB (*1*). Approximately 500,000 cases of multidrug-resistant TB (MDR TB) are diagnosed annually, but the true magnitude of the MDR TB problem is not known because adequate laboratory tools are lacking. Multiple factors contribute to low cure rates, treatment failures, and relapses: poor-quality guidance regarding treatment, HIV co-infection, transmission of resistant forms of TB, underdeveloped laboratory services, and unavailability of alternative drug treatments. However, the evolution of *M. tuberculosis* is an additional factor that presumably fuels the worldwide problem of emerging resistance. The Beijing genotype is significantly associated with drug resistance (*2*,*3*), especially in geographic areas where prevalence of resistance to anti-TB drugs is high, and it is associated with recent TB transmission (*2*–*6*). There are also indications that the population structure of *M. tuberculosis* in areas with a high prevalence of anti-TB drug resistance is changing rapidly toward an increase in Beijing genotype strains (*2*,*6*–*8*).

The World Health Organization target rates for detecting and curing TB in Vietnam have been met; however, the rate of TB infection is not decreasing as expected (*4*,*5*). Earlier in this country, the Beijing genotype was strongly correlated with MDR TB and treatment failures (*9*). Extensive molecular epidemiologic studies showed that the Beijing and East-African/Indian (EAI) genotypes are predominating in Vietnam; each lineage causes ≈40% of the TB cases. According to the single-nucleotide polymorphism typing described by Hershberg et al. (*10*), the Beijing genotype is a representative of the modern lineage, and the EAI genotype is believed to represent an evolutionary lineage more closely related to the common ancestor of the *M. tuberculosis* complex.

We compared the in vitro activity of anti-TB drugs against susceptible Beijing and EAI *M. tuberculosis* isolates from Vietnam and determined the in vitro mutation frequency of these strains during drug exposure. We also determined time-kill kinetics of anti-TB drugs and assessed the emergence of resistant mutants and the concentration range within which resistant mutants and no susceptible mycobacteria were selected. The concentration at which resistant mutants did not emerge (the mutant prevention concentration) was also ascertained. By using this approach, we established an in vitro model for determining differences in the ability of *M. tuberculosis* strains to resist anti-TB drug treatment.

The Study

Results of a liquid culturing system (BD BACTEC MGIT 960 System; BD Diagnostics, Sparks, MD, US) (for details, see the online Technical Appendix, wwwnc.cdc.gov/EID/pdfs/11-0912-Techapp.pdf) showed that all 5 Beijing and 5 EAI genotype strains were susceptible to isoniazid (INH), rifampin (RIF), moxifloxacin (MXF), and amikacin (AMK). MICs were determined by using the agar proportion method (*11*), which showed that ranges were small for the Beijing and EAI genotype strains: INH, 0.062–0.125 mg/L; RIF, 0.125–1 mg/L; MXF, 0.125–0.5 mg/L; and AMK, 0.5–2 mg/L. Duplicate values showed only minor differences.

We determined the mutation frequencies of the Beijing and EAI genotype strains by using previously defined critical drug concentrations of 1 mg/L for INH, RIF, and

Author affiliations: Erasmus University Medical Center, Rotterdam, the Netherlands (J.E.M. de Steenwinkel, M.T. ten Kate, G.J. de Knegt, H.A. Verbrugh, I.A.J.M. Bakker-Woudenberg); National Institute of Public Health and the Environment Center for Infectious Disease Control (RIVM), Bilthoven, the Netherlands (K. Kremer, D. van Soolingen); World Health Organization Regional Office for Europe, Copenhagen, Denmark (K. Kremer); and Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands (R.E. Aarnoutse, M.J. Boeree, D. van Soolingen)

DOI: http://dx.doi.org/10.3201/eid1804.110912

Table 1. Mutation frequency of *Mycobacterium tuberculosis* genotype strains originating from Vietnam, by antituberculosis drug

Genotype	Frequency of mutation among strains*			
	Isoniazid	Rifampin	Moxifloxacin	Amikacin
Beijing				
1585	5.7×10^{-6}, 6.2×10^{-6}	3.0×10^{-3}, 4.3×10^{-3}	4.3×10^{-8}, 6.1×10^{-8}	2.3×10^{-8}, 3.2×10^{-8}
1607	8.6×10^{-6}, 1.4×10^{-5}	1.5×10^{-3}, 5.4×10^{-3}	6.9×10^{-8}, 2.4×10^{-7}	8.6×10^{-8}, 3.0×10^{-7}
2115	7.3×10^{-6}, 1.1×10^{-5}	1.0×10^{-3}, 9.2×10^{-5}	1.0×10^{-8}, 4.3×10^{-8}	1.4×10^{-8}, 2.8×10^{-8}
2121	6.8×10^{-5}, 2.9×10^{-4}	2.9×10^{-5}, 1.9×10^{-4}	1.1×10^{-7}, 1.6×10^{-7}	9.3×10^{-8}, 1.1×10^{-7}
2145	9.1×10^{-4}, 5.0×10^{-4}	1.6×10^{-5}, 5.5×10^{-5}	7.9×10^{-8}, 1.0×10^{-7}	7.6×10^{-7}, 1.1×10^{-6}
East-African/Indian				
1627	3.7×10^{-6}, 6.5×10^{-6}	4.1×10^{-6}, 2.8×10^{-6}	9.3×10^{-9}, 1.5×10^{-7}	5.6×10^{-8}, 4.5×10^{-9}
1606	8.7×10^{-6}, 1.6×10^{-4}	3.8×10^{-4}, 2.7×10^{-5}	3.2×10^{-8}, 1.0×10^{-7}	7.5×10^{-9}, 1.5×10^{-9}
1592	1.8×10^{-5}, 2.6×10^{-5}	3.0×10^{-4}, 2.4×10^{-5}	9.9×10^{-8}, 4.5×10^{-8}	9.4×10^{-8}, 1.5×10^{-9}
1596	3.9×10^{-5}, 2.8×10^{-5}	1.4×10^{-5}, 3.9×10^{-6}	1.7×10^{-7}, 2.0×10^{-7}	3.7×10^{-8}, 3.2×10^{-7}
2113	1.3×10^{-5}, 4.1×10^{-5}	6.7×10^{-8}, 6.3×10^{-8}	1.5×10^{-8}, 1.0×10^{-7}	4.4×10^{-8}, 3.3×10^{-7}

*Determined in duplicate.

MXF and 5 mg/L for AMK (*11*,*12*) (for details, see the online Technical Appendix). The mutation frequencies of the Beijing and EAI genotype strains were similar for INH, MXF, and AMK, but they were significantly different for RIF (1.6×10^{-5} to 5.4×10^{-3} for Beijing strains vs. 6.3×10^{-8} to 3.8×10^{-4} for EAI strains; $p = 0.003$, unpaired Mann-Whitney test) (Table 1; Figure 1). Because rifamycin drugs are widely used to treat TB, the difference in the mutation frequencies of Beijing and EAI genotype strains for RIF is a major finding.

For Beijing genotype strains, the increase in mutation frequency during exposure to RIF could be due to described missense mutations in the *mut* genes (*13*). Such mutations in the *mut* genes can change the DNA repair mechanism; as a consequence, the frequency of resistant mutant formation might increase. However, a direct correlation between the occurrence of particular mutations in *mut* genes and altered mutation frequency has not been proven. Furthermore, Werngren and Hoffner (*14*) found an equal mutation frequency for Beijing (3.6×10^{-8}) and non-Beijing (4.4×10^{-8}) genotypes. A possible explanation for the discrepancy in findings might be the concentration of RIF used in the subculture plates. In our study, the critical concentration of 1 mg/L RIF was used (*11*), whereas Werngren and Hoffner used a concentration of 2 mg/L RIF. In addition, Werngren and Hoffner compared the Beijing and non-Beijing genotypes of several genotype families, whereas we compared Beijing and EAI genotype strains that were selected from the same tuberculosis-endemic area and during the same period.

We determined the time-kill kinetics of RIF toward 2 strains with significantly different mutation frequencies: Beijing-1585 (3.7×10^{-3} [3.0×10^{-3} and 4.3×10^{-3}, duplicates]) and EAI-1627 (3.5×10^{-6} [2.8×10^{-6} and 4.1×10^{-6}, duplicates]). Cultures with low and high densities of Beijing-1585 and EAI-1627 were investigated as described (*15*). RIF showed strong time- and concentration-dependent activity toward low-density cultures of the 2 strains (Figure 2). Low concentrations of RIF were needed to achieve ≥99% mycobacterial killing; differences between Beijing-1585 and EAI-1627 were minor (Table 2). However, to achieve 100% killing, especially for Beijing-1585, RIF concentrations had to be increased substantially (Table 2). Compared with the low-density culture for Beijing-1585, a substantial increase in RIF concentrations was needed to achieve 100% killing of the high-density culture (Table 2). This finding may be relevant in the clinical context because high-density mycobacteria populations are expected to exist in infected tissues of TB patients.

RIF-resistant mutants did not emerge in low-density cultures of Beijing-1585 and EAI-1627. However, RIF-resistant mutants were selected at relatively high numbers from high-density Beijing-1585 cultures compared with high-density EAI-1627 cultures. In Beijing-1585 cultures,

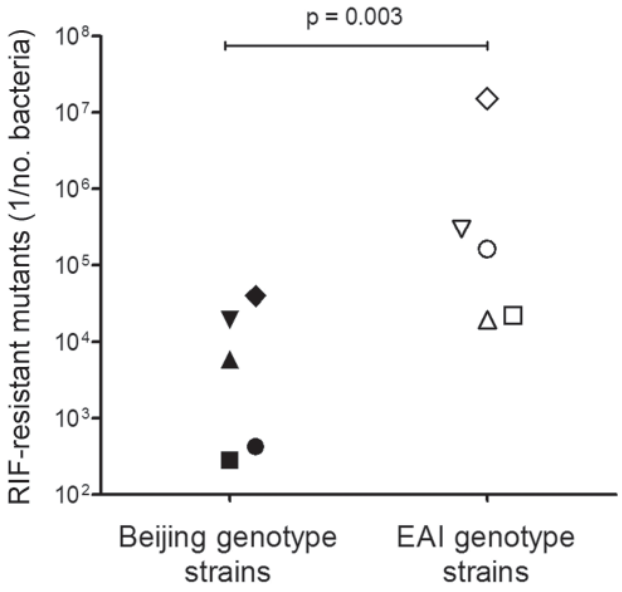


Figure 1. Frequency of rifampin (RIF)-resistant mutants in *Mycobacterium tuberculosis* Beijing and East-African/Indian (EAI) genotype strains (5 strains each) originating from Vietnam. Mutation frequencies were determined in duplicate. Statistical analysis was performed by using an unpaired Mann-Whitney test.

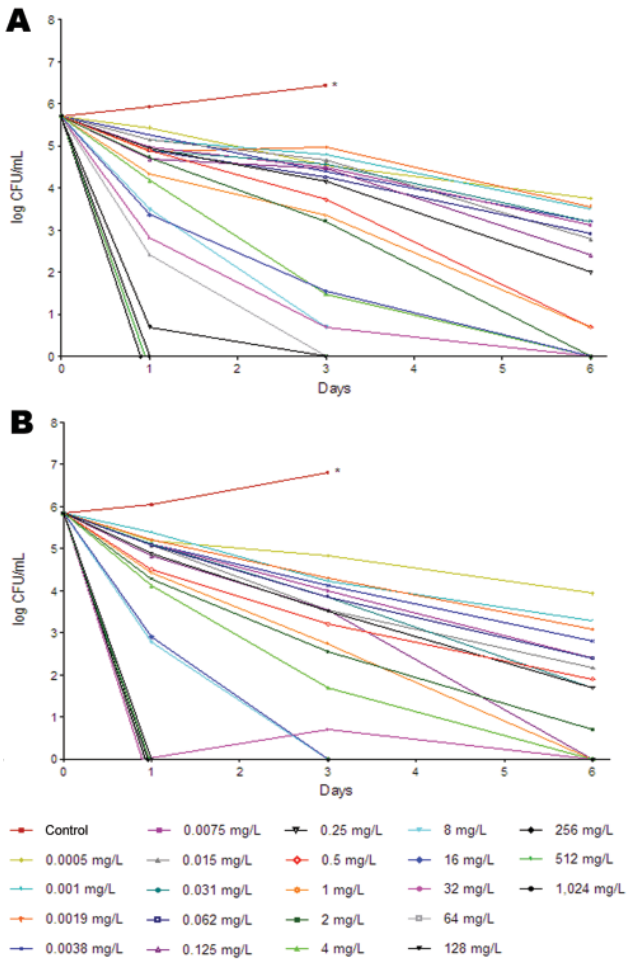


Figure 2. Concentration- and time-dependent bactericidal effect of rifampin (RIF) toward low-density cultures of *Mycobacterium tuberculosis* BE-1585 (5.1 × 10^5 CFU/mL) (A) and *M. tuberculosis* EAI-1627 (6.8 × 10^5 CFU/mL) (B). Cultures were exposed to RIF at 2-fold increasing concentrations for 6 days at 37°C. After 1, 3, or 6 days of exposure, subcultures were performed on solid media to count CFUs. *Accurate CFU counting could not be performed because complete outgrowth of mycobacteria occurred on the sixth day of RIF exposure, leading to aggregation.

exposure to RIF concentrations of 2–32 mg/L selected resistant mutants only; this was not observed in EAI-1627 cultures. Analysis of RIF-resistant Beijing mutants showed the following altered *rpoB* gene sequences: CAC→GAC (H526D), CAC→TAC (H526Y), and TCG→TTG (S531L), as assessed by using the GenoType MTBDR*plus* (Hain Lifescience, Nehren, Germany) assay (for details, see the online Technical Appendix).

For 3 of the 4 anti-TB drugs, the difference in the range of mutant prevention concentrations for the Beijing and EAI genotype strains was small: INH, 128–256 mg/L; RIF, 256–1,024 mg/L; and MXF, 2–8 mg/L. The mutant prevention concentration for AMK was >1,024 mg/L for all strains tested.

Conclusions

We showed that the currently used anti-TB drug susceptibility assays do not discriminate between the in vitro susceptibility, as determined by the methods used in this study, of the *M. tuberculosis* Beijing and EAI genotype strains. We also showed that the determination of mutation frequencies might be more informative than results of anti-TB drug susceptibility assays. For RIF, mutation frequencies in Beijing genotype strains were high compared with those in EAI genotype strains, and the selection of RIF-resistant mutants among Beijing strains, but not EAI strains, occurred within a wide range of RIF concentrations. In addition, the killing capacity of RIF toward the Beijing genotype is dependent on the density of mycobacteria: high concentrations of RIF are required to achieve 100% killing of high-density Beijing genotype populations but not of high-density EAI genotype populations. These in vitro characteristics might contribute to the less favorable treatment outcome of Beijing genotype TB infections and their significant association with drug resistance. Our findings demonstrate the need for anti-TB drug treatments that will prevent resistance among *M. tuberculosis* Beijing genotype TB cases, and they suggest that the development of genotype-specific TB therapy might be justified.

Table 2. Concentration- and time-dependent bactericidal effect of rifampin toward *Mycobacterium tuberculosis* genotypes in low- and high-density cultures*

	Lowest RIF concentration resulting in killing of *M. tuberculosis*, mg/L							
	Beijing-1585 genotype				EAI-1627 genotype			
	≥99% killing		100% killing		≥99% killing		100% killing	
Day	Low†	High‡	Low†	High‡	Low§	High¶	Low§	High¶
1	8	ND	256	ND	8	ND	32	ND
3	1	0.008	64	1024	0.125	0.03	8	32
6	0.001	0.008	2	64	0.001	0.06	1	2

*Cultures were exposed to RIF at 2-fold increasing concentrations for 6 days at 37°C; at indicated time-points, subcultures were performed on solid media for counting. Low, low-density culture; high, high-density culture; ND, not determined.
†Density of 5.1 × 10^5 CFU/mL.
‡Density of 4.4 × 10^6 CFU/mL.
§Density of 6.8 × 10^5 CFU/mL.
¶Density of 3.0 × 10^6 CFU/mL.

Acknowledgments

We acknowledge RIVM for technical assistance and the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam, and the Pham Ngoc Thach Tuberculosis and Lung Disease Hospital, Ho Chi Minh City, Vietnam, for sharing their *M. tuberculosis* isolates.

Financial support was provided by Erasmus University Medical Center and RIVM.

Dr de Steenwinkel is a medical doctor, resident in training for medical microbiologist, and a PhD student in clinical microbiology and antimicrobial therapy at Erasmus University Medical Center. His interests include research on improving therapy for TB and fundamental exploration of resistance formation.

References

1. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response: 2010 global report on surveillance and response. 2010 Mar [cited 2011 Jun 11]. http://www.who.int/tb/publications/2010/9789245991 91/en/index.html
2. European Concerted Action on New Generation Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis. Beijing/W genotype *Mycobacterium tuberculosis* and drug resistance. Emerg Infect Dis. 2006;12:736–43. http://dx.doi.org/10.3201/eid1205.050400
3. Parwati I, van Crevel R, van Soolingen D. Possible underlying mechanisms for successful emergence of the *Mycobacterium tuberculosis* Beijing genotype strains. Lancet Infect Dis. 2010;10:103–11. http://dx.doi.org/10.1016/S1473-3099(09)70330-5
4. Buu TN, Huyen MN, Lan NN, Quy HT, Hen NV, Zignol M, et al. *Mycobacterium tuberculosis* genotype and case notification rates, rural Vietnam, 2003–2006. Emerg Infect Dis. 2009;15:1570–7.
5. Buu TN, Huyen MN, Lan NT, Quy HT, Hen NV, Zignol M, et al. The Beijing genotype is associated with young age and multidrug-resistant tuberculosis in rural Vietnam. Int J Tuberc Lung Dis. 2009;13:900–6.
6. van der Spuy GD, Kremer K, Ndabambi SL, Beyers N, Dunbar R, Marais BJ, et al. Changing *Mycobacterium tuberculosis* population highlights clade-specific pathogenic characteristics. Tuberculosis (Edinb). 2009;89:120–5. http://dx.doi.org/10.1016/j.tube.2008.09.003
7. Anh DD, Borgdorff MW, Van LN, Lan NT, van Gorkom T, Kremer K, et al. *Mycobacterium tuberculosis* Beijing genotype emerging in Vietnam. Emerg Infect Dis. 2000;6:302–5. http://dx.doi.org/10.3201/eid0603.000312
8. Cowley D, Govender D, February B, Wolfe M, Steyn L, Evans J, et al. Recent and rapid emergence of W-Beijing strains of *Mycobacterium tuberculosis* in Cape Town, South Africa. Clin Infect Dis. 2008;47:1252–9. http://dx.doi.org/10.1086/592575
9. Lan NT, Lien HT. Tung le B, Borgdorff MW, Kremer K, van Soolingen D. *Mycobacterium tuberculosis* Beijing genotype and risk for treatment failure and relapse, Vietnam. Emerg Infect Dis. 2003;9:1633–5.
10. Hershberg R, Lipatov M, Small PM, Sheffer H, Niemann S, Homolka S, et al. High functional diversity in *Mycobacterium tuberculosis* driven by genetic drift and human demography. PLoS Biol. 2008;6:e311. http://dx.doi.org/10.1371/journal.pbio.0060311
11. National Committee for Clinical Laboratory Standards. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard—second edition. CLSI document M24-A; 2011 [cited 2011 Feb 11]. http://www.clsi.org/source/orders/free/m24-a2.pdf
12. Gumbo T. New susceptibility breakpoints for first-line antituberculosis drugs based on antimicrobial pharmacokinetic/pharmacodynamic science and population pharmacokinetic variability. Antimicrob Agents Chemother. 2010;54:1484–91. http://dx.doi.org/10.1128/AAC.01474-09
13. Ebrahimi-Rad M, Bifani P, Martin C, Kremer K, Samper S, Rauzier J, et al. Mutations in putative mutator genes of *Mycobacterium tuberculosis* strains of the W-Beijing family. Emerg Infect Dis. 2003;9:838–45.
14. Werngren J. *Hoffner SE*. Drug-susceptible *Mycobacterium tuberculosis* Beijing genotype does not develop mutation-conferred resistance to rifampin at an elevated rate. J Clin Microbiol. 2003;41:1520–4. http://dx.doi.org/10.1128/JCM.41.4.1520-1524.2003
15. de Steenwinkel JE, de Knegt GJ, ten Kate MT, van Belkum A, Verbrugh HA, Kremer K, et al. Time-kill kinetics of anti-tuberculosis drugs, and emergence of resistance, in relation to metabolic activity of *Mycobacterium tuberculosis.* J Antimicrob Chemother. 2010;65:2582–9. http://dx.doi.org/10.1093/jac/dkq374

Address for correspondence: Jurriaan E.M. de Steenwinkel, Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, PO Box 2040, 3000 CA Rotterdam, the Netherlands; email: j.desteenwinkel@erasmusmc.nl

Ikoma Lyssavirus, Highly Divergent Novel Lyssavirus in an African Civet[1]

Denise A. Marston, Daniel L. Horton, Chanasa Ngeleja, Katie Hampson, Lorraine M. McElhinney, Ashley C. Banyard, Daniel Haydon, Sarah Cleaveland, Charles E. Rupprecht, Machunde Bigambo, Anthony R. Fooks, and Tiziana Lembo

Evidence in support of a novel lyssavirus was obtained from brain samples of an African civet in Tanzania. Results of phylogenetic analysis of nucleoprotein gene sequences from representative *Lyssavirus* species and this novel lyssavirus provided strong empirical evidence that this is a new lyssavirus species, designated Ikoma lyssavirus.

Eleven *Lyssavirus* species have been classified: *Rabies virus* (RABV), *Lagos bat virus* (LBV), *Mokola virus* (MOKV), *Duvenhage virus* (DUVV), *European bat lyssavirus* types -1 and -2, *Australian bat lyssavirus*, *Aravan virus*, *Khujand virus*, *Irkut virus*, and *West Caucasian bat virus* (WCBV) (*1*). All these viruses except MOKV have been detected in bats. Two newly identified lyssaviruses, Shimoni bat virus (SHIBV) (*2*) and Bokeloh bat lyssavirus (*3*), both detected in bats, have not yet been classified. The presence of numerous lyssaviruses in bat species has led to increasing research efforts toward lyssavirus discovery in bat populations globally. However, lyssavirus surveillance in terrestrial mammals remains limited across most of Africa.

Of the 13 lyssaviruses, 5 circulate in Africa (RABV, LBV, MOKV, DUVV, and SHIBV). LBV, MOKV, DUVV, and SHIBV are detected exclusively in Africa, whereas RABV is detected worldwide. The predominant RABV variants circulating in Africa are the mongoose and canine biotypes. In South Africa, canine RABV is considered to have been introduced in the eastern Cape Province after importation of an infected dog from England in 1892 and subsequently spread, infecting domestic and wild carnivores (*4*). Separate introductions of canine RABV (particularly in northern Africa) have been suggested (*5*). In addition, molecular clock analysis indicates that mongoose RABV was present in southern Africa ≈200 years before the introduction of canine RABV (*6*).

In Tanzania, canine RABV is endemic and widespread throughout the country. In the Serengeti ecosystem, detailed studies have shown a single variant of canine RABV circulating in multiple host species (*7*). However, annual mass rabies vaccination campaigns have been conducted for dogs in villages surrounding Serengeti National Park since 2003, and rabies has not been detected in the park since 2000 (*8*). Enhanced laboratory-based surveillance in support of this canine rabies elimination program has been running concurrently in the region.

The Study

On May 11, 2009, an African civet (*Civettictis civetta*) displaying clinical signs consistent with rabies was killed by rangers in Ikoma Ward within Serengeti National Park (Figure 1). Rangers were contacted because the civet had bitten a child on the right leg in an unprovoked attack. The wound was washed with soap and water, and the child received postexposure rabies vaccination but no rabies immunoglobulin. Brain samples from the civet were tested multiple times (as part of a training course) at the Central Veterinary Laboratory in Tanzania. Results of the fluorescent antibody test and a direct rapid immunohistochemistry test were positive for lyssavirus-specific antigen. When testing was complete, the samples were sent to the Animal Health and Veterinary Laboratories Agency (AHVLA, Weybridge, UK) for additional confirmation of results and molecular analysis.

RNA was extracted by using TRIzol Reagent (Invitrogen, Paisley, UK), and a pan-lyssavirus reverse transcription PCR yielded a specific 606-bp amplicon (*9*). The amplicon (GenBank accession no. JN800509) was sequenced by using standard primers and protocols (*10*). Bayesian reconstructions were used for phylogenetic analysis of the nucleoprotein gene region (405 bp) and included representatives of all species from the lyssavirus genus; results showed that the sequence was unique and most closely related to WCBV (Figure 2). A canine RABV biotype from Tanzania and a mongoose RABV biotype from southern Africa were included in the dataset. Nucleotide comparisons indicated similar divergence from all

Author affiliations: Animal Health and Veterinary Laboratories Agency, Addlestone, UK (D.A. Marston, D.L. Horton, L.M. McElhinney, A.C. Banyard, A.R. Fooks); Central Veterinary Laboratory, Dar es Salaam, Tanzania (C. Ngeleja); University of Glasgow, Glasgow, Scotland, UK (K. Hampson, D. Haydon, S. Cleaveland, T. Lembo); National Consortium for Zoonosis Research, Neston, UK (L.M. McElhinney, A. R. Fooks); Centers for Disease Control and Prevention, Atlanta, Georgia, USA (C.E. Rupprecht); and Lincoln Park Zoo Tanzania Program, Arusha, Tanzania (M. Bigambo)

DOI: http://dx.doi.org/10.3201/eid1804.111553

[1]These data were presented in part at the XXII Rabies in the Americas meeting in San Juan, Puerto Rico, October 16–21, 2011.

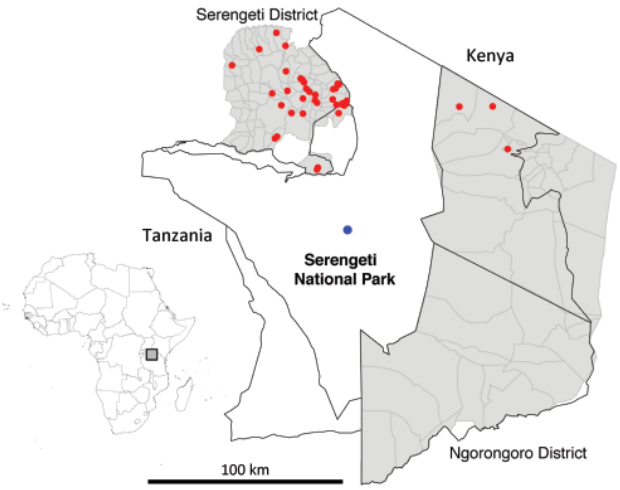


Figure 1. Serengeti National Park and surrounding districts (Serengeti and Ngorongoro). Blue dot indicates location of Ikoma lyssavirus–infected African civet within Ikoma Ward in northwest Tanzania. Red dots indicate cases of rabies confirmed during 2003–2011. Top left, map of Africa indicating study area in Tanzania (gray box).

lyssavirus species (minimum identity 62.2% *Australian bat lyssavirus*, maximum identity 68.6% WCBV), including 12 canine RABV sequences from domestic and wild animals in the Serengeti ecosystem (64.1%–65.1% identity). The posterior probabilities indicated that the IKOV and WCBV grouping was strongly supported, despite low sequence identity. Further phylogenetic analysis of representatives from other rhabdoviruses demonstrated that IKOV is a member of the *Lyssavirus* genus (41.6%–50.9% identity to representative rhabdovirus sequences that are available for this region of the genome) (data not shown).

Conclusions

We describe evidence, based on genomic sequences obtained from the brain sample of an African civet with clinical signs consistent with rabies, for the existence of a novel lyssavirus designated IKOV (Ikoma lyssavirus). The sample was frozen and thawed several times before being sent to AHVLA, which had a detrimental effect on the sample quality and resulted in viral RNA degradation and loss in viral viability. The results of confirmatory FATs performed at AHVLA were inconclusive, and attempts to isolate virus by using the rabies tissue culture inoculation test and the mouse inoculation test were unsuccessful. Despite the lack of isolated virus, the pan-lyssavirus sensitivity and specificity of the fluorescent antibody test (the test prescribed by the World Organisation for Animal Health as the standard for rabies testing) and direct rapid immunohistochemistry test support the assertion that a novel lyssavirus exists in the region. In addition, despite the poor quality of the sample, molecular techniques identified a lyssavirus-specific amplicon that was confirmed to be unique by phylogenetic analysis and to be highly divergent from known circulating RABV strains. A real-time PCR also detected this unique lyssavirus sequence, confirming that both molecular tests are pan-lyssavirus specific and are sufficient for the detection of highly divergent novel lyssaviruses (*11*).

The child who was bitten by the African civet received appropriate wound care and postexposure rabies vaccination. At the time of this report, the child remained well. We cannot, however, draw any conclusions as to whether the African civet was shedding virus when it bit the child or whether postexposure vaccinations are effective against IKOV.

This case of rabies in an African civet in the center of Serengeti National Park was highly unexpected. Since

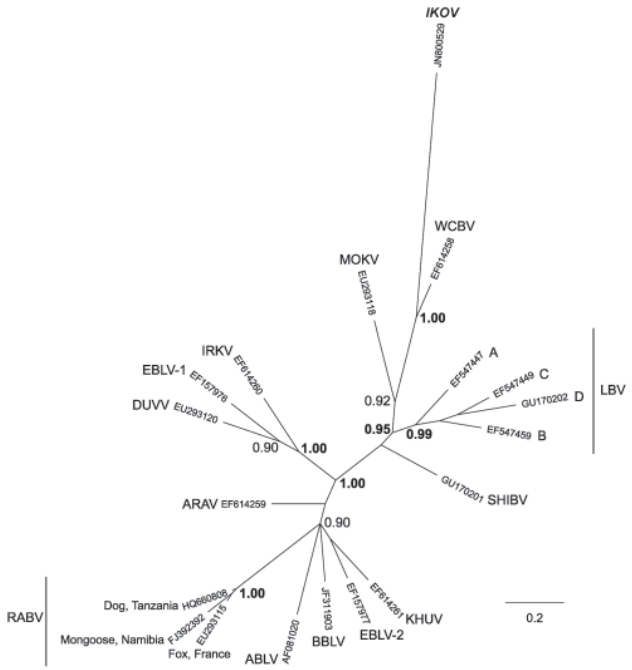


Figure 2. Phylogenetic relationships between all currently identified lyssaviruses compared with Ikoma lyssavirus (IKOV; shown in ***boldface italics***), as determined on the basis of partial nucleoprotein gene sequences (405 bp). Relationships are presented as an unrooted phylogram based on Bayesian Markov chain Monte Carlo analysis. Posterior probability values represent the degree of support for each node on the tree: only values ≥0.90 are shown; values ≥0.95 are shown in **boldface**. Scale bar indicates branch length, expressed as the expected number of substitutions per site. ARAV, Aravan virus; ABLV, Australian bat lyssavirus; BBLV, Bokeloh bat lyssavirus; DUVV, Duvenhage virus; EBLV-1 and EBLV-2, European bat lyssavirus type 1 and 2; IRKV, Irkut virus; KHUV, Khujand virus; LBV, Lagos bat virus (lineages A, B, C and D); MOKV, Mokola virus; RABV, rabies virus; SHIBV, Shimoni bat virus; WCBV, West Caucasian bat virus. Complete methods are described online (wwwnc.cdc.gov/EID/article/18/4/11-1553-F2.htm).

2000, the park had been free of rabies and no cases had been detected within a 30-km radius. This case of rabies in wildlife implied a major breach in the vaccination program. However, subsequent molecular characterization demonstrated that this case of rabies had not been caused by a RABV from a canine source. Thus, a breach had not occurred; instead, a novel lyssavirus with an unknown reservoir had caused the infection. Previously published data on lyssavirus infection in African civets (n = 6) was restricted to the RABV mongoose lineage (*12*). Although African civets can be infected with RABV and IKOV, infrequent detection of lyssaviruses in this species suggests that they are more likely to be incidental hosts. The nocturnal, opportunistic foraging behaviors of African civets imply that contact with bats is possible, particularly at roosts where interactions with a grounded rabid bat are more likely to occur. In the absence of virus isolates, the origin of IKOV is difficult to determine. Surveillance for rabies in bats and other mammals in Tanzania and typing of all lyssavirus-positive samples is necessary to determine the distribution and prevalence of IKOV.

The detection of WCBV cross-reacting neutralizing antibodies in gregarious *Miniopterus* spp. bats in neighboring Kenya could be informative, given the strong posterior probability values on the grouping of IKOV and WCBV in the Bayesian analysis (Figure 2) (*13*). Additional genomic and evolutionary analysis is underway to support IKOV as a new *Lyssavirus* species, potentially grouping with WCBV in phylogroup III (*14*), and to determine the antigenic diversity of the glycoprotein (*15*). Given that IKOV is highly distinct from RABV (more genetically distinct than WCBV from RABV) and that a human has been bitten by an infected animal, the effectiveness of current rabies vaccines needs to be further investigated.

Acknowledgments

We are grateful to the Tanzania Wildlife Research Institute, Tanzania Commission for Science and Technology, Tanzania National Parks, the Ngorongoro Conservation Area Authority, and the National Institute for Medical Research for permissions. We thank Tanzania National Parks, Tanzania Wildlife Research Institute, and Frankfurt Zoological Society for field support and the Ministry of Livestock and Fisheries Development for laboratory support in Tanzania. We also thank Sarah Devery, Stacey Leech, Emma Wise, Colin Black, Lillian Orciari, and Michael Niezgoda for their technical support.

This work was partially supported by The Department for Environment, Food and Rural Affairs ROAME SE0423/SV3500, by the EU FP7–funded Research Infrastructure Grant European Virus Archive (no. 228292), and by the Research and Policy for Infectious Disease Dynamics program of the Science and Technology Directorate (US Department of Homeland Security), and the Fogarty International Center. In Tanzania, the work was funded by Lincoln Park Zoo (Chicago, IL, USA), the Wellcome Trust, UBS Optimus Foundation, and the Medical Research Council.

Mrs Marston is a research scientist at AHVLA. Her research interests include viral genetics, molecular epidemiology, and virus–host interactions.

References

1. Dietzgen R, Calisher CH, Kurath G, Kuzmin IV, Rodriguez LL, Stone DM, et al. *Rhabdoviridae*. In: King A, Adams MJ, Carstens EB, Lefkowitz, EJ, editors. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. San Diego (CA): Elsevier; 2011. p. 654–81.
2. Kuzmin IV, Mayer AE, Niezgoda M, Markotter W, Agwanda B, Breiman RF, et al. *Shimoni bat virus*, a new representative of the *Lyssavirus* genus. Virus Res. 2010;149:197–210. http://dx.doi.org/10.1016/j.virusres.2010.01.018
3. Freuling CM, Beer M, Conraths FJ, Finke S, Hoffmann B, Keller B, et al. Novel lyssavirus in Natterer's bat, Germany. Emerg Infect Dis. 2011;17:1519–22.
4. Swanepoel R, Barnard BJ, Meredith CD, Bishop GC, Bruckner GK, Foggin CM, et al. Rabies in southern Africa. Onderstepoort J Vet Res. 1993;60:325–46.
5. Talbi C, Lemey P, Suchard MA, Abdelatif E, Elharrak M, Nourlil J, et al. Phylodynamics and human-mediated dispersal of a zoonotic virus. PLoS Pathog. 2010;6:e1001166. http://dx.doi.org/10.1371/journal.ppat.1001166
6. Van Zyl N, Markotter W, Nel LH. Evolutionary history of African mongoose rabies. Virus Res. 2010;150:93–102. http://dx.doi.org/10.1016/j.virusres.2010.02.018
7. Lembo T, Haydon DT, Velasco-Villa A, Rupprecht CE, Packer C, Brandao PE, et al. Molecular epidemiology identifies only a single rabies virus variant circulating in complex carnivore communities of the Serengeti. Proc Biol Sci. 2007;274:2123–30. http://dx.doi.org/10.1098/rspb.2007.0664
8. Lembo T, Hampson K, Haydon DT, Craft M, Dobson A, Dushoff J, et al. Exploring reservoir dynamics: a case study of rabies in the Serengeti ecosystem. J Appl Ecol. 2008;45:1246–57. http://dx.doi.org/10.1111/j.1365-2664.2008.01468.x
9. Heaton PR, Johnstone P, McElhinney LM, Cowley R, O'Sullivan E, Whitby JE. Heminested PCR assay for detection of six genotypes of rabies and rabies-related viruses. J Clin Microbiol. 1997;35:2762–6.
10. Hayman DT, Johnson N, Horton DL, Hedge J, Wakeley PR, Banyard AC, et al. Evolutionary history of rabies in Ghana. PLoS Negl Trop Dis. 2011;5:e1001. http://dx.doi.org/10.1371/journal.pntd.0001001
11. Hayman DT, Banyard AC, Wakeley PR, Harkess G, Marston D, Wood JL, et al. A universal real-time assay for the detection of *Lyssaviruses.* J Virol Methods. 2011;177:87–93. http://dx.doi.org/10.1016/j.jviromet.2011.07.002
12. Sabeta CT, Shumba W, Mohale DK, Miyen JM, Wandeler AI, Nel LH. Mongoose rabies and the African civet in Zimbabwe [letter]. Vet Rec. 2008;163:580. http://dx.doi.org/10.1136/vr.163.19.580
13. Kuzmin IV, Niezgoda M, Franka R, Agwanda B, Markotter W, Beagley JC, et al. Possible emergence of West Caucasian bat virus in Africa. Emerg Infect Dis. 2008;14:1887–9. http://dx.doi.org/10.3201/eid1412.080750
14. Fooks A. The challenge of new and emerging lyssaviruses. Expert Rev Vaccines. 2004;3:333–6. http://dx.doi.org/10.1586/14760584.3.4.333

15. Horton DL, McElhinney LM, Marston DA, Wood JL, Russell CA, Lewis N, et al. Quantifying antigenic relationships among the lyssaviruses. J Virol. 2010;84:11841–8. http://dx.doi.org/10.1128/JVI.01153-10

Address for correspondence: Anthony R. Fooks, Wildlife Zoonoses and Vector-borne Diseases Research Group, Animal Health and Veterinary Laboratories Agency, New Haw, Addlestone, Surrey, KT15 3NB, UK; email: tony.fooks@ahvla.gsi.gov.uk

Coccidioides posadasii Infection in Bats, Brazil

Rossana de Aguiar Cordeiro, Kylvia Rocha de Castro e Silva, Raimunda Sâmia Nogueira Brilhante, Francisco Bergson Pinheiro Moura, Naylê Francelino Holanda Duarte, Francisca Jakelyne de Farias Marques, Rebecca de Aguiar Cordeiro, Renato Evando Moreira Filho, Roberto Wagner Bezerra de Araújo, Tereza de Jesus Pinheiro Gomes Bandeira, Marcos Fábio Gadelha Rocha, and José Júlio Costa Sidrim

To analyze the eco-epidemiologic aspects of *Histoplasma capsulatum* in Brazil, we tested 83 bats for this fungus. Although *H. capsulatum* was not isolated, *Coccidioides posadasii* was recovered from *Carollia perspicillata* bat lungs. Immunologic studies detected coccidioidal antibodies and antigens in *Glossophaga soricina* and *Desmodus rotundus* bats.

Studies have demonstrated that bats (order Chiroptera) are reservoirs for many infectious agents, including protozoa, bacteria, viruses, and fungi (*1*). Several studies confirm that bats have a great effect on human health because they can transmit numerous infectious agents and provide a reservoir for emerging pathogens (*1*,*2*). The interaction between these animals and pathogenic fungi is well illustrated by the occurrence of histoplasmosis outbreaks in humans who are exposed to bat droppings in the environment (*3*,*4*). In Brazil, histoplasmosis is an endemic disease that occurs mainly in patients with AIDS (*5*), but *Histoplasma capsulatum* var. *capsulatum* has also been isolated from bats captured in urban areas (*4*).

To analyze the eco-epidemiologic aspects of *H. capsulatum* in northeast Brazil, we captured bats from urban and rural areas of Ceará State. However, the research revealed the existence of a bat that was naturally infected with *Coccidioides posadasii* and 2 other chiropterans with coccidioidal immunologic responses. This fungal pathogen can cause coccidioidomycosis, a serious infection in humans and animals. The mycosis is presently considered to be endemic to Northeast Brazil, as evidenced by human autochthonous cases (*6*–*8*), positive coccidioidin skin-test results (*7*), and isolation of the fungus from soil (*7*,*9*). We describe the isolation of *C. posadasii* in bats and discuss the epidemiologic effects of this finding.

Author affiliations: Universidade Federal do Ceará, Fortaleza-Ceará, Brazil (R.A. Cordeiro, K.R.C. Silva, R.S.N. Brilhante, F.J.F. Marques, R.A. Cordeiro, R.E. Moreira Filho, R.W.B. Araújo, T.J.P.G. Bandeira, M.F.G. Rocha, J.J.C. Sidrim); Instituto Federal de Educação, Ciência e Tecnologia, Ceará, Brazil (K.R.C. Silva) Universidade Estadual do Ceará, Fortaleza- Ceará (M.F.G. Rocha); and Secretaria da Saúde do Estado do Ceará, Ceará (F.B.P. Moura, N.F.H. Duarte)

DOI: http://dx.doi.org/10.3201/eid1804.111641

The Study

From August 2010 to March 2011, a total of 83 bats of 7 species were captured in 6 cities in Ceará State, Northeast Brazil, where patients with histoplasmosis are seen: Ubajara, Itapiúna, Quixadá, Russas, Aracoiaba, and Baturité. The animals were captured during the day (nonhematophagous bats) or night (hematophagous bats) by using nylon mist nets with 36-mm mesh. The study was part of the rabies control surveillance program headed by the Ceará State Health Department and was approved by the ethics committee of the State University of Ceará (process 07381395–8).

Immediately after capture, the bats were euthanized by an overdose of diethyl ether by inhalation, and their spleen, liver, and lungs were analyzed for *H. capsulatum* isolation. Fragments of each organ were homogenized by maceration in saline supplemented with 200 mg/L chloramphenicol. Aliquots of 100 μL were seeded onto plates containing brain–heart infusion agar, supplemented with 1% glucose, 0.1% L-cysteine, 200 mg/L chloramphenicol, and 0.05% cycloheximide, and incubated at 25°C or 35°C for as long as 6 weeks (*10*). Remaining aliquots of each homogenate, as well as organ fragments, were kept at –20°C.

Although none of the samples were positive for *H. capsulatum*, a colony (Figure) suggestive of *Coccidioides* spp. (Figure, panel A) was isolated from lung homogenates (incubated at 35°C) from *Carollia perspicillata* bats. Microscopic analysis showed hyaline septate hyphae and arthroconidia alternating with empty disjunctor cells (Figure, panel B). Lung fragments from the infected bat were then removed from storage and examined by direct microscopy, revealing coccidioidal spherules (Figure, panel C). The suspected *Coccicioides* colony was evaluated through the in vivo reversion test (*9*). In brief, 5 mL of 0.9% saline was added to a well-sporulating slant culture (15 days old) that was then gently scraped with a cotton swab. Two mice were injected intraperitoneally with 1 mL of the homogeneous supernatant and then were held under biosafety level 3 conditions for 4 weeks. After this period, the animals were euthanized and their spleen, liver, and lungs were removed. Fragments were examined for coccidioidal spherules by direct microscopy with 10% potassium hydroxide and

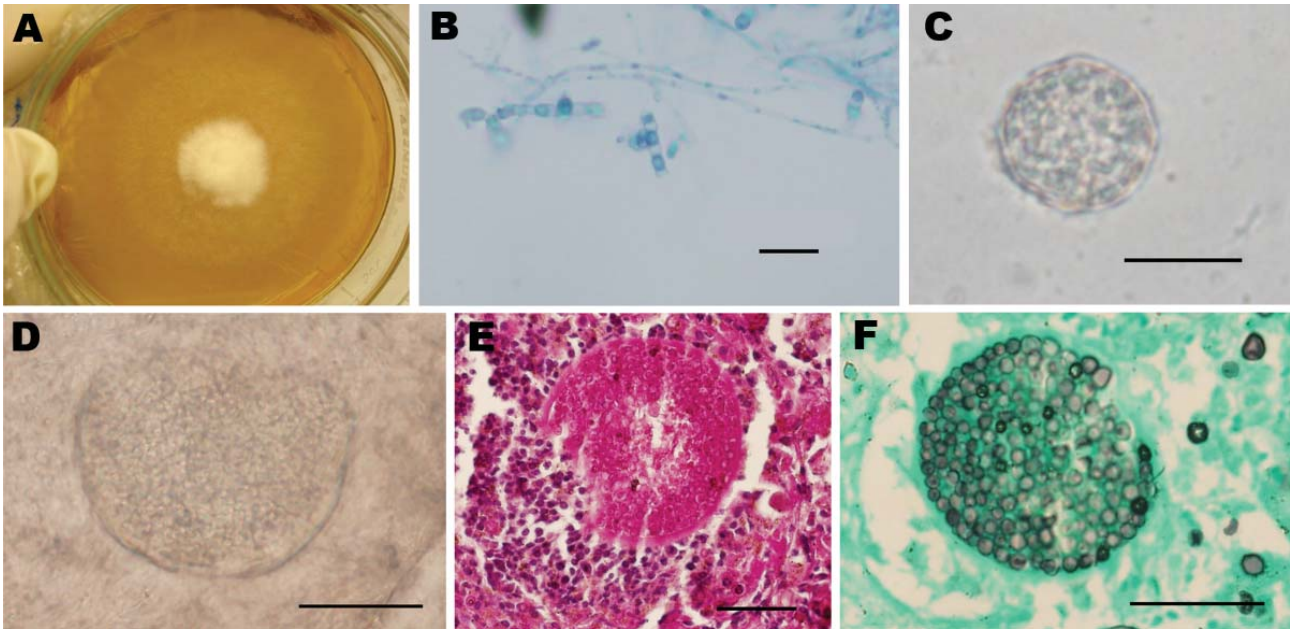


Figure. Coccidioidal structures obtained from a naturally infected *Carollia perspicillata* bat (upper images) and experimentally infected mice (lower images). A) Macroscopic aspect of *Coccidioides posadasii* culture recovered from homogenate of bat lungs. B) Microscopic view of *C. posadasii* culture from bat lungs showing hyaline hyphae with arthroconidia and disjunctor cells (lactophenol cotton blue staining). C) Mature spherule filled with endospores in lung tissue (10% KOH) of bat. D) Bursting spherule with endospores in mouse lung tissue (10% KOH). E) Histopathologic features of mouse lungs revealing parasitic coccidioidal forms by periodic acid-Schiff staining. F) Coccidioidal forms on mouse lungs shown by Grocott-Gomori methenamine-silver staining. Scale bars = 20 μm

also cultured on BBL Mycosel Agar (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Additional histopathologic analyses of each organ were performed. Spherules with endospores were found in the lungs of the infected animals (Figure, panel D), and histopathologic analysis supported the identification of *Coccidioides* spp. (Figures 1, panels E, F). Fragments of the spleen, liver, and lungs cultured on Mycosel Agar yielded mold colonies that produced typical coccidioidal arthroconidia. An additional test was performed by specific PCR reaction (*11*) with Coi9–1F (5′-TACGGTGTAATCCCGATACA-3′) and Coi9–1R (5′-GGTCTGAATGATCTGACGCA-3′) primers to confirm the identity of the pathogen. A voucher of the fungal strain was deposited in the Specialized Medical Mycology Center Culture Collection at the Federal University of Ceará under code CEMM 05–5-059.

Homogenates of lungs, spleen, and liver of all bats were removed from storage and assayed by immunodiffusion tests specific for *H. capsulatum* and *C. posadasii* antigens (*12*) (ID Antigen H & M and IDCF Antigen; Immy Immunodiagnostics, Inc., Norman, OK, USA) according to the manufacturer's instructions. None of the homogenates showed positive reactions in *H. capsulatum* immunodiffusion tests. However, positive antibodies against *Coccidioides* spp. were found in 1 sample of lung from *Glossophaga soricina* bats. Positive antigen reactions were seen in homogenate liver samples from 2 animals, identified as *G. soricina* and *Desmodus rotundus* bats. These results suggest natural coccidioidal infection among the animals evaluated.

Positive *C. perspicillata* and *G. soricina* bats were captured in the same place, a deserted house in the urban area of Aracoiaba (4°21′59.1″S and 38°48′51.9″W) that has a semi-arid climate, with a rainy season from February through April and an average rainfall of 1,010.3 mm per year. The vampire bat, *D. rotundus,* was captured inside a cave in Ubajara (3°48′14.3″S and 40°52′46.2″W), a city characterized by a warm, subhumid tropical climate, with a rainy period from January through April and rainfall of 1,483.5 mm per year.

Conclusions

Coccidioides spp. can infect many mammal species (*13*). In this study, *C. posadasii* was isolated from the lungs of *C. perspicillata* bats, a colonial species that can cohabitate with different species of chiropterans (*14*,*15*). We propose 3 hypotheses for this finding. First, we hypothesize that the ability to travel long distances daily in their search for food and the social behavior of chiropterans may promote the acquisition and dispersion of *C. posadasii*. As a result, infected bats may have migrated from areas where *Coccidioides* infections are endemic and introduced the fungus in previously non–disease-endemic areas. A second hypothesis involves the possible existence

of other animals that cohabitate with bats in artificial or natural shelters as the primary source of *C. posadasii* infections. Our third hypothesis is that climate changes in recent decades, mainly the increasing temperature in South America, along with the desertification process, which affects approximately one third of Ceará State, might have contributed to this unusual finding. Hypothetical links between climate changes and the epidemiology of other fungal diseases have been described. Studies need to be performed to investigate the role of chiropterans in the epidemiologic cycle of coccidioidomycosis.

Acknowledgments

We thank the Secretaria de Saúde do Estado do Ceará for technical support.

This work was supported by grants from the National Scientific and Technological Development Council (CNPq–process no. 306637/2010-3 and Programa de Capacitação em Taxonomia 562296/2010-7).

Dr Cordeiro is professor of medical microbiology at Universidade Federal do Ceará, Brazil. Her research focuses on pathogenic fungi in humans and animals.

References

1. Wibbelt G, More MS, Schountz T, Voigt CC. Emerging diseases in Chiroptera: why bats? Biol Lett. 2010;6:438–40. http://dx.doi.org/10.1098/rsbl.2010.0267
2. Chaturvedi V, Springer DJ, Behr MJ, Ramani R, Li X, Peck MK, et al. Morphological and molecular characterizations of psychrophilic fungus *Geomyces destructans* from NewYork bats with white nose syndrome (WNS). PLoS ONE. 2010;5:e10783. http://dx.doi.org/10.1371/journal.pone.0010783
3. Emmons CW, Klite PD, Baer GM, Hill WB Jr. Isolation of *Histoplasma capsulatum* from bats in the United States. Am J Epidemiol. 1966;84:103–9.
4. Galvão Dias MA, Zancopé Oliveira RM, Giudice MC, Montenegro Netto H, Jordão LR, Grigorio IM, et al. Isolation of *Histoplasma capsulatum* from bats in the urban area of São Paulo State, Brazil. Epidemiol Infect. 2010. Epub ahead of print.
5. Daher EF, Silva GB Jr, Barros FAS, Takeda CFV, Mota RMS, Ferreira MT, et al. Clinical and laboratory features of disseminated histoplasmosis in HIV patients from Brazil. Trop Med Int Health. 2007;12:1108–15. http://dx.doi.org/10.1111/j.1365-3156.2007.01894.x
6. Sidrim JJC, Silva LCI, Nunes JMA, Rocha MFG, Paixão GC. Le nord-est Brésilien, région d'endémie de coccidioïdomycose? J Mycol Med. 1997;7:37–9.
7. Wanke B, Lazera M, Monteiro PC, Lima FC, Leal MJ, Ferreira Filho PL, et al. Investigation of an outbreak of endemic coccidioidomycosis in Brazil's northeastern state of Piauí with a review of the occurrence and distribution of *Coccidioides immitis* in three other Brazilian states. Mycopathologia. 1999;148:57–67. http://dx.doi.org/10.1023/A:1007183022761
8. Cordeiro RA, Brilhante RSN, Rocha MFG, Bandeira SP, Fechine MAB, Camargo ZP, et al. Twelve years of coccidioidomycosis in Ceará State, Northeast Brazil: epidemiologic and diagnostic aspects. Diagn Microbiol Infect Dis. 2010;66:65–72. http://dx.doi.org/10.1016/j.diagmicrobio.2008.09.016
9. Cordeiro RA, Brilhante RSN, Rocha MFG, Fechine MAB, Camara LMC, Camargo ZP, et al. Phenotypic characterization and ecological features of *Coccidioides* spp. from Northeast Brazil. Med Mycol. 2006;44:631–9. http://dx.doi.org/10.1080/13693780600876546
10. Taylor ML, Chávez-Tapia CB, Vargas-Yañez R, Rodríguez-Arellanes G, Peña-Sandoval GR, Toriello C, et al. Environmental conditions favoring bat infection with *Histoplasma capsulatum* in Mexican shelters. Am J Trop Med Hyg. 1999;61:914–9.
11. Umeyama T, Sano A, Kamei K, Niimi M, Nishimura K, Uehara Y. Novel approach to designing primers for identification and distinction of the human pathogenic fungi *Coccidioides immitis* and *Coccidioides posadasii* by PCR amplification. J Clin Microbiol. 2006;44:1859–62. http://dx.doi.org/10.1128/JCM.44.5.1859-1862.2006
12. Brilhante RSN, Cordeiro RA, Rocha MFG, Fechine MAB, Furtado FM, Nagao-Dias AT, et al. Coccidioidal pericarditis: a rapid presumptive diagnosis by an in-house antigen confirmed by mycological and molecular methods. J Med Microbiol. 2008;57:1288–92. http://dx.doi.org/10.1099/jmm.0.2008/002428-0
13. Laniado-Laborin R. Expanding understanding of epidemiology of coccidioidomycosis in the Western Hemisphere. Ann N Y Acad Sci. 2007;1111:19–34. http://dx.doi.org/10.1196/annals.1406.004
14. Cloutier D, Thomas DW. Carollia perspicillata. Mamm Species. 1992;417:1–9.
15. Alvarez J, Willing MR, Jones JK Jr, Webster D. Glossophaga soricina. Mamm Species. 1991;379:1–7. http://dx.doi.org/10.2307/3504146

Address for correspondence: Rossana Cordeiro, 10 50 Av. Domingos Olimpio, Apto. 501, Bairro Jose Bonifacio, CEP 60040-080, Fortaleza, Ceará, Brazil; email: rossanacordeiro@ufc.br

Surveillance for West Nile, Dengue, and Chikungunya Virus Infections, Veneto Region, Italy, 2010

Federico Gobbi, Luisa Barzon, Gioia Capelli, Andrea Angheben, Monia Pacenti, Giuseppina Napoletano, Cinzia Piovesan, Fabrizio Montarsi, Simone Martini, Roberto Rigoli, Anna M. Cattelan, Roberto Rinaldi, Mario Conforto, Francesca Russo, Giorgio Palù, Zeno Bisoffi, and the Veneto Summer Fever Study Group[1]

In 2010, in Veneto Region, Italy, surveillance of summer fevers was conducted to promptly identify autochthonous cases of West Nile fever and increase detection of imported dengue and chikungunya in travelers. Surveillance highlighted the need to modify case definitions, train physicians, and when a case is identified, implement vector control measures

In 2010, a special surveillance for West Nile virus (WNV), dengue virus (DENV), and chikungunya virus (CHIKV) was initiated in the Veneto Region of northeastern Italy. The surveillance had 2 main objectives. First, we aimed to increase the detection rate of imported chikungunya and dengue in travelers from areas to which these diseases are endemic, including in new immigrants and settled immigrants visiting relatives and friends, and to promptly identify potential autochthonous cases. Second, we aimed to detect autochthonous cases of West Nile fever (WNF) and West Nile neuroinvasive disease (WNND), which were already included in regular surveillance, to acquire a more reliable picture of disease transmission in the region.

Author affiliations: Ospedale Sacro Cuore-Don Calabria, Negrar, Verona, Italy (F. Gobbi, A. Angheben, Z. Bisoffi); Università di Padova, Padua, Italy (L. Barzon, M. Pacenti, G. Palù); Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua (G. Capelli, F. Montarsi); Unità Locale Sanitaria 20–Regione Veneto, Verona (G. Napoletano); Unità Locale Sanitaria 9–Treviso, Treviso, Italy (C. Piovesan); Entostudio, Brugine, Padua (S. Martini); Ospedale Cà Foncello, Treviso (R. Rigoli); Ospedale di Rovigo, Rovigo, Italy (A. M. Cattelan); Ospedale di Padova, Padua (R. Rinaldi); Ospedale San Bortolo, Vicenza, Italy (M. Conforto); and Regione Veneto–Servizio Promozione e Sviluppo Igiene e Sanità Pubblica, Venice, Italy (F. Russo)

DOI: http://dx.doi.org/10.3201/eid1804.110753

The Study

In accordance with the study protocol, possible cases detected by general physicians and emergency department physicians had to be referred within 24 hours to the closest Unit of Infectious or Tropical Diseases. Serum samples from persons with possible cases were sent to the regional reference laboratory (Padua, Italy) for confirmation. If neuroinvasive disease was present, the specific protocol for WNND was followed (*1*).

We defined a possible case of DENV or CHIKV infection as fever ≥38°C during the past 7 days in a traveler who had returned within the previous 15 days from countries to which these viruses are endemic, absence of leucocytosis (leukocyte count <10,000 μL), and absence of other obvious causes of fever. After malaria was ruled out, cases were further classified as probable if rapid tests yielded positive results for dengue and chikungunya viruses. Rapid tests included detection of anti-CHIKV IgM by using the OnSite Chikungunya IgM Combo Rapid Test (CTK Biotech, Inc., San Diego, CA, USA) and of DENV nonstructural protein (NS) 1 antigen by using the Dengue NS1 Ag STRIP (Bio-Rad Laboratories, Hercules, CA, USA) on serum samples. Samples from persons with possible cases were sent to the regional reference laboratory for second-line laboratory testing and confirmation. Second-line laboratory testing consisted of detection of DENV and CHIKV nucleic acids in plasma specimens by using real-time PCR and endpoint PCR, respectively, and detection of serum IgM and IgG by using an anti-CHIKV indirect immunofluorescence assay (Euroimmun AG, Lübeck, Germany), DENV IgG DxSelect (Focus Diagnostics, Cypress, CA, USA), and DENV IgM Capture DxSelect (Focus Diagnostics). Samples with DENV -positive results by ELISA were further tested by plaque-reduction neutralization test to confirm specificity of antibody response. Confirmed cases were defined as the presence of viral nucleic acid in blood specimens or by seroconversion or detection of increasing serum levels of specific IgM and IgG. Possible autochthonous cases of WNF were defined as fever ≥38°C for ≤7 days, age ≥15 years, no recent travel history, rash, and absence of other obvious causes of fever (Figure 1).

In instances of high clinical suspicion for DENV and CHIKV in patients with autochthonous fever, laboratory tests for these 2 diseases also were performed. Moreover,

[1]Additional members of the Veneto Summer Fever Study Group who contributed data: Margherita Cattai, Ercole Concia, Riccardo Cusinato, Roberto Ferretto, Ermenegildo Francavilla, Elisa Franchin, Giampietro Pellizzer, Francesca Pozza, Enzo Raise, Pierangelo Rovere, Piergiorgio Scotton, and Filippo Viviano.

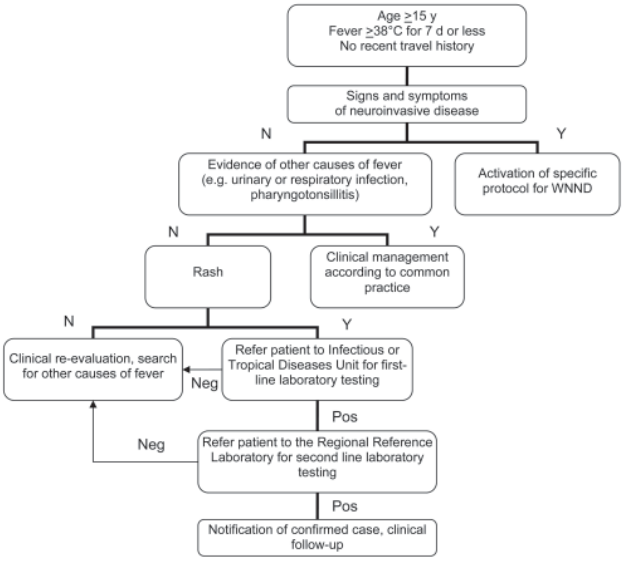


Figure 1. Algorithm for detection of possible cases of West Nile fever, Veneto Region, Italy, 2010. N, no; Y, yes; WNND, West Nile neuroinvasive disease; neg, negative; pos, positive.

all travelers tested for DENV and CHIKV also were tested for WNV.

Of 79 possible cases, we detected 14 cases of DENV infection and 1 case of CHIKV infection among travelers with fever (Table; online Appendix Table, wwwnc.cdc.gov/EID/article/18/4/11-0753-TA1.htm). No cases were severe.

Four (11%) of 38 possible cases of autochthonous WNF were confirmed. All were positive for WNV IgM and/or IgG and confirmed by plaque-reduction neutralization test, but none were WNV RNA positive. Clinical descriptions of WNF and WNND cases are reported elsewhere (*1*).

Conclusions

DENV, CHIKV, and WNV infections are arboviral diseases that find potentially suitable vectors in Italy, particularly in Veneto. No autochtonous case of fever caused by DENV has been documented in Italy, but the possible role of the *Aedes albopictus* mosquito as a vector has been demonstrated by recent cases in France (*2*) and Croatia (*3*).

CHIKV caused the well-known outbreak in Emilia Romagna Region (northern Italy) in 2007, which was detected, by coincidence a few days after the imported cases in Italy had been reported (*4*); the published report concluded that "the possibility of introducing CHIKV into Italy cannot be ruled out on the basis of current evidence." The index case had occurred ≈2 months before the first case was diagnosed (*5*). The recent occurrence of 2 locally transmitted cases of chikungunya in France, despite a low number of imported cases (*6*), shows that the risk remains high.

Since summer 2008, WNV has caused WNND in humans, first in Emilia Romagna Region (*7*), then in Veneto Region (*8*). In contrast, the more common presentation, WNF, has been detected in only 1 patient; the casewas identified retrospectively (*9*,*10*), despite the expected WNF:WNND ratio of 20:1 (*11*).

Because we were concerned about being overwhelmed by an unmanageable number of case reports of unspecific fevers, we chose a selective case definition, particularly for WNF, with the obligatory presence of a rash, and thereby lowered the sensitivity of the surveillance. However, the proportion of virus-positive patients was strikingly high: ≈20% of persons tested who had imported fever were positive for DENV or CHIKV, as were 10% of persons with locally acquired fevers for WNV. Compared with the 2 previous years, the special surveillance enabled detection of substantially more cases, showing that you only find what you are looking for (Table). WNV circulation has now been documented in many areas of Italy, from north to south, through retrospective screening of solid organ donors (*12*) and through entomologic (*13*) and animal surveillance (*14*); nevertheless, in 2010, no human clinical cases were detected outside Veneto.

The success of this pilot phase prompted regional authorities to propose a 3-year plan, which the Ministry of Health has approved and funded, as part of the integrated surveillance of arboviral diseases, along with animal and entomologic surveillance. Relying only on the latter 2 would not be sensible. However, mosquito surveillance was able to predict cases in animals and humans (Figure 2). Expected rates of WNV infection in mosquitoes at the only site with repeated positivity in animals, humans, and vectors (Venice Province) are shown together with the time of exposure of animals and humans in the same province. Time of exposure was estimated as 1 week before onset of symptoms (incubation range 2–14 days) (*15*). When the expected rate of mosquito infection was low (i.e., 0.06%), no clinical cases were recorded; when the expected

Table. Reported cases of West Nile, dengue, and chikungunya virus infections, Veneto Region, Italy, June 15–October 31, 2008–2010*

Year	Autochthonous WNF cases	Autochthonous WNND cases	Imported dengue cases	Imported chikungunya cases
2008	1 (retrospective)	5 (4 retrospective)	2	1
2009	0	6 (1 fatal)	4	0
2010†	4	3	14	1

*WNF, West Nile fever; WNND, West Nile neuroinvasive disease.
†Surveillance started during the last week of July.

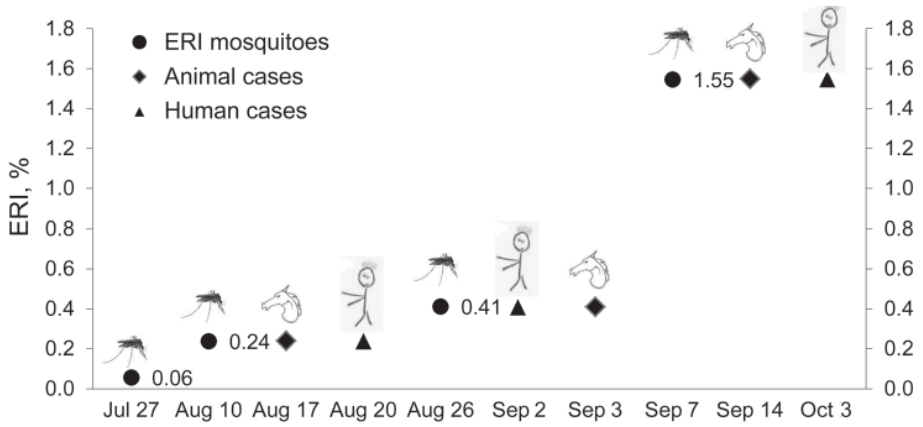


Figure 2. Expected rates of infection (ERI) in mosquitoes in the West Nile virus–positive site and hypothetical time from exposure to infected mosquitoes to clinical cases in animals and humans (calculated 1 week before symptom onset) recorded in the same province, Venice Province, Italy, 2010.

rate of infection was higher (≥0.24%), clinical cases were observed in animals and humans.

Concerning the new plan for human surveillance of summer fevers, the case definition, particularly for WNF, has been modified by removing the compulsory presence of rash, to enhance sensitivity. Training and sensitization of general practitioners and emergency department physicians play a fundamental role. On the basis of a predefined threshold of vector intensity in an area where a new case has been identified, immediate vector control measures will be started when necessary.

Dr Gobbi is an infectious diseases consultant with at the Centre for Tropical Diseases of Sacro Cuore-Don Calabria Hospital, Negrar (Verona), Italy. His primary research interests are travel-related infectious diseases and rapid diagnostic tests for malaria.

References

1. Barzon L, Pacenti M, Cusinato R, Cattai M, Franchin E, Pagni S, et al. Human cases of West Nile virus infection in north-eastern Italy, 15 June to 15 November 2010. Euro Surveill. 2011;16:pii:19949.
2. La Ruche G, Souarès Y, Armengaud A, Peloux-Petiot F, Delaunay P, Desprès P, et al. First two autochthonous dengue virus infections in metropolitan France, September 2010. Euro Surveill. 2010;15:19676.
3. Gjenero-Margan I, Aleraj B, Krajcar D, Lesnikar V, Klobučar A, Pem-Novosel I, et al. Autochthonous dengue fever in Croatia, August–September 2010. Euro Surveill. 2011;16:pii: 19805.
4. Beltrame A, Angheben A, Bisoffi Z, Monteiro G, Marocco S, Calleri G, et al. Imported chikungunya infection, Italy. Emerg Infect Dis. 2007;13:1264–6.
5. Rezza G, Nicoletti L, Angelini R, Romi R, Finarelli AC, Panning M, et al. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet. 2007;370:1840–6. http://dx.doi.org/10.1016/S0140-6736(07)61779-6
6. Grandadam M, Caro V, Plumet S, Thiberge JM, Souares Y, Failloux AB, et al. Chikungunya virus, southeastern France. Emerg Infect Dis. 2011;17:910–3.
7. Rossini G, Cavrini F, Pierro A, Macini P, Finarelli A, Po C, et al. First human case of West Nile virus neuroinvasive infection in Italy, September 2008—case report. Euro Surveill. 2008;13:pii: 19002.
8. Barzon L, Squarzon L, Cattai M, Franchin E, Pagni S, Cusinato R, et al. West Nile virus infection in Veneto Region, Italy, 2008–2009. Euro Surveill. 2009;14:pii:19289.
9. Gobbi F, Napoletano G, Piovesan C, Russo F, Angheben A, Rossanese A, et al. Where is West Nile fever? Lessons learnt from recent human cases in northern Italy. Euro Surveill. 2009;14:pii:19143.
10. Rizzo C, Vescio F, Declich S, Finarelli AC, Macini P, Mattivi A, et al. West Nile virus transmission with human cases in Italy, August–September 2009. Euro Surveill. 2009;14:pii:19353.
11. Hayes EB, Sejvar JJ, Zaki SR, Lanciotti RS, Bode AV, Campbell GL. Virology, pathology, and clinical manifestations of West Nile virus disease. Emerg Infect Dis. 2005;11:1174–9.
12. Capobianchi MR, Sambri V, Castilletti C, Pierro AM, Rossini G, Gaibani P, et al. Retrospective screening of solid organ donors in Italy, 2009, reveals unpredicted circulation of West Nile virus. Euro Surveill. 2010;15:pii:19648.
13. Calzolari M, Bonilauri P, Bellini R, Caimi M, Defilippo F, Maioli G, et al. Arboviral survey of mosquitoes in two northern Italian regions in 2007 and 2008. Vector Borne Zoonotic Dis. 2010;10:875–84. http://dx.doi.org/10.1089/vbz.2009.0176
14. Calistri P, Monaco F, Savini G, Guercio A, Purpari G, Vicari D, et al. Further spread of West Nile virus in Italy. Vet Ital. 2010;46:467–74.
15. Rizzo C, Esposito S, Azzari C, Bartolozzi G, Fara GM, Lo Giudice M, et al. West Nile virus infections in children: a disease pediatricians should think about. Pediatr Infect Dis J. 2011;30:65–6. http://dx.doi.org/10.1097/INF.0b013e31820591dc

Address for correspondence: Federico Gobbi, Centre of Tropical Diseases, Sacro Cuore Hospital, Negrar Verona, Italy; email: federico.gobbi@sacrocuore.it

De Novo Daptomycin-Nonsusceptible Enterococcal Infections

Theodoros Kelesidis, Romney Humphries, Daniel Z. Uslan, and David Pegues

Potential emergence of enterococcal daptomycin nonsusceptibility among patients with no prior exposure to daptomycin poses clinical and public health challenges. We found that development of infections with daptomycin-nonsusceptible enterococci in these patients could be associated with sporadic emergence and clonal spread.

The development of daptomycin resistance by enterococci poses treatment and infection control challenges. Emergence of daptomycin-nonsusceptible enterococci (DNSE) during treatment with daptomycin has been reported (*1*). We describe patient characteristics, clinical presentation, and outcome of 9 cases of DNSE infections in patients with no history of daptomycin treatment.

The Study

We defined DNSE, by using the criteria of the Clinical and Laboratory Standards Institute (*2*), as enterococci with an MIC >4 ug/mL (*1*), as determined by in-house prepared reference broth microdilution (*3*) testing. We identified cases of DNSE infection by reviewing microbiology records from UCLA Health System during January 1, 2007–March 1, 2011. Patients with no history of daptomycin exposure and ≈1 positive clinical culture for DNSE were included in this study.

During the study period, we isolated 3,600 unique enterococci from adult inpatients at our facility and tested for antimicrobial drug susceptibility; 25 isolates were DNSE, 16 of which were recovered from patients who had received prior daptomycin therapy. We isolated DNSE from an additional 9 patients with no history of daptomycin use. Six (66.7%) patients were male and mean age was 58.9 years (range 26–75 years). Seven (77.8%) patients were immunosuppressed, of whom 5 had solid malignant tumors and 3 had diabetes. All patients in our case series had complicated concurrent medical conditions, and all but one had undergone surgery in the 3 months before isolation of DNSE (Table 1). For 4 patients, DNSE were isolated on the day of admission. For the remaining 5 patients, the average length of hospitalization before isolation of the first DNSE isolate was 45.4 days (Table 1).

Use of antimicrobial drugs associated with presence of vancomycin-resistant enterococci, such as recent use of vancomycin, third-generation cephalosporins, or agents with activity against anaerobic bacteria (*4*), was associated with 5 (55.6%; mean duration 31.6 days, range 5–58 days), 1 (11.1%; duration 12 days), and 6 (66.7%; mean duration 33 days, range 5–65 days) patients, respectively. Recently, we suggested that the interplay between anaerobes and enterococci might have a possible role in the dissemination of daptomycin resistance (*5*). However, DNSE were also found in 3 (33.3%) patients (patients 3, 4, and 5; Table 1) who had no recent exposure to any antimicrobial agent. In addition, 2 patients (patients 4 and 5; Table 1) had no hospitalization or other health care exposure in the 12-month period before first isolation of DNSE, and DNSE were isolated on the first day of hospitalization, strongly suggesting community acquisition of DNSE.

Further support for a possible community reservoir of DNSE was provided by the identification of clonally related DNSE isolates. Of 9 DNSE isolates, 6 (66.7%) were *Enterococcus faecium*, 2 (22%) were *E. faecalis*, and 1 (11%) was *E. gallinarum*. Five isolates were available for further study (patients 4, 5, 6, 7, and 8) (Table 2). The daptomycin MICs were confirmed for these isolates by in-house prepared broth microdilution in cation-adjusted Muller Hinton broth plus calcium and by Etest (bioMérieux, Durham, NC, USA). Using strain typing by repPCR (DiversiLabTM; bioMérieux), we found that there was no genetic relatedness between the 2 *E. faecium* isolates available for typing, but the 2 *E. faecalis* were 97.7% similar by repPCR. These 2 isolates were also 98.5% related to a third daptomycin-nonsusceptible *E. faecalis* isolate from a patient who had received 90 days of daptomycin treatment before isolation of the DNSE. No epidemiologic link was found between these 3 patients, and 2 of the cases were identified on the first day of hospitalization, 6 months (patient 5) and 1 year (case 4) after the isolation of the original DNSE in the third patient (not included in this case series).

Of the 9 patients, 8 showed evidence of clinical infection, including 3 bloodstream (33.3%), 3 intraabdominal (33.3%: 2 bile and 1 abscess), 1 urinary tract (22.2%), and 1 soft tissue (11.1%) infections. The remaining patient (patient 4) had asymptomatic bacteriuria with normal urinalysis. A potential nidus of infection, including a central venous catheter or inadequately drained abscess, was identified in 5 patients (55.6%) (Table 1).

Author affiliations: David Geffen School of Medicine at UCLA, Los Angeles, California, USA

DOI: http://dx.doi.org/10.3201/eid1804.110932

Table 1. Patient and treatment characteristics for 9 patients with DNSE infection and colonization, Los Angeles, California, USA, 2007–2011*

Patient no., age, y/sex	Concurrent conditions†	Recent surgery	Site of isolation	*Enterococcus* species	Hospital day of DNSE isolation	Source of *Enterococcus* infection	Other pathogens isolated at DNSE site	Tx	Outcome of Enterococcus infection
1/69/M	RA on steroids, CHF, s/p AVR, s/p CABG	BKA, vascular surgery	Blood	*E. faecium*	47	CLABSI	None	DAP	Death
2/70/M	Diabetes, bowel disease, ILD on steroids, lung transplant	Thoracic	Blood, pleural fluid empyema	*E. faecium*	75	CLABSI, empyema	None	LZD, Q-D, GEN	Death
3/51/M	Colitis, TCC, severe hypocalcemia	No	Blood	*E. faecium*	17	CLABSI	None	VAN	Death
4/53/F	CNS tumor	No	Urine	*E. faecalis*	1	Asymptomatic bacteriuria	None	None	Undetermined
5/75/M	CVA	No	Urine	*E. faecalis*	1	UTI	None	LZD, VAN	Recovered
6/62/M	AIDS	Soft tissue	Wound	*E. faecium*	23	Chronic decubitus ulcerations	*E. coli*, MRSA, *Candida* spp.	LZD	Undetermined
7/64/F	Diabetes, bowel disease, splenectomy	GI tract	Abscess	*E. gallinarum*	1	Intra-abdominal abscess	Anaerobes, *S. viridans*	PIP/TAZ, ERT	Recovered
8/26/F	Cirrhosis, ESRD, breast cancer	GI tract	Abscess	*E. faecium*	65	Liver abscess	*E. coli, Klebsiella* spp., Stenotro.	LZD	Death
9/60/M	Diabetes, pancreatic cancer	No	Bile	*E. faecium*	1	Cholangitis	Fungi (*C. glabrata, A. fumigatus, S. cerevisiae*)	PIP/TAZ	Undetermined

*DNSE, daptomycin-nonsusceptible enterococcus; Tx, treatment; RA, rheumatoid arthritis; CHF, congestive heart failure; AVR, aortic valve replacement; CABG, coronary artery bypass graft; BKA, below knee amputation; CLABSI, central line–associated bloodstream infection; DAP, daptomycin; ILD, interstitial lung disease; LZD, linezolid; Q-D, quinupristin/dalfopristin; GEN, gentamicin; TCC, transitional cell carcinoma; VAN, vancomycin; CNS, central nervous system; CVA, cerebrovascular accident; UTI, urinary tract infection; *E. coli*, *Escherichia coli* ; MRSA, methicillin-resistant *Staphylococcus aureus*; GI, gastrointestinal; *S. viridans, Streptococcus viridans*; PIP/TAZ, piperacillin/tazobactam;ESRD, end-stage renal disease; Stenotro., Stenotrophomonas; *C. glabrata, Candida glabrata; A. fumigatus, Aspergillus fumigatus; S. cerevisiae, Saccharomyces cerevisiae*
†Bowel disease was defined as presence of colitis, ischemic colitis, colonic ulcers, obstruction, or bowel surgery within 3 mo.

Susceptibilities of the DNSE isolates to antimicrobial drugs with activity against the enterococci are summarized in Table 2. Fluoroquinolones, nitrofurantoin, doxycycline, and quinupristin/dalfopristin had activity against 3 (33.3%), 8 (88.9%), 7 (77.7%), and 7 (77.7%) of DNSE isolates, respectively. All isolates were susceptible in vitro to tigecycline and linezolid (Table 2). All but 1 *E. faecium* isolate were vancomycin resistant (83%, MIC ≥32 μg/mL) (Table 2), and all *E. faecalis* isolates were susceptible to vancomycin. As this series demonstrates, vancomycin-resistant enterococci and vancomycin-susceptible enterococci may be nonsusceptible to daptomycin. Although all laboratories are encouraged to investigate unusual MIC results by confirming organism identification and MIC result by the Clinical and Laboratory Standards Institute standards, the unusual MIC (e.g., daptomycin nonsusceptible) might or might not be relayed to the clinician in the instance of vancomycin-susceptible isolates.

Four (44.4%) patients died while receiving therapy for DNSE (Table 1). Each patient had multiple concurrent conditions, and cause of death could not be attributed solely to DNSE. (Table 1). Of the 5 patients who survived, the clinical response to treatment of DNSE infection could not be determined for 3 patients because of multiple concurrent conditions and polymicrobial infection. However, 1 patient with cholangitis, from whom DNSE were isolated from a bile culture, improved clinically despite receiving antimicrobial agents that were inactive against DNSE. The second clinically evaluable patient had asymptomatic bacteriuria associated with DNSE, did not receive any antimicrobial therapy, and remained clinically stable. Follow-up urine samples were cultured for 1 of the 5 surviving patients (patient 5); the result was negative.

Table 2. Antimicrobial susceptibilities of DNSE isolates from 9 patients to antimicrobial drugs with activity against *Enterococcus* spp., Los Angeles, California, USA, 2007–2011*

Patient no.	*Enterococcus* species	MIC, μg/mL									Synergy test	
		DAP†	VAN	AMP	DOX	NIT	LZD	Q-D	TGC	CIP	GEN	STR
1	*E. faecium*	ND	>32 (R)	>64 (R)	<1 (S)	128 (R)	2 (S)	<0.5 (S)	≤0.25 (S)	>4 (R)	S	S
2	*E. faecium*	ND	>32 (R)	>64 (R)	8 (I)	64 (I)	2 (S)	<0.5 (S)	≤0.25 (S)	>4 (R)	R	S
3	*E. faecium*	ND	2 (S)	>64 (R)	16 (R)	32 (S)	1 (S)	<0.5 (S)	≤0.25 (S)	>4 (R)	R	R
4	*E. faecalis*	4	1 (S)	<2 (S)	16 (R)	<16 (S)	2 (S)	4 (R)	≤0.25 (S)	1 (S)	S	S
5	*E. faecalis*	12	2 (S)	<2 (S)	8 (I)	<16 (S)	2 (S)	4 (R)	≤0.25 (S)	1 (S)	S	S
6	*E. faecium*	8	>32 (R)	>64 (R)	8 (I)	32 (S)	1 (S)	1(S)	≤0.25 (S)	>4 (R)	S	S
7	*E. gallinarum*	4	<0.5 (S)	<2 (S)	<1(S)	32 (S)	1 (S)	1(S)	≤0.25 (S)	<0.5 (S)	S	S
8	*E. faecium*	8	>32 (R)	>64 (R)	4 (S)	64 (I)	2 (S)	<0.5 (S)	≤0.25 (S)	>4 (R)	R	S
9	*E. faecium*	ND	>32 (R)	>64 (R)	4 (S)	64 (I)	1 (S)	1 (S)	≤0.25 (S)	>4 (R)	R	S

*DNSE, daptomycin-nonsusceptible enterococci; DAP, daptomycin; VAN, vancomycin; AMP, ampicillin; DOX, doxycycline; NIT, nitrofurantoin; LZD, linezolid; Q-D, quinupristin/dalfopristin; TGC, tigecycline; CIP, ciprofloxacin; GEN, gentamicin; STR, streptomycin; NS, nonsusceptible; ND, not done; R, resistant; S, sensitive.
†By Etest.

Conclusions

Two existing studies of de novo development of daptomycin nonsusceptibility describe 1 case each (*6*,*7*). To our knowledge, this study analyzes one of the largest series of DNSE isolates in patients with no prior exposure to daptomycin.

The mechanism for daptomycin nonsusceptibility in enterococci is poorly understood (*8*). A recent study found that 25% of *Enterococcus* spp. isolated from beef products were DNSE (*9*). Spread of these DNSE from agriculture to humans through the food chain may be a mechanism by which DNSE are emerging (*10–12*). Of note, in our case series, 3 patients (patients 2, 7, and 8) had a history of exposure to livestock: 1 (patient 2) was a veterinarian; 2 (patients 7 and 8) had histories of farm exposure. Three (patients 4, 5, and 7) reported frequent ingestion of beef. We recommend further investigation of these observations by case–control study.

The limitations of our study include the retrospective observational study design, the small number of cases identified, and lack of a comparison group. Case–control studies could better define risk factors associated with emergence of DNSE. Clinicians should be aware of the possibility of serious infections associated with DNSE even when there is no history of prior daptomycin therapy.

Dr Kelesidis is an infectious disease specialist at the UCLA Medical Center, David Geffen School of Medicine at UCLA, Los Angeles, USA. His research interests include microbial pathogenesis and HIV/AIDS.

References

1. Kelesidis T, Humphries R, Uslan DZ, Pegues DA. Daptomycin nonsusceptible enterococci: an emerging challenge for clinicians. Clin Infect Dis. 2011;52:228–34. http://dx.doi.org/10.1093/cid/ciq113
2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: twenty-first supplement. Wayne (PA): The Institute; 2011.
3. Clinical and Laboratory Standards Institute. Methods for dilution in antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. 8th ed. Wayne (PA): The Institute; 2009.
4. Tacconelli E, De Angelis G, Cataldo MA, Mantengoli E, Spanu T, Pan A, et al. Antibiotic usage and risk of colonization and infection with antibiotic-resistant bacteria: a hospital population-based study. Antimicrob Agents Chemother. 2009;53:4264–9. http://dx.doi.org/10.1128/AAC.00431-09
5. Kelesidis T. Comment on: successful therapy of treatment-emergent, non-clonal daptomycin-non-susceptible *Enterococcus faecium* infections. J Antimicrob Chemother. 2012;67:515–6. http://dx.doi.org/10.1093/jac/dkr465
6. Lesho EP, Wortmann GW, Craft D, Moran KA. De novo daptomycin nonsusceptibility in a clinical isolate. J Clin Microbiol. 2006;44:673. http://dx.doi.org/10.1128/JCM.44.2.673.2006
7. Fraher MH, Corcoran GD, Creagh S, Feeney E. Daptomycin-resistant *Enteroccoccus faecium* in a patient with no prior exposure to daptomycin. J Hosp Infect. 2007;65:376–8. http://dx.doi.org/10.1016/j.jhin.2007.01.002
8. Palmer KL, Daniel A, Hardy C, Silverman J, Gilmore MS. Genetic basis for daptomycin resistance in enterococci. Antimicrob Agents Chemother. 2011;55:3345–56. http://dx.doi.org/10.1128/AAC.00207-11
9. Zhang J, Wall SK, Xu L, Ebner PD. Contamination rates and antimicrobial resistance in bacteria isolated from "grass-fed" labeled beef products. Foodborne Pathog Dis. 2010;7:1331–6. http://dx.doi.org/10.1089/fpd.2010.0562
10. Lester CH, Frimodt-Moller N, Sorensen TL, Monnet DL, Hammerum AM. In vivo transfer of the vanA resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. Antimicrob Agents Chemother. 2006;50:596–9. http://dx.doi.org/10.1128/AAC.50.2.596-599.2006
11. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG Jr. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. Proc Natl Acad Sci U S A. 2002;99:6434–9. http://dx.doi.org/10.1073/pnas.082188899
12. Waters AE, Contente-Cuomo T, Buchhagen J, Liu CM, Watson L, Pearce K, et al. Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. Clin Infect Dis. 2011;52:1227–30. http://dx.doi.org/10.1093/cid/cir181

Address for correspondence: Theodoros Kelesidis, Department of Medicine, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, 10833 Le Conte Ave, CHS 37-121, Los Angeles, CA 90095, USA; email: tkelesidis@mednet.ucla.edu

Dengue in Patients with Central Nervous System Manifestations, Brazil

Fernanda Araújo, Rita Nogueira, Maurício de Sousa Araújo, Anne Perdigão, Luciano Cavalcanti, Raimunda Brilhante, Marcos Rocha, Dina Feitosa Vilar, Suzana Silveira Holanda, Deborah de Melo Braga, and José Sidrim

We investigated the prevalence of dengue in patients with suspected viral meningitis/meningoencephalitis in a dengue-endemic area. Cerebrospinal fluid analysis showed positive results and a 6.74× greater likelihood of identifying positive fluid in patients who died. Our findings support testing patients with neurologic manifestations for the virus in dengue-endemic areas.

Dengue is the most prevalent arboviral infection in humans (*1*). Since the reintroduction of dengue virus (DENV) into Brazil in the 1980s, >60% of the reported dengue cases in this region of the Western Hemisphere have occurred there (*2*). As the disease has become more common, unusual clinical signs, some of which involve the central nervous system, have been observed in dengue patients (*2*–*4*). We therefore assessed prevalence of dengue neurologic cases from Ceará State, Brazil, a region where dengue is endemic.

The Study

We enrolled 183 patients with suspected viral meningitis/meningoencephalitis admitted to São José Hospital of Infectious Disease and 26 deceased patients with suspected fatal meningitis who had been sent to the city of Fortaleza Coroner's Office. Cerebrospinal fluid (CSF) was collected from all 209 patients. Study inclusion criteria were suspicion of viral meningitis/meningoencephalitis, a CSF cell count <500 cells/mm^3, and negative results of culture and microscopic examination for bacteria and fungi. The CSF samples were not contaminated with blood. The study was performed retrospectively and used samples from patients who had been treated for meningitis during 2005–2008, a period during which a dengue epidemic may have occurred in Ceará. This study was approved by the Ethics Committee of São José Hospital of Infectious Disease (protocol no. 005/2009; Certificado de Apresentação para Apreciação Ética [Proof of Application for Ethical Review] 0005.0.042.000–09).

Dengue meningitis was suspected when a patient had fever and symptoms of irritation of the meninges, such as headache and neck stiffness; a diagnosis of dengue meningoencephalitis was established when the patient showed signs of focal involvement of the central nervous system (CNS). A diagnosis of dengue was confirmed with a DENV-positive CSF result by reverse transcription PCR (RT-PCR), nonstructural protein (NS) 1, or IgM against DENV (*3*,*4*).

Samples were analyzed by using RT-PCR, ELISA for NS1, and IgM monoclonal antibody and a rapid immunochromatography test for IgG (*3*–*5*).Viral RNA for the nested RT-PCR was extracted from 140 μL of the CSF samples by using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, CA, USA), following the manufacturer's protocol, and stored at −80°C until tested. The RT-PCR for DENV was performed on 209 CSF samples, as described (*5*).

The NS1Ag Pan-E Dengue Early ELISA kit (Panbio Diagnostics, Brisbane, Queensland, Australia) was used to detect the dengue NS1 in 209 CSF specimens in accordance with the manufacturers' instructions (*4*). The Dengue IgM Capture ELISA (Panbio Diagnostics) was performed on 209 CSF samples, according to the manufacturer's instructions. The Panbio Dengue Duo Cassette rapid test was performed, according to the manufacturer's instructions, with CSF specimens that were positive for DENV in any of the other tests used.

Of 209 CSF samples studied, 8 (3.8%) showed positive results in ≥1 test: 5 from the group admitted to São José Hospital of Infectious Disease and 3 deceased patients examined at the Fortaleza Coroner's Office (Table 1). Reviewed literature showed that the etiologic agents of most cases of viral meningitis in Brazil are enterovirus and herpesvirus; cytomegalovirus and dengue viruses are each responsible for 10% (2/20) (*6*).

Conclusions

DENV as a causal agent for meningitis has been rarely reported, although some cases have been described in the

Author affiliations: State Health Secretariat of Ceará, Fortaleza, Ceará, Brazil (F.M.C. Araújo, A.C.B. Perdigão, D.C.L. Feitosa Vilar, S.G. Silveira Holanda and D.N. de Melo Braga); Oswaldo Cruz Institute, Rio de Janeiro, Rio de Janeiro, Brazil (R.M.R. Nogueira); Dr. José Frota Institute, Fortaleza (M. de Sousa Araújo); Federal University of Ceará, Fortaleza (L.P.G., Cavalcanti, F.M. de Carvalho Araújo, J.J. da Costa Sidrim, R.S.N. Brilhante, M.F.G. Rocha); State University of Ceará, Fortaleza (A.C.B. Perdigão); College Christus, Fortaleza (L.P.G. Cavalcanti)

DOI: http://dx.doi.org/10.3201/eid1804.111522

Table 1. Clinical features and virologic findings for 8 patients with meningitis/meningoencephalitis and confirmed cases of dengue, Brazil, 2005–2008*

Patient no.	Age, y/sex	Initial symptoms and signs	Progress and outcome	RT-PCR	NS1Ag	IgM	IgG	ND
1	45/M	Fever, headache, sweating, thorax pain, seizure, coma, chronic hypertension.	Cerebral edema and congestion; mononuclear cells in meninges; death after 6 d	–	+	–	–	ME
2	32/F	Fever, vomiting, neck stiffness, myalgia, abdominal pain, asthenia, somnolence, confusion	Meningitis, sixth nerve palsy; death after 14 d	DENV-3	+	–	–	ME
3	1/M	Fever, tremors, rigidity of limbs, otitis	Intracranial hypertension, meningitis; death after 24 h	–	+	+	–	M
4	6/F	Fever, headache, malaise, vomiting, drowsiness, neck stiffness	CSF: clear, 133 cells/mm^3, 42% lymphocytes, 2% monocytes, 53% neutrophils, 3% eosinophils; protein 58 g/L, glucose 54 g/L; recovery after 9 d	–	–	+	+	M
5	58/M	Fever, headache, severe malaise, vomiting, lowering of consciousness, delirium	CSF: 300 cells/mm^3; lymphocytes, 87%, monocytes 5%, neutrophils 4%, protein 112 g/L, glucose 59 g/L; serum: AST 127 U/L, ALT 74 U/L; CT scan: expansible lesion measuring 4 × 2 x 2.3 cm; referred for surgical treatment	–	–	+	+	Brain tumor; M
6	5/F	Fever, headache, vomiting, neck stiffness	CSF: 490 cells/mm^3, 2% monocytes, 5% lymphocytes, 93% neutrophils, protein 45 g/L, glucose 110 g/L; recovery after 8 d	–	–	+		M
7	15/M	Fever, headache, arthralgia, severe malaise, dry cough, dyspnea, epigastric pain	IHC result positive for dengue. CSF: clear; cerebrum and cerebellum with marked edema and vasocongestion of meninges and nerve tissue; death after 5 d	–	+	–	–	ME
8	24/M	Fever, headache, vomiting, and neck stiffness	CSF: 426 cells/mm^3; protein 136 g/L, glucose 55 g/L; recovery	–	–	+	+	M

*RT-PCR, reverse transcription PCR; NS1Ag, nonstructural protein 1 antigen; ND, neurologic diagnosis; –, negative; +, positive; ME, meningoencephalitis; DENV, dengue virus; M, meningitis; CSF, cerebrospinal fluid; AST, aspartate aminotransferase; ALT, alanine aminotransferase; CT, computed tomography; IHC, immunohistochemical test.

literature. In Jamaica, a study of 401 patients with suspected cases of viral infection of the CNS showed that 54 (13.5%) were positive for dengue; 18 (33.3%) of those patients showed clinical signs of meningitis (*7*). However, when we included patients in the cohort who were initially suspected of having CNS infection, the frequency of meningitis in this study was 18/401 (4.5%).

An investigation of dengue patients with suspected CNS infection conducted in Vietnam found 4.2% (16/378) of persons positive for DENV; 1 (0.3%) patient had meningitis (*3*). The frequency of finding dengue virus in patients with suspected cases of meningitis found in this study corroborated what was hypothesized in the literature: neurologic manifestations in patients with DENV have been reported in Ceará, but previous studies based laboratory diagnosis on serum, not on CSF as in our study, which indicated a relationship between dengue and CNS manifestations (*8*).

Of 5 patients treated at São José Hospital of Infectious Disease (Table 1), 3 recovered, 1 was given a diagnosis of a brain tumor, and 1 died. The patient who died was the only person of 5 with dengue fever who had signs and symptoms of fatal dengue hemorrhagic fever (DHF) (such as intense malaise, dry cough with dyspnea, and abdominal pain) (*9*). Of the 3 deceased patients (Table 1), only 1 had signs of severe dengue, including myalgia, abdominal pain, asthenia, somnolence, and confusion. Suspected cases of meningitis with other pathologic changes might also be confused with dengue cases with CNS involvement (*10*). Of 8 dengue patients, 2 had signs and symptoms of dengue infection. In Brazil, meningitis was confirmed for patients with oligosymptomatic dengue infection in the cities of Vitoria and Rio de Janeiro (*10*,*11*).

The presence of DENV NS1 antigen (NS1Ag) has been associated with virus replication and viremia with the risk for development of DHF (*12*). The NS1Ag was detected in 4 of the fatal cases reported here, but because none fulfilled the World Health Organization criteria for DHF, they were considered to have been cases of severe dengue because the patients died (*1*) (Table 1). Detection of dengue IgM in CSF has shown a high specificity (97%) for diagnosing neurologic dengue and might be associated with the neurovirulence of DENV and its ability to cause encephalitis (*13*). Prior to the 1996 publication of findings

Table 2. Risk for death among patients with meningitis/meningoencephalitis with DENV+ versus DENV– cerebrospinal fluid test results, Brazil, 2005–2008*

Outcome	DENV+	DENV–	Total
Death	4 (14.8)	23 (85.2)	27 (100)
Recovery	4 (2.2)	178 (97.8)	182 (100)

*Values are no. (%) patients. Relative risk 6.74 (95% CI 1.79–25.38); p<0.0109. DENV, dengue virus; –, negative; +, positive.

by Lum et al., involvement of the CNS in dengue infection had been thought to be secondary to vasculitis only; direct involvement of the brain by DENV was thought to be unlikely (*14*),. The literature has reported detection of DENV in the brain and CSF by PCR and virus isolation and detection of NS1 and dengue IgM, providing strong evidence that DENV has neurovirulent properties (*3*,*4*,*11*,*13*–*15*). Meningeal lesions, neuronal damage, and evidence of DENV in CSF by RT-PCR and ELISA (NS1/IgM) found in this study are consistent with CNS infection (Table 1).

The prevalence of CNS involvement in patients with dengue infection seems to vary with severity of dengue cases (*11*). Mortality rates also vary among studies; the reported rate of neurologic dengue was found to be 3.7% (2/54) in a study in Jamaica (*7*). In another study conducted in Vietnam, no patients with the neurologic form of dengue died (*3*); our study found a mortality rate of 1.9% (4/209). However, the proportional positivity was higher for the group of patients who died (4/27, 14.8%) than for those who recovered (4/182, 2.2%) (Table 2). The relative risk for identifying DENV-positive CSF in patients who died was 6.74× greater than that for patients who recovered (95% CI 1.79×–25.38×; p<0.0109). No patients had DHF or a concurrent condition to predict deterioration to death, thus suggesting that patients with meningitis/meningoencephalitis and DENV-positive CSF may have higher risk for development of severe forms of dengue infection.

The high risk for death among patients with dengue meningitis/meningoencephalitis in this study supports the need for increased surveillance. Dengue should be suspected in patients with neurologic manifestations in dengue-endemic areas, and appropriate treatment should be given to prevent death.

This study was supported by the Brazilian National Research Council, process MCT/CNPq 14/2009, and by the Ceará State Scientific Development Foundation, process FUNCAP 09100097-1.

Dr Araújo is a researcher in the dengue reference laboratory in the State Health Secretariat in Ceara. Her research interest and work for the past 23 years is in dengue viruses in Brazil.

References

1. World Health Organization. Dengue hemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva: The Organization; 1997.
2. Nogueira RMR, Araújo JMG, Schatzmayr HG. Dengue virus in Brazil, 1986–2006. Rev Panam Salud Publica. 2007;22:358–63. http://dx.doi.org/10.1590/S1020-49892007001000009
3. Solomon T, Dung NM, Vaughn DW, Kneen R, Thao LT, Raengsakulrach B, et al. Neurological manifestations of dengue infection. Lancet. 2000;355:1053–9. http://dx.doi.org/10.1016/S0140-6736(00)02036-5
4. Araújo FMC, Brilhante RSN, Cavalcanti LPG, Rocha MFG, Cordeiro RA, Perdigão ACB, et al. Detection of the dengue non-structural 1 antigen in cerebral spinal fluid samples using a commercially available enzyme-linked immunosorbent assay. J Virol Methods. 2011;177:128–31. http://dx.doi.org/10.1016/j.jviromet.2011.07.003
5. Lanciotti RS, Calisher CH, Gubler DJ, Chang GJ, Vorndam V. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol. 1992;30:545–51.
6. Soares CN, Cabral-Castro MJ, Peralta JM, Freitas MRG, Puccioni-Sohler M. Review of the etiologies of viral meningitis and encephalitis in a dengue endemic region. J Neurol Sci. 2011;303:75–9. http://dx.doi.org/10.1016/j.jns.2011.01.012
7. Jackson ST, Mullings A, Bennett F, Khan C, Gordon-Strachan G, Rhoden T. Dengue infection in patients presenting with neurological manifestations in a dengue endemic population. West Indian Med J. 2008;57:373–6.
8. Vasconcelos PFC, Travassos Da Rosa APA, Coelho ICB, Menezes DB, Travassos Da Rosa ES, Rodrigues SG, et al. Involvement of the central nervous system in dengue fever: three serologically confirmed cases from Fortaleza, Ceará, Brazil. Rev Inst Med Trop Sao Paulo. 1998;40:35–9. http://dx.doi.org/10.1590/S0036-46651998000100008
9. Guzmán MG, Kouri G, Morier L, Soler M, Fernández A. A study of fatal hemorrhagic dengue cases in Cuba, 1981. Bull Pan Am Health Organ. 1984;18:213–20.
10. Soares CN, Cabral-Castro MJ, Peralta JM, Freitas MRG, Puccioni-Sohler M. Meningitis determined by oligosymptomatic dengue virus type 3 infection: report of a case. Int J Infect Dis. 2010;14:e150–2. http://dx.doi.org/10.1016/j.ijid.2009.03.016
11. Domingues RB, Kuster GW, Onuki-Castro FL, Souza VA, Levi JE, Pannuti CS. Involvement of the central nervous system in patients with dengue virus infection. J Neurol Sci. 2008;267:36–40. http://dx.doi.org/10.1016/j.jns.2007.09.040
12. Duong V, Ly S, Ong S, Cgroeung N, Try PL, Deubel V, et al. Variation of dengue NS1 antigen measured by commercial ELISA kit in various forms of dengue infections and assessment of the association between NS1 level and disease severity. BMC Proceedings. 2011;5:47. http://dx.doi.org/10.1186/1753-6561-5-s1-p47
13. Soares CN, Faria LC, Puccioni-Sohler M, Peralta JM, Freitas MRG. Dengue infection: neurological manifestations and cerebrospinal fluid (CSF) analysis. J Neurol Sci. 2006;249:19–24. http://dx.doi.org/10.1016/j.jns.2006.05.068
14. Lum LCS, Lam SK, Choy YS, George R, Harun F. Dengue encephalitis: a true entity? Am J Trop Med Hyg. 1996;54:256–9.
15. de Araújo JMG, Schatzmayr HG, Filippis AMB, Santos FB, Cardoso MA, Britto C, et al. Retrospective survey of dengue virus infection in fatal cases from an epidemic in Brazil. J Virol Methods. 2009;155:34–8. http://dx.doi.org/10.1016/j.jviromet.2008.09.023

Address for correspondence: Fernanda Araújo, Av. Barão de Studart, 2405, 60120-002, Fortaleza, CE, Brazil; email: fernanda.montenegro@lacen.ce.gov.br

Human Parvovirus 4 Infection, Cameroon

Myriam Lavoie, Colin P. Sharp, Jacques Pépin, Christopher Pennington, Yacouba Foupouapouognigni, Oliver G. Pybus, Richard Njouom, and Peter Simmonds

In a post hoc analysis of samples collected in 2009, we determined seroprevalence of parvovirus 4 (PARV4) among elderly Cameroonians. PARV4 seropositivity was associated with receipt of intravenous antimalarial drugs, intramuscular streptomycin, or an intramuscular contraceptive, but not hepatitis C virus seropositivity. Findings suggest parenteral acquisition of some PARV4 infections.

Human parvovirus 4 (PARV4), also known as partetravirus, was identified in 2005 from the plasma of an intravenous drug user (IDU) (*1*). In separate studies that used PCR, PARV4 was subsequently documented in autopsy tissues from IDUs and persons with hemophilia; in bone marrow aspirates from patients with AIDS; and in the blood of transplant recipients, hemodialysis patients, and infants in Ghana (*2*–*5*).

In 2007, 199 (32.4%) of 626 adults tested in Burkina Faso, Democratic Republic of the Congo, and Cameroon were seropositive by first-generation serologic assay for PARV4 (*6*). In South Africa, prevalence was 36% among HIV-infected blood donors but only 4% among their HIV-seronegative counterparts (*6*). Although PARV4 presence in IDUs and hemophilia patients suggests parenteral transmission (*7*,*8*), this route has not yet been studied and other modes of transmission have not been ruled out. The pathogenicity of PARV4 remains unclear, but PARV4 DNA recently was found in the cerebrospinal fluid of 2 children from India who had unexplained encephalitis (*9*).

During 2010, to investigate the epidemiology of PARV4 in Africa, we tested for PARV4 antibodies in serum samples collected during a 2009 study of a defined population of elderly Cameroonians among whom prevalence of hepatitis C virus (HCV) infection was high. Previous exposures to parenteral and sexual risk factors had been documented for this population (*10*–*12*), indicating that this population had been excessively exposed to improperly sterilized syringes and needles and that the main risk factor for HCV was the administration of intravenous antimalarial drugs, mostly before 1960.

Author affiliations: Université de Sherbrooke, Sherbrooke, Quebec, Canada (M. Lavoie, J. Pépin); University of Edinburgh, Edinburgh, Scotland, UK (C.P. Sharp, C. Pennington, P. Simmonds); Centre Pasteur du Cameroun, Yaoundé, Cameroon (Y. Foupouapouognigni, R. Njouom); and University of Oxford, Oxford, UK (O. Pybus)

DOI: http://dx.doi.org/10.3201/eid1804.110628

The Study

The ethics committees of the Cameroonian Ministry of Health and the Centre Hospitalier Universitaire de Sherbrooke (Sherbrooke, Quebec, Canada) approved the 2009 study and 2010 follow-up specimen testing. The study was conducted in Ebolowa, southern Cameroon (*10*). Inclusion criteria were age ≥60 years and consent. Exclusion criteria were dementia or inability to communicate. With cooperation from community leaders, we visited a convenience sample of houses to identify participants. We obtained venous samples from participants and gathered sociodemographic data and information about past intravenous treatment for any disease, past parenteral treatment for infectious diseases, transfusions, scarifications, and circumcision. Vaccine scars were documented.

We performed PARV4 IgG detection on each sample in replicate by indirect ELISA by using baculovirus-expressed viral protein 2 and control antigens (*8*); arbitrary unit (AU) values were calculated relative to a control sample. Because of a high background reactivity observed for this cohort, we additionally stipulated that for positive samples, the optical density ratio (ODR) of viral protein 2 to control must be >1.2; ODRs below this threshold were considered negative.

Serologic assays for HCV and treponemal antibodies were described in the original study by Pepin et al. (*10*). We detected antibodies against hepatitis B core antigen (HBcAg) by using AxSYM (Abbott, Montreal, Quebec, Canada) and analyzed data by using Stata 10.0 (StataCorp LP, College Station, TX, USA). Proportions were compared by using either the χ^2 or Fisher exact test. Variables associated with PARV4 seropositivity in univariate analysis were tested in logistic regression models through nonautomated forward selection, continuing until no other variable reached significance. Each variable was then eliminated to assess its effect by using likelihood ratio tests. We retained in the final model variables that enhanced the fit at the $p<0.05$ level.

The study comprised 451 persons 60–102 years of age (median 70 years); 56% were HCV seropositive, 74% had antibodies against *Treponema* (10), and 95% were anti-HBcAg seropositive. Seventy-nine (17.5%) persons carried PARV4 antibodies.

PARV4 antibodies were more prevalent among persons 60–64 years of age than among older persons (Table 1). Prevalence did not vary by sex or by presence of anti-HCV, anti-HBcAg, or treponemal antibodies. The prevalence of anti-PARV4 increased, but not significantly, with

Table 1. Prevalence of human parvovirus 4 by patient characteristics, Cameroon, 2009*

Characteristic	No. virus positive/ no. tested (%)	p value
Age, y		0.04
60–64	32/125 (26)	
65–69	13/96 (14)	
70–74	17/103 (17)	
≥75	17/127 (13)	
Sex		0.15
M	25/178 (14)	
F	54/273 (20)	
HCV serologic results		0.61
Negative	29/178 (16)	
Positive	47/252 (19)	
Anti-HBcAg		1.00
Negative	3/21 (14)	
Positive	76/430 (18)	
Treponemal antibodies		0.06
Absent	28/119 (24)	
Present	51/332 (15)	
Intravenous treatment for malaria		0.04
No	29/216 (13)	
Yes	50/235 (21)	
Intravenous treatment for other diseases		0.93
No	42/239 (18)	
Yes	37/212 (17)	
No. past intravenous treatments		0.38
0	12/88 (14)	
1–3	33/206 (16)	
≥4	25/116 (22)	
Unknown	9/41 (22)	
Tuberculosis		0.04
No	72/433 (17)	
Yes, treated with oral drugs only	4/12 (33)	
Yes, treatment included streptomycin	3/6 (50)	
Transfusion		0.09
No	76/408 (19)	
Yes	3/43 (7)	
Depo-Provera injections†		0.006
No	50/268 (19)	
Yes	4/5 (80)	
Scarifications		0.88
No	30/165 (18)	
Yes	49/286 (17)	
Vaccine scar, left arm		0.005
Absent	17/53 (32)	
Present	61/397 (15)	
Vaccine scar, right arm		0.76
Absent	27/165 (16)	
Present	51/284 (18)	
Circumcision (males only)		0.74
Medical	9/73 (12)	
Traditional	16/105 (15)	

*HCV, hepatitis C virus, HBcAg, hepatitis B core antigen.
†Pharmacia & Upjohn Company, New York, NY, USA.

exposure to intravenous treatments in general. Receipt of intravenous antimalarial drugs was associated with PARV4 seropositivity, which was also more frequent among persons treated for tuberculosis and among the few women who had received injections of the contraceptive Depo-Provera (Pharmacia & Upjohn Company, New York, NY, USA). PARV4 seropositivity was not associated with treatments delivered by injection against yaws, syphilis, leprosy, or trypanosomiasis (data not shown) or with sexually transmitted infections. PARV4 seropositivity was less common among persons who had a vaccine scar on the left arm.

In multivariate analysis (Table 2), PARV4 seropositivity was associated with younger age, intravenous receipt of antimalarial drugs, and parenteral receipt of antituberculosis treatment (the latter was of borderline significance) and was less common among persons with a left-sided vaccine scar. In that model, Depo-Provera injections were associated with PARV-4 seropositivity among women (adjusted odds ratio 17.27, 95% CI 1.57–189.78; p = 0.02).

To confirm that associations were not biased by assay sensitivity, we conducted a secondary analysis that excluded 81 borderline PARV4-negative persons (AU >0.5 and ODR <1.2) and 35 borderline PARV4-positive persons (AU 0.5–2.0, ODR >1.2) (Table 2). The same factors as in the main analysis were associated with PARV4 seropositivity; receipt of intravenous antimalarial drugs was not significant in the smaller sample.

Conclusions

We retrospectively analyzed samples obtained during a study of elderly Cameroonians from an area where HCV infection was hyperendemic and in which we had collected much information about potential parenteral modes of transmission of blood-borne viruses but less information about other routes (*10*). Because this was a cross-sectional study, the time sequence of exposure routes and PARV4 infection could not be determined. Thus, our results should be considered exploratory.

The sensitivity, specificity, and ability of our assay to identify seroconversions are comparable to those of PCR-based methods for determining active infections and past exposure (*7–9,13*). Exclusion of samples showing low antibody levels that might represent nonspecific reactivity had little effect on the analysis of risk factors.

The results provide some evidence for parenteral transmission of PARV4 in the study community. As was HCV infection (*10*), PARV4 infection was associated with receipt of intravenous antimalarial therapy. This risk factor was found for half of the population we studied, whereas intramuscular Depo-Provera and streptomycin were administered to few patients. In univariate analysis, PARV4 seropositivity was also more common in patients treated with oral antituberculosis drugs. Although the seroprevalence of PARV4 increased with past exposure to intravenous treatments in general, this finding was not statistically significant because antibodies against PARV4 were common among persons who reported no such treatments. This finding, and the lack of association between PARV4 and HCV seropositivity, suggests that other, nonparenteral modes of transmission existed.

Table 2. Correlates of study participants and human parvovirus 4 infection in multivariate analysis, Cameroon, 2009

Participant characteristic	All participants		After exclusions*	
	Adjusted odds ratio (95% CI)	p value	Adjusted odds ratio (95% CI)	p value
Age group, y				
60–64	2.21 (1.13–4.31)	0.02	2.88 (1.16–7.17)	0.02
65–69	1.01 (0.46–2.24)	0.98	1.20 (0.39–3.70)	0.76
70–74	1.16 (0.54–2.46)	0.71	1.51 (0.55–4.16)	0.42
≥75	1.00		1.00	
Tuberculosis				
No	1.00		1.00	
Yes, treated with oral drugs only	2.09 (0.58–7.54)	0.26	2.91 (0.63–13.51)	0.17
Yes, treatment included streptomycin	5.21 (0.99–27.37)	0.05	20.96 (1.67–262.99)	0.02
Vaccine scar, left arm				
Absent	1.00		1.00	
Present	0.37 (0.19–0.71)	0.003	0.32 (0.13–0.78)	0.01
Intravenous treatment for malaria				0.06
No	1.00		1.00	
Yes	1.92 (1.13–3.24)	0.015	1.98 (0.97–4.03)	0.06

*After exclusion of 81 participants with borderline negative results and 35 with borderline positive results.

PARV4 seropositivity was more common in persons 60–64 years of age than in older persons. This finding has 3 potential explanations. First, exposure to the virus might have fluctuated over time. Second, titers of antibodies against PARV4 might progressively wane, eventually leading to false negative results. Third, PARV4 infection might increase long-term risk for death, although this explanation seems unlikely.

Absence of a vaccine scar on the left arm was associated with PARV4 seropositivity. Historical and epidemiologic data suggest that in Cameroon, the left side was used for smallpox vaccine and the right side for *Mycobacterium bovis* BCG (*14*,*15*). Failure of scar development after smallpox vaccination might reflect immunologic characteristics associated with greater susceptibility to PARV4 infection.

Our findings suggest that some parenteral transmission of PARV4 occurred among elderly Cameroonians, but parenteral transmission might not have been the main route of infection. The association with past tuberculosis, although perhaps coincidental, is intriguing and deserves further study.

Initial data collection was funded by the Canadian Institutes for Health Research. The current work was supported solely by funding from The Roslin Institute, University of Edinburgh, Scotland, UK.

Dr Lavoie is a senior resident in infectious diseases and medical microbiology at the Université de Sherbrooke. Her principal research interest is the epidemiology of bloodborne viruses in Cameroon.

References

1. Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E. DNA viruses identified in patients with acute viral infection syndrome. J Virol. 2005;79:8230–6. http://dx.doi.org/10.1128/JVI.79.13.8230-8236.2005
2. Longhi E, Bestetti G, Acquaviva V, Foschi A, Piolini R, Meroni L, et al. Human parvovirus 4 in the bone marrow of Italian patients with AIDS. AIDS. 2007;21:1481–3. http://dx.doi.org/10.1097/QAD.0b013e3281e38558
3. Vallerini D, Barozzi P, Quadrelli C, Bosco F, Potenza L, Riva G, et al. Parvoviruses in blood donors and transplant patients, Italy. Emerg Infect Dis. 2008;14:185–6. http://dx.doi.org/10.3201/eid1401.070610
4. Touinssi M, Reynaud-Gaubert M, Gomez C, Thomas P, Dussol B, Berland Y, et al. Parvovirus 4 in French in-patients: a study of hemodialysis and lung transplant cohorts. J Med Virol. 2011;83:717–20. http://dx.doi.org/10.1002/jmv.22003
5. Panning M, Kobbe R, Vollbach S, Drexler JF, Adjei S, Adjei O, et al. Novel human parvovirus 4 genotype 3 in infants, Ghana. Emerg Infect Dis. 2010;16:1143–6. http://dx.doi.org/10.3201/eid1607.100025
6. Sharp CP, Vermeulen M, Nébié Y, Djoko CF, LeBreton M, Tamoufe U, et al. Epidemiology of human parvovirus 4 infection in sub-Saharan Africa. Emerg Infect Dis. 2010;16:1605–7.
7. Simmonds P, Manning A, Kenneil R, Carnie FW, Bell JE. Parenteral transmission of the novel human parvovirus PARV4. Emerg Infect Dis. 2007;13:1386–8.
8. Sharp CP, Lail A, Donfield S, Simmons R, Leen C, Klenerman P, et al. High frequencies of exposure to the novel human parvovirus, PARV4 in haemophiliacs and injecting drug users detected by a serological assay for PARV4 antibodies. J Infect Dis. 2009;200:1119–25. http://dx.doi.org/10.1086/605646
9. Benjamin LA, Lewthwaite P, Vasanthapuram R, Zhao G, Sharp C, Simmonds P, et al. Human parvovirus 4 (PARV4) as potential cause of encephalitis in children, India. Emerg Infect Dis. 2011;17:1484–7.
10. Pépin J, Lavoie M, Pybus OG, Pouillot R, Foupouapouognigni Y, Rousset D, et al. HCV transmission during medical interventions and traditional practices in colonial Cameroon: potential implications for the emergence of HIV-1. Clin Infect Dis. 2010;51:768–76.
11. Njouom R, Nerrienet E, Dubois M, Lachenal G, Rousset D, Vessière A, et al. The hepatitis C virus epidemic in Cameroon: genetic evidence for rapid transmission between 1920 and 1960. Infect Genet Evol. 2007;7:361–7. http://dx.doi.org/10.1016/j.meegid.2006.10.003
12. Pépin J, Labbé AC. Noble goals, unforeseen consequences: the control of tropical diseases in colonial central Africa and the iatrogenic transmission of blood-borne viruses. Trop Med Int Health. 2008;13:744–53. http://dx.doi.org/10.1111/j.1365-3156.2008.02060.x

13. Lahtinen A, Kivela P, Hedman L, Kumar A, Kantele A, Lappalainen M, et al. Serodiagnosis of primary infections with human parvovirus 4, Finland. Emerg Infect Dis. 2011;17:79–82. http://dx.doi.org/10.3201/eid1701.100750
14. Blanchard M. Précis d'épidémiologie. Médecine préventive et hygiène coloniales. Paris: Vigot Frères; 1938.
15. Brunel M. La tuberculose pulmonaire au Cameroun en 1958, endémie tuberculeuse, formes cliniques, traitement, prophylaxie. Bull Soc Pathol Exot. 1958;51:920–35.

Address for correspondence: Jacques Pépin, CHUS, 3001, 12ème Ave Nord, Sherbrooke, Québec J1H 5N4, Canada; email: jacques.pepin@usherbrooke.ca

Neuroinvasive Disease and West Nile Virus Infection, North Dakota, USA, 1999–2008

Paul J. Carson, Stephanie M. Borchardt, Brian Custer, Harry E. Prince, Joan Dunn-Williams, Valerie Winkelman, Leslie Tobler, Brad J. Biggerstaff, Robert Lanciotti, Lyle R. Petersen, and Michael P. Busch

To determine risk for West Nile virus (WNV) neuroinvasive disease in North Dakota, we tested plasma samples from blood donors for WNV IgG and compared infection rates with reported WNV neuroinvasive disease incidence. We estimate that 1 in 244 WNV infections leads to neuroinvasive disease; risk is substantially increased among men and older persons.

Human infection with West Nile virus (WNV) was first identified in North America in 1999 during an outbreak in New York City (NYC), New York, USA (*1*). Since that time, the ArboNET surveillance system housed in the Centers for Disease Control and Prevention (Atlanta, GA, USA) has documented the virus' spread across the United States (*2*); incidence of disease was highest in the central plains (*3*). Because most persons with WNV infection remain asymptomatic or have West Nile fever, for which routine diagnostic testing is not recommended, the number of infections vastly exceeds the number of cases reported (*4*).

On the basis of an initial study of the 1999 outbreak in NYC, it was estimated that 1 case of neuroinvasive disease (meningitis, encephalitis, or acute flaccid paralysis) occurred for 140 WNV infections (*5*). This ratio is frequently quoted in the literature and has been used to develop national estimates for incidence of WNV infection (*6*). More recent data derived from blood donor screening suggested that WNV neuroinvasive disease (WNND) might occur as frequently as 1 case for 256–353 infections (*7*). Furthermore, surveillance data suggest that the proportion of infected persons in whom WNND develops varies markedly by age and sex (*3*). Previous studies have not clearly identified these differences or used age- and sex-adjusted proportions to estimate the cumulative incidence.

Understanding the full incidence of disease, including asymptomatic infections, is essential for preventing transfusion- and transplantation-associated infections. The purposes of this study were to define the cumulative WNV infection incidence spanning a decade in a highly disease-endemic area and to correlate this with the reported incidence of WNND.

The Study

Plasma from 6,999 sequential volunteer blood donations collected from November through December 2008 at North Dakota donation centers was archived at Creative Testing Solution's donor screening laboratory in Tempe, Arizona, USA. Samples were selected to include only those from donors with a North Dakota residence as determined on the basis of postal codes. The resulting 4,514 samples were tested for WNV-specific IgG, and IgM if IgG test results were reactive, by using commercially available ELISAs (*8*) at Focus Laboratories (Cypress, CA, USA).

The cumulative number of infections by age and sex strata in the North Dakota population was estimated by multiplying the corresponding stratum-specific WNV-specific IgG seroprevalence of blood donors by the North Dakota population (US Census Bureau data, 2010). Because blood donors include only persons ≥16 years of age, the seroprevalence among persons <16 years of age could not be accurately estimated. Therefore, estimates were only calculated for the adult population. For the purposes of this report, adolescents 16–17 years of age are considered adults. The corresponding ratio of WNND cases to infections for each age and sex stratum was determined by dividing the estimated number of infections by the number of WNND cases reported in North Dakota with onset from the first reported case during 2002–2008.

We assessed specificity of the IgG response by examining a subset of 54 samples across the range of IgG responses (every 6th sample) of antibody levels in seropositive donors. We used WNV 90% plaque-reduction neutralization tests and Vero cells as described (*9*).

We calculated relative risks (RRs) and 95% CIs by using standard, asymptotic methods. We performed statistical

Author affiliations: University of North Dakota School of Medicine and Health Sciences, Grand Forks, North Dakota, USA (P.J. Carson, S.M. Borchardt); Sanford Health, Fargo, North Dakota, USA (P.J. Carson); Fargo Veterans Affairs Medical Center, Fargo (S.M. Borchardt); Wisconsin Department of Health, Madison, Wisconsin, USA (S.M. Borchardt); Blood Systems Research Institute, San Francisco, California, USA (B. Custer, L. Tobler, M.P. Busch); Focus Diagnostics, Cypress, California, USA (H.E. Prince); Creative Testing Solutions, Tempe, Arizona, USA (J. Dunn-Williams, V. Winkelman); Centers for Disease Control and Prevention, Fort Collins, Colorado, USA (B.J. Biggerstaff, R. Lanciotti, L.R. Petersen); and University of California San Francisco, San Francisco (M.P. Busch)

DOI: http://dx.doi.org/10.3201/eid1804.111313

Table. Correlation of WNV IgG seroprevalence among blood donors with WNND, by sex and age group, North Dakota, USA, 2002–2008*

Sex and age group, y	No. donors	Seroprevalence, % (95% CI)	Seropositivity, RR (95% CI)	Total population†	WNV-infected population‡	No. WNND cases§	Ratio of WNND cases to WNV infections (95% CI)	WNND, RR (95% CI)	Inverse of ratio¶
Male	2,349	9.2 (6.1–8.3)		279,252	26,122	119	0.0045 (0.0038–0.0054)		220
16–24	321	12.8 (9.1–16.4)	Referent	56,156	7,188	10	0.0014 (0.0008–0.0026)	Referent	719
25–44	562	8.7 (6.4–11.1)	0.7 (0.5–1.0)	89,968	7,827	22	0.0028 (0.0018–0.0042)	2.0 (1.0–4.2)	356
45–64	1,132	9.3 (7.6–11.0)	0.7 (0.5–1.0)	90,701	8,435	34	0.0040 (0.0029–0.0056)	2.9 (1.4–5.9)	248
≥65	334	6.3 (3.7–8.9)	0.5 (0.3–0.8)	42.427	2,672	53	0.0198 (0.0152–0.0258)	14.3 (7.3–28.0)	50
Female	2,165	7.2 (6.2–8.3)		271,968	18,353	63	0.0034 (0.0027–0.0044)		291
16–24	316	9.8 (6.5–13.1)	Referent	50,274	4,926	4	0.0008 (0.0003–0.09)	Referent	1,231
25–44	600	6.7 (4.7–8.7)	0.7 (0.4–1.1)	78,869	5,284	16	0.0030 (0.0018–0.0049)	3.7 (1.2–11.1)	330
45–64	1,010	7.5 (5.9–9.2)	0.8 (0.5–1.1)	87,775	6,583	17	0.0026 (0.0016–0.0042)	3.2 (1.1–9.4)	387
≥65	239	2.9 (0.8–5.1)	0.3 (0.1–0.7)	55,050	1,596	26	0.0163 (0.0111–0.0241)	20.1 (7.0–57.4)	61
Total	4,514	8.2 (7.4–9.0)		551,220	44,511	182	0.0041 (0.0035–0.0047)		244

*WNV, West Nile virus; WNND, WNV neuroinvasive disease; RR, relative risk.
†Source: US Census Bureau, 2010.
‡Seroprevalence × population.
§Data from Centers for Disease Control and Prevention; ArboNET.
¶No. WNV infections/no. WNND cases.

analyses by using SAS version 9.2 (SAS Institute, Cary, NC, USA), Epi Info (Centers for Disease Control and Prevention), and R version 2.11.1 (www.r-project.org).

Of the 4,514 North Dakota blood donors whose plasma samples were tested, 2,349 (52%) were male. Data on race were available for 4,166 (92%) donors: 98% were white/non-Hispanic, 1% (40) were American Indian, and 0.2% (*10*) were black/non-Hispanic. Among 370 (8.2%) donors who were positive for WNV-specific IgG, 28 (7.5%) were positive for WNV-specific IgM, suggesting recent infection. All 54 representative IgG-reactive samples tested by plaque-reduction neutralization tests had WNV-specific neutralizing antibodies, confirming the specificity of the IgG assay results.

We estimate a total of 44,511 WNV infections in North Dakota residents from its 1999 introduction to the United States through 2008. Seroprevalence was highest among persons 16–24 years of age compared with all other ages combined (RR 1.8, 95% CI 1.2–2.6) and men (9.2%) compared with women (7.2%; RR 1.3, 95% CI 1.1–1.6), possibly reflecting differences in mosquito exposure (Table). Comparison of seroprevalence data with reported numbers of WNND cases in North Dakota indicated that WNND was more likely to develop in infected men than women (RR 1.3, 95% CI 1.0–1.8) and that age was a strong predictor for development of WNND (Table). Among persons ≥65 years of age, the risk was 1 in 54 (95% CI 1:43–1:67), ≈16.0× (95% CI 9.1–28.2) as high as that for persons in the youngest age group. Overall, the risk of WNND development after WNV infection was 1 in 244 (95% CI 1:213–1:286).

Conclusions

We estimated that during 1999 through 2008, >40,000 North Dakota residents were infected with WNV. WNND was ≈30% more likely in WNV-infected male than in WNV-infected female donors, and the risk for WNND markedly increased with age. We estimated that the chance of WNND development in WNV-infected persons ≥65 years of age was ≈1 in 50.

The findings of this large study of WNV expand on findings from previous seroprevalence studies (*5*, *10*–*15*) and suggest that the incidence of WNND cases related to WNV infection in North Dakota is nearly half that estimated during the 1999 NYC epidemic (*5*). These findings are more consistent with those of our previous study (*7*), which estimated 256 infections for every case of WNND nationwide. That study was based on projections of infection rates from the yield of WNV nucleic acid amplification screening in blood donors across the United States, and it correlated those rates with the number of WNND cases nationwide reported to ArboNET in 2003.

The NYC study (*5*) might have overestimated the proportion of infections that resulted in WNND because of a

smaller sample size with wider CIs and selection bias resulting from symptomatic persons preferentially enrolling in the survey. Similar to our study, the NYC study indicated that WNND developed in 1 of every 50 infected persons ≥65 years of age (*5*). Published studies have not stratified the risk for WNND by age and sex, which our data indicate are major considerations in projecting overall incidence of WNV infection rates from the number of WNND case reports. The ratio of cases of WNND to cases of WNV infections ranged from 1 in 50 to 1 in 1,231 from the groups with the highest risk (men ≥65 years of age) to lowest risk (women 16–24 years of age).

Our study has potential limitations. Blood donors might not represent the general population with regard to mosquito exposure. Also, blood donation is limited to persons ≥16 years of age; therefore, our analysis is limited to the adult population. The completeness of reporting of WNND cases to ArboNET is also unknown and could vary over time. In addition, differences in WNND rates based on race or ethnicity have not been reported in previous studies, and we were unable to explore these differences because the North Dakota blood donor pool was predominantly white.

Results of our study indicate that ≈1 in 12 North Dakota residents has been infected with WNV. The rate of WNV infection was greatest for younger persons and men. Among those infected with WNV, male sex and older age markedly increased the risk for development of WNND; WNND developed in ≈1 of 50 of the infected men ≥65 years of age. Prevention measures should be particularly targeted to this group during the summer WNV transmission season.

Acknowledgment

We thank Abigail Schrock for assistance with composing the table and formatting the paper.

This work was funded in part by grants from the Centers for Disease Control and Prevention (R01-CI-000214) and the National Heart, Lung, and Blood Institute (RC2-HL-101632).

Dr Carson is chairman of the Department of Infectious Disease and director of Clinical Research at Sanford Health, Fargo, ND. His research interests include West Nile virus infection, antimicrobial drug stewardship, and nosocomial infections.

References

1. Nash D, Mostashari F, Fine A, Miller J, O'Leary D, Murray K, et al. The outbreak of West Nile virus infection in the New York City area in 1999. N Engl J Med. 2001;344:1807–14. http://dx.doi.org/10.1056/NEJM200106143442401
2. Petersen LR, Hayes EB. Westward ho?—The spread of West Nile virus. N Engl J Med. 2004;351:2257–9. http://dx.doi.org/10.1056/NEJMp048261
3. Lindsey NP, Staples JE, Lehman JA, Fischer M. Surveillance for human West Nile virus disease—United States, 1999–2008. MMWR Surveill Summ. 2010;59:1–17.
4. Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. West Nile fever characteristics among viremic persons identified through blood donor screening. J Infect Dis. 2010;202:1354–61. http://dx.doi.org/10.1086/656602
5. Mostashari F, Bunning ML, Kitsutani PT, Singer DA, Nash D, Cooper MJ, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. Lancet. 2001;358:261–4. http://dx.doi.org/10.1016/S0140-6736(01)05480-0
6. Lindsey NP, Kuhn S, Campbell GL, Hayes EB. West Nile virus neuroinvasive disease incidence in the United States, 2002–2006. Vector Borne Zoonotic Dis. 2008;8:35–40. http://dx.doi.org/10.1089/vbz.2007.0137
7. Busch MP, Wright DJ, Custer B, Tobler LH, Stramer SL, Kleinman SH, et al. West Nile virus infections projected from blood donor screening data, United States, 2003. Emerg Infect Dis. 2006;12:395–402. http://dx.doi.org/10.3201/eid1203.051287
8. Hogrefe WR, Moore R, Lape-Nixon M, Wagner M, Prince HE. Performance of immunoglobulin G (IgG) and IgM enzyme-linked immunosorbent assays using a West Nile virus recombinant antigen (preM/E) for detection of West Nile virus– and other flavivirus-specific antibodies. J Clin Microbiol. 2004;42:4641–8. http://dx.doi.org/10.1128/JCM.42.10.4641-4648.2004
9. Oceguera LF III, Patiris PJ, Chiles RE, Busch MP, Tobler LH, Hanson CV. Flavivirus serology by Western blot analysis. Am J Trop Med Hyg. 2007;77:159–63.
10. Mandalakas AM, Kippes C, Sedransk J, Kile JR, Garg A, McLeod J, et al. West Nile virus epidemic, northeast Ohio, 2002. Emerg Infect Dis. 2005;11:1774–7. http://dx.doi.org/10.3201/eid1111.040933
11. Meyer TE, Bull LM, Cain Holmes K, Pascua RF, Travassos da Rosa A, Gutierrez CR, et al. West Nile virus infection among the homeless, Houston, Texas. Emerg Infect Dis. 2007;13:1500–3.
12. Schweitzer BK, Kramer WL, Sambol AR, Meza JL, Hinrichs SH, Iwen PC. Geographic factors contributing to a high seroprevalence of West Nile virus–specific antibodies in humans following an epidemic. Clin Vaccine Immunol. 2006;13:314–8. http://dx.doi.org/10.1128/CVI.13.3.314-318.2006
13. Schellenberg TL, Anderson ME, Drebot MA, Vooght MT, Findlater AR, Curry PS, et al. Seroprevalence of West Nile virus in Saskatchewan's Five Hills Health Region, 2003. Can J Public Health. 2006;97:369–73.
14. Murphy TD, Grandpre J, Novick SL, Seys SA, Harris RW, Musgrave K. West Nile virus infection among health-fair participants, Wyoming 2003: assessment of symptoms and risk factors. Vector Borne Zoonotic Dis. 2005;5:246–51. http://dx.doi.org/10.1089/vbz.2005.5.246
15. Michaels SR, Balsamo GA, Kukreja M, Anderson C, Straif-Bourgeois S, Talati G, et al. Surveillance for West Nile virus cases in Louisiana 2001–2004. J La State Med Soc. 2005;157:269–72.

Address for correspondence: Michael P. Busch, Blood Systems Research Institute, University of California San Francisco, 270 Masonic Ave, San Francisco, CA 94118, USA; email: mbusch@bloodsystems.org

Leaving the Hospital

By Anya Silver

As the doors glide shut behind me,
the world flares back into being—
I exist again, recover myself,
sunlight undimmed by dark panes,
the heat on my arms the earth's breath.
The wind tongues me to my feet
like a doe licking clean her newborn fawn.
At my back, days measured by vital signs,
my mouth opened and arm extended,
the nighttime cries of a man withered
child-size by cancer, and the bells
of emptied IVs tolling through hallways.
Before me, life—mysterious, ordinary—
holding off pain with its muscular wings.
As I step to the curb, an orange moth
dives into the basket of roses
that lately stood on my sickroom table,
and the petals yield to its persistent
nudge, opening manifold and golden.

Poem reprinted from the New Ohio Review, No. 9, Spring, 2011, by permission of Anya Silver.

Dr. Silver is associate professor of literature at Mercer University in Atlanta, Georgia.

DOI: http://dx.doi.org/10.3201/eid1804.AD1804

West Nile Virus Lineage 2 from Blood Donor, Greece

To the Editor: West Nile virus (WNV) is a mosquito-borne flavivirus that primarily causes an asymptomatic or mild disease in humans; however, in <1% of infected persons, it causes neurologic disease. The virus has received increased attention since 2002 when it was established that WNV is transmissible by blood transfusion and organ transplantation (*1*).

A major WNV outbreak occurred in 2010 in Greece; most cases occurred in the northern part of the country (*2*). Of the 197 WNV neuroinvasive cases reported, 33 were fatal (*3*). Many nonneuroinvasive cases were observed (*4*). A lineage 2 WNV (Nea Santa-Greece-2010 strain) was detected in *Culex pipiens* mosquitoes collected at 2 locations where WNV cases had been reported (*5*). Although this strain shows high genetic identity to a Hungarian WNV strain isolated from birds in 2004, it has the amino acid substitution $H_{249}P$ in nonstructural protein 3 (NS3) (*6*). This mutation has been associated with increased virulence in WNV lineage 1 strains (*7*). Clinical WNV disease in humans had not been previously documented in Greece, and surveillance of blood donors in 2006 and 2007 did not show any WNV-positive result (*8*).

On August 11, 2010, shortly after confirmation of the outbreak of WNV infections in humans in Greece, an action plan for the protection of blood safety was initiated. All donors were asked to report any fever-like illness up to 15 days after donation. Individual donation nucleic acid testing (NAT) of all blood donors living in the WNV-affected areas was implemented on August 22, 2010.

The first WNV-positive by NAT result was obtained from a sample donated on August 22. Testing was performed by using the automated Procleix TIGRIS System (Chiron Corporation, Emeryville, CA, USA). The WNV-positive blood donor was a 40-year-old immunocompetent woman, a resident of a village in northern Greece. The village is located between 2 lakes, and the area is one of Europe's major wetlands. The 2 locations where the WNV-positive mosquitoes were collected are near each other (online Appendix Figure, wwwnc.cdc.gov/EID/article/18/4/11-0771-FA1.htm). The woman was working in an open-air fish market and reported numerous mosquito bites. At the time of blood donation, she was asymptomatic; 2 days later she had myalgia, arthralgia, and severe retro-orbital pain, lasting 2–3 days each. A second blood sample taken on August 26 was also NAT positive.

Serologic testing for WNV IgM and IgG was performed by ELISA (WNV IgM Capture DxSelect and WNV IgG DxSelect; Focus Diagnostics Inc., Cypress, CA, USA). An index ≈1.5 for IgM and ≈1.1 for IgG was considered positive. No antibodies were detected in the initial and second serum samples; however, a third sample taken on September 20 was positive for WNV IgM and IgG (indices 4.7 and 3.8, respectively).

Nested reverse transcription PCRs with the initial blood sample gave positive results (*9*,*10*). WNV lineage 2 sequences were obtained and were identical to those of the Nea Santa-Greece-2010 strain (*6*). One milliliter of each 1:10 and 1:100 dilution of whole blood in minimum essential medium containing antimicrobial drugs and 2% fetal bovine serum was placed on Vero E6 cell monolayers in 25-cm^2 cell culture flasks. The procedure was performed in a biosafety level 3 laboratory, in which WNV lineage 2 had never been handled. After the sample was incubated 1 hour at 37°C in a 5% CO_2 incubator, 9 mL of fresh medium containing 4% fetal bovine serum was added.

Cytopathic effects were observed in the flask inoculated with the 1:100 dilution on day 3 after infection. An aliquot of supernatant was used to infect fresh cell monolayers. WNV growth in the cell culture was demonstrated by reverse transcription PCR and immunofluorescence assay. Sequences of the cell culture isolate were identical to those of the directly detected virus.

To check whether the isolate possessed the $H_{249}P$ substitution, a set of nested primers spanning the region 5140–5660 from the WNV genome was designed: NS3a-5′-GCTGGCTTCGAACCTGA-3′and NS3b-5′-CAATCATCGTTCTTGC-3′ for the first round PCR and NS3c-5′-GCTGCTGAGATGTCTGA-3′and NS3d-5′-TCATATCCAGTGTTCCA-3′ for the nested PCR. The $H_{249}P$ substitution was present. Sequences were submitted to GenBank (accession nos. JF917091, JF917092).

Virus isolation from WNV patients is usually unsuccessful because viremia levels are low and last only a short time. WNV strains are usually isolated from immunocompromised patients, blood donors, IgM-negative immunocompetent patients who seroconverted, or autopsy brain samples. For the donor reported here, WNV was isolated 2 days before illness onset, when no antibodies were present. The WNV-positive blood donor was detected 1 day after the introduction of blood screening.

The early diagnosis of the initial human WNV cases in Greece, which resulted in prompt implementation of NAT testing, had a substantial positive impact on the safety of the blood supply in the affected areas. The risk for virus transmission was reduced for blood recipients, in particular those who receive multiple transfusions and immunocompromised patients in need of transfusion.

This study was supported by the Hellenic Centre for Disease Control and Prevention.

Anna Papa, Constantina Politis, Athina Tsoukala, Aikaterini Eglezou, Vassiliki Bakaloudi, Maria Hatzitaki, and Katerina Tsergouli

Author affiliations: Aristotle University of Thessaloniki, Thessaloniki, Greece (A. Papa, K. Tsergouli); National Coordinating Haemovigilance Centre of the Hellenic Centre for Disease Control and Prevention, Athens, Greece (C. Politis); AHEPA University General Hospital Blood Centre, Thessaloniki (A. Tsoukala, V. Bakaloudi); Papanikolaou General Hospital Blood Bank, Thessaloniki (A. Eglezou); and Koutlibaneio General Hospital Blood Centre, Larissa, Greece (M. Hatzitaki)

DOI: http://dx.doi.org/10.3201/eid1804.110771

References

1. Centers for Disease Control and Prevention. Update: investigations of West Nile virus infections in recipients of organ transplantation and blood transfusion. MMWR Morb Mortal Wkly Rep. 2002;51:833–6.
2. Papa A, Danis K, Baka A, Bakas A, Dougas G, Lytras T, et al. Ongoing outbreak of West Nile virus infections in humans in Greece, July–August 2010. Euro Surveill. 2010;15(34):pii:19644.
3. Danis K, Papa A, Theocharopoulos G, Dougas G, Athanasiou M, Detsis M, et al. Outbreak of West Nile virus infection in Greece, 2010. Emerg Infect Dis. 2011;17:1868–72.
4. Anastasiadou A, Economopoulou A, Kakoulidis I, Zilidou R, Butel D, Zorpidou D, et al. Non-neuroinvasive West Nile virus infections during the outbreak in Greece. Clin Microbiol Infect. 2011;17:1681–3. http://dx.doi.org/10.1111/j.1469-0691.2011.03642.x
5. Papa A, Xanthopoulou K, Gewehr S, Mourelatos S. Detection of West Nile virus lineage 2 in mosquitoes during a human outbreak in Greece. Clin Microbiol Infect. 2011;17:1176–80. http://dx.doi.org/10.1111/j.1469-0691.2010.03438.x
6. Papa A, Bakonyi T, Xanthopoulou K, Vazquez A, Tenorio A, Nowotny N. Genetic characterization of West Nile virus lineage 2, Greece, 2010. Emerg Infect Dis. 2011;17:920–2.
7. Brault AC, Huang CY, Langevin SA, Kinney RM, Bowen RA, Ramey WN, et al. A single positively selected West Nile viral mutation confers increased virogenesis in American crows. Nat Genet. 2007;39:1162–6. http://dx.doi.org/10.1038/ng2097
8. Kantzanou MN, Moschidis ZM, Kremastinou G, Levidiotou S, Karafoulidou A, Politis C, et al. Searching for West Nile virus (WNV) in Greece. Transfus Med. 2010;20:113–7. http://dx.doi.org/10.1111/j.1365-3148.2009.00964.x
9. Sánchez-Seco MP, Rosario D, Domingo C, Hernandez L, Valdes K, Guzman MG, et al. Generic RT-nested-PCR for detection of flaviviruses using degenerated primers and internal control followed by sequencing for specific identification. J Virol Methods. 2005;126:101–9. http://dx.doi.org/10.1016/j.jviromet.2005.01.025
10. Shi PY, Kauffman EB, Ren P, Felton A, Tai JH, Dupuis AP II, et al. High-throughput detection of West Nile virus RNA. J Clin Microbiol. 2001;39:1264–71. http://dx.doi.org/10.1128/JCM.39.4.1264-1271.2001

Address for correspondence: Anna Papa, First Department of Microbiology, Medical School, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece; email: annap@med.auth.gr

Tuberculosis Screening before Anti–Hepatitis C Virus Therapy in Prisons

To the Editor: Prisons represent a crucial setting for tuberculosis (TB) control. Worldwide, reported TB rates for correctional system populations have been 10–100× higher than rates for the local civilian populations, and TB outbreaks with a high number of TB multidrug-resistant cases have been documented (*1*,*2*). Prisons are known as social and sanitary pathology reservoirs in which TB is often associated with chronic infectious diseases caused by HIV, hepatitis B virus (HBV), or hepatitis C virus (HCV) (*2*).

HCV prevalence among inmates is 30%–40% (range 2%–58%), which is higher than that in the general population and is related to injection drug use (*3*). For these reasons, effective anti-HCV therapeutic approaches are recommended by national and international guidelines for decreasing illness, death rates, and reservoirs of infection in prisons (*4*,*5*).

The standard of care for patients with chronic hepatitis C infection is represented by pegylated interferon-α (Peg-IFN) and ribavirin. These drugs determine complex antiviral, immunomodulatory, and antiproliferative actions, which can cause serious side effects such as leukopenia/neutropenia and alterations in the cytokine network (*3*). Although severe cellular immunodeficiency can often facilitate the development of many infections, only 4 clinical cases of TB in patients undergoing HCV antiviral therapy have been described in the literature (*6*–*8*), and only 1 of these was clearly described as a TB reactivation (*7*).

We describe a case of pulmonary TB reactivation during therapy with Peg-IFN and ribavirin in a 44-year-old white male inmate, affected by genotype 1b/4a chronic hepatitis C. After prison admission in 2009, he underwent routine screening tests for infectious diseases, which indicated HCV antibody, HBV surface antibody, HBV core IgG antibody, and tuberculin skin test positivity. Results of chest radiograph and HIV screening were negative.

His previous history involved injection drug use, smoking, and alcohol consumption. Anti-HCV therapy of directly observed administration of Peg-INF α-2a (180 μg/wk) and ribavirin (1,200 mg/d) was started. During therapy, the patient had only mild musculoskeletal pain and temporary

irritability. During the 12th week of treatment, HCV-RNA decreased by 1 $\log_{10}$; therefore, the ribavirin dose was increased to 1,600 mg per day. Even after the therapy modification, no virologic suppression was found. Although during the 33rd week of therapy the patient had weakness, cough, and 2 episodes of hemoptysis, the results of a physical examination were unremarkable. Therapy was immediately discontinued. Sputum specimens collected on 3 consecutive days were positive for acid-fast bacilli. Nucleic acid amplification assays and cultures performed on mycobacteria growth indicator tube (Bactec MGIT; Becton Dickinson, Franklin Lakes, NJ, USA) and on Lowenstein-Jensen medium were positive for *Mycobacterium tuberculosis* isolates that later showed sensitivity to streptomycin, isoniazid, rifampin, and ethambutol.

The patient was isolated at the Institute of Respiratory Diseases, University of Sassari–Faculty of Medicine, Sassari, Italy. A chest radiograph showed opacity in the upper right lung, and a high-resolution computed tomography scan (Figure) showed multiple lesions that were considered compatible with TB. CD4+ cell count (52.4%; 669 cells/mm^3) was within reference range.

TB treatment with rifampin, isoniazid, pyrazinamide, and ethambutol with pyridoxine was started. After 4 weeks of therapy, 3 sputum specimens were negative for acid-fast bacilli, but a bacterial culture was still positive; mycobacteria indicator growth tube culture was negative after 5 weeks.

The interaction process between the IFN-α/β system and *M. tuberculosis* is not well known; nevertheless, Peg-IFN, alone and in combination with ribavirin, is considered poten-tially immunosuppressive (*4*,*9*). Immuno-deficiency caused by Peg-IFNs and ribavirin may cause lower leukopenia/lymphopenia values than expected during anti-HCV treatment and may also lower CD4+ cell count and function (*10*).

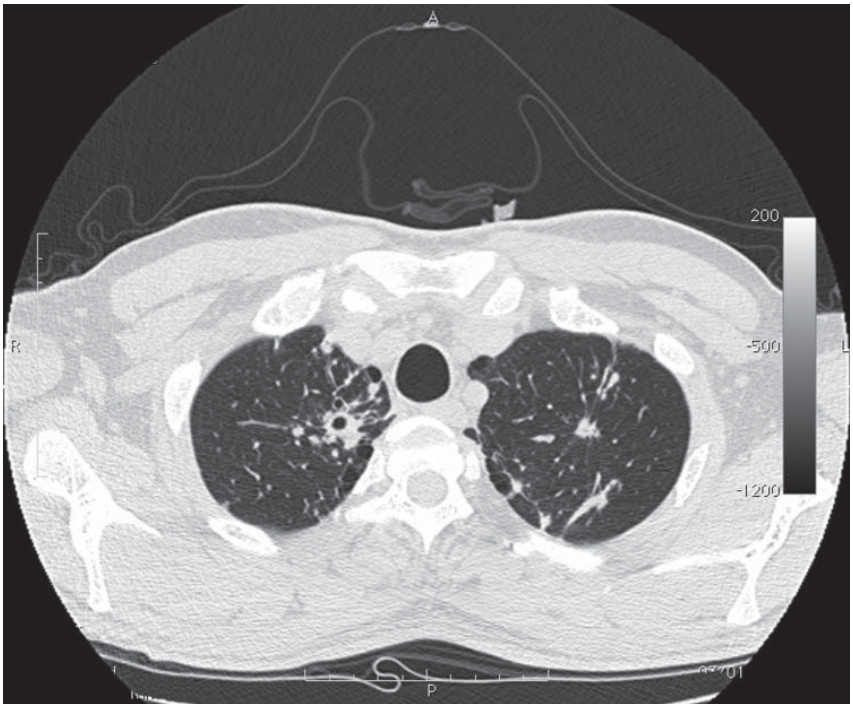


Figure. Computed tomography image of chest of patient with tuberculosis after anti–hepatitis C virus therapy. A parenchymal distortion 32 mm in diameter is shown in the upper right lung with initial central excavation 10 mm in diameter. Similar lesions 8 mm in diameter without central excavation are shown in the upper left lung.

In the patient reported here, CD4+ cell count was within the reference range, and lung TB with excavations developed after 34 weeks of therapy. Before TB diagnosis, the patient had not shown any signs or symptoms of other infections and had not mentioned serious adverse effects from Peg-IFN and ribavirin treatment. However, the initial symptoms of TB and the common side effects of Peg-IFN therapy can be similar, which could have led to a delay in the diagnosis of TB.

In conclusion, even if only a few cases of active TB have been reported in the literature, it is well known that standard anti-HCV treatment increases the risk for infections. A high proportion of patients with positive purified protein derivative results, isolation of >30% of multidrug-resistant strains of *M. tuberculosis*, and high prevalence of HCV antibody are concomitant among inmates. These data, together with current recommendations for increasing use of Peg-IFN and ribavirin in marginalized populations in correctional facilities, show the need to consider TB risk before starting HCV antiviral therapy. The management of simultaneous HCV and *M. tuberculosis* infections in prisons presents particular difficulties and pitfalls to overcome. In prisons, the clinical history of inmates should be carefully evaluated, a tuberculin skin test or Quantiferon TB in Tube test (Cellestis, Melbourne, Australia) should be performed, and, if those results are positive, a chest radiograph should be taken. Before receiving Peg-IFN, purified protein derivative–positive patients should receive anti-TB chemoprophylaxis. The case described here underscores the need for a careful and multidisciplinary evaluation of inmate patients for latent TB before administration of Peg-IFN and ribavirin therapy, thus avoiding reactivation.

Sergio Babudieri, Andrea Soddu, Monica Murino, Paola Molicotti, Alberto A. Muredda, Giordano Madeddu, Alessandro G. Fois, Stefania Zanetti, Pietro Pirina, and Maria Stella Mura

Author affiliations: University of Sassari, Sassari, Italy (S. Babudieri, A. Soddu, P. Molicotti, G. Madeddu, M.S. Mura, S. Zanetti, A.G. Fois, P. Pirina); and Ministry of Justice, Sassari (M. Murino, A.A. Muredda)

DOI: http://dx.doi.org/10.3201/eid1804.111016

References

1. World Health Organization. Tuberculosis control in prisons. A manual for programme managers. WHO/CDS/TB/2000.281. Geneva: The Organization; 2000 [cited 2012 Jan 31]. http://whqlibdoc.who.int/hq/2000/WHO_CDS_TB_2000.281.pdf
2. Carbonara S, Babudieri S, Longo B, Starnini G, Monarca R, Brunetti B, et al. Correlates of *Mycobacterium tuberculosis* infection in a prison population. Eur Respir J. 2005;25:1070–6. http://dx.doi.org/10.1183/09031936.05.00098104
3. Vescio MF, Longo B, Babudieri S, Starnini G, Rezza G, Monarca R. Correlates of HCV seropositivity in prison inmates: a meta-analysis. J Epidemiol Community Health. 2008;62:305–13. http://dx.doi.org/10.1136/jech.2006.051599
4. National Institutes of Health. Consensus statement on management of hepatitis C. NIH Consens State Sci Statements. 2002;19:1–46 [cited 2012 Jan 31]. http://consensus.nih.gov/2002/2002HepatitisC2002116html.htm
5. Almasio PL, Babudieri S, Barbarini G, Brunetto M, Conte D, Dentico P, et al. Recommendations for the prevention, diagnosis, and treatment of chronic hepatitis B and C in special population groups (migrants, intravenous drug users and prison inmates). Dig Liver Dis. 2011;43:589–95. http://dx.doi.org/10.1016/j.dld.2010.12.004
6. Sabbatani S, Manfredi R, Marinacci G, Pavoni M, Cristoni L, Chiodo F. Reactivation of severe acute pulmonary tuberculosis during treatment with pegylated interferon-alpha and ribavirin for chronic HCV hepatitis. Scand J Infect Dis. 2006;38:205–8. http://dx.doi.org/10.1080/00365540500263268
7. Belkahla N, Kchir H, Maamouri N, Ouerghi H, Haritz FB, Chouaib S. Reactivation of tuberculosis during dual therapy with pegylated interferon and ribavirin for chronic hepatitis C [in French]. Rev Med Interne. 2010;31:e1–3. Epub 2010 Jun 3. http://dx.doi.org/10.1016/j.revmed.2009.11.017
8. Farah R, Awad J. The association of interferon with the development of pulmonary tuberculosis. Int J Clin Pharmacol Ther. 2007;45:598–600.
9. Puoti M, Babudieri S, Rezza G, Viale P, Antonini MG, Maida I, et al. Use of pegylated interferons is associated with an increased incidence of infections during combination treatment of chronic hepatitis C: a side effect of pegylation? Antivir Ther. 2004;9:627–30.
10. Fried MW. Side effects of therapy of hepatitis C and their management. Hepatology. 2002;36(Suppl1):S237–44. http://dx.doi.org/10.1002/hep.1840360730

Address for correspondence Sergio Babudieri U.O. di Malattie Infettive Università degli Studi di Sassari via Enrico De Nicola n. 1 07100, Sassari, Italy; email: babuder@uniss.it

Deficient Reporting in Avian Influenza Surveillance, Mali

To the Editor: In response to influenza outbreaks caused by highly pathogenic avian influenza virus (HPAIV) throughout western Africa as of 2006, the National Veterinary Epidemiologic Surveillance Network of Mali (EPIVET-Mali) started conducting domestic and wild bird surveillance. No HPAI outbreaks were reported to the World Organisation for Animal Health. An evaluation survey conducted in 2009 enabled identification and correction of some weaknesses in the organization and functioning of the network (*1*). However, no attempt was made to assess how much information on bird health in backyard poultry farms (which account for ≈95% of the total poultry population in Mali) actually reached EPIVET-Mali veterinarians and technicians. Therefore, we quantified reporting of clinical signs of avian diseases, especially those suggesting HPAI, by poultry owners in Mali.

We used a pilot-tested standardized quantitative and qualitative questionnaire to conduct face-to-face interviews in 32 randomly selected villages in the southern half of Mali (which accounts for 98% of the poultry population). In each village, we conducted interviews in 4 randomly chosen households. No eligibility criteria were used for household selection because all village households had poultry. Interviews were repeated 6 times (approximately every 3 months) during November 2009–February 2011 in the same villages and whenever possible in the same households. If it was not possible to repeat an interview in a previously interviewed household (absence of the household chief), the neighboring household was interviewed.

For each household, data were collected on number of sick and dead birds in the previous 3 months, clinical signs observed, and their notification or lack thereof to veterinary authorities. Households in which birds showed ≥3 of the following clinical signs (diarrhea, respiratory disorder, nervous signs, cyanosis of the combs or wattles, and mortality rate >50%) were considered as having clinical signs suggesting HPAI. The study was approved by the Direction Nationale des Services Vétérinaires and traditional authorities in all 32 villages, and oral consent was obtained from the poultry owners before interviews.

A total of 110–128 households were investigated at each study interval, depending on village accessibility and presence or absence of household chiefs (Table). We conducted 738 household investigations in 152 households (80 households were

Table. Observations and reporting of sick poultry and signs of influenza suggesting HPAI virus infection, Mali, 2009–2011*

Date of investigation	Total no. households investigated	No. with sick poultry/ no. with information on sick poultry (%)	No. with HPAI signs/ no. with information on signs (%)	No. with reported sick poultry/ no. with sick poultry and information on reporting (%)	No. with reported HPAI signs/no. with signs and information on reporting (%)
2009 Nov	128	67/128 (52.3)	25/124 (20.2)	7/66 (10.6)	5/25 (20.0)
2010 Feb	128	68/128 (53.1)	21/123 (17.1)	4/68 (5.9)	0/21 (0.0)
2010 May	127	47/127 (37.0)	6/118 (5.1)	9/45 (20.0)	2/6 (33.3)
2010 Sep	110	37/110 (33.6)	17/107 (15.9)	3/34 (8.8)	2/17 (11.8)
2010 Nov	124	53/124 (42.7)	7/119 (5.9)	12/51 (23.5)	4/7 (57.1)
2011 Feb	121	57/121 (47.1)	10/115 (8.7)	8/55 (14.5)	2/10 (20.0)
Total	738	329/738 (44.6)	86/706 (12.2)	43/319 (13.5)	15/86 (17.4)

*Information was obtained for 3 months before the interview. HPAI, highly pathogenic avian influenza.

interviewed 6 times, 26 five times, 11 four times, 21 three times, 7 two times, and 7 one time). Observation of sick poultry in the 3 months before the interview was reported in 44.6% of household investigations, and observation of signs suggesting HPAI was reported in 12.2% (Table). Notification of veterinary authorities was reported in 13.5% of household investigations with sick poultry and in 17.4% of household investigations with signs suggesting HPAI (Table).

When we considered the 80 households interviewed 6 times, observation of sick poultry and signs suggesting HPAI varied over time ($p = 0.043$ and $p = 0.018$, respectively, by Cochran Q test), whereas variation over time could not be tested for notification because of an insufficient number of observations. When we considered all 738 household investigations as independent investigations, observation of sick poultry and signs suggesting HPAI varied over time ($p = 0.008$ and $p<0.001$, respectively, by χ^2 test), but reporting of sick poultry did not vary over time ($p = 0.06$, by χ^2 test). Reporting of signs of HPAI could not be tested.

These results illustrate gaps in reporting signs suggesting HPAI by backyard poultry owners. Although these signs could also be those of Newcastle disease, which is present in Mali (*2*), these signs should be reported because HPAI and Newcastle disease are officially targeted by EPIVET-Mali. One survey attempted to similarly quantify the level of HPAI reporting in Africa. In Kwara State in Nigeria, 56.5% of respondents indicated that they would not notify officials if they suspected HPAI in their flocks (*3*). Reluctance of poultry owners to comply with notification and culling obligations has also been reported in Indonesia (*4*). Several studies that assessed knowledge and practices of poultry workers with regard to avian influenza have been conducted in different countries, including developing countries (*5*,*6*). These studies were useful for better defining content of risk mitigation advice messages and the audience they should primarily target.

In our survey, occurrence of disease in Mali varied over time, which was expected because of the seasonal pattern of many avian diseases, especially Newcastle disease, in western Africa (*7*). However, reporting of sick poultry did not vary over time despite seasonality of activities in rural areas. Lack of awareness of who to report to, fatalistic attitudes toward animal diseases, and mistrust toward the government and its compensation schemes are among the major constraints affecting the likelihood of HPAI signs being reported (*3*,*6*,*8*). However, approaches associating socioanthropology and epidemiology have recently been developed to help solve the problem posed by deficient reporting (*9*).

Acknowledgments

We thank Abass Diarra, Kadiatou Diarra Sissoko, Souleymane Magassa, Idrissa Traoré, and Issa Traoré for participating in field investigations; the Laboratoire Central Vétérinaire and the Direction Nationale des Services Vétérinaires for facilitating the survey; and all poultry farmers for participating in the study.

This study was supported by the French Ministry of Foreign Affairs through the Ecology and Epidemiology of Avian Influenza and Newcastle Diseases in Developing Countries Project.

Sophie Molia, Badian Kamissoko, Maimouna Sanogo Sidibé, Adama Diakité, Mahmoudou Diall, and Mamadou Racine N'Diaye

Author affiliations: Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier, France (S. Molia); Laboratoire Central Vétérinaire, Bamako, Mali (B. Kamissoko, A. Diakité); Direction Nationale des Services Vétérinaires, Bamako (M.S. Sidibé); and Pan African Programme for the Control of Epizootics–Mali, Bamako (M. Diall, M.R. N'Diaye)

DOI: http://dx.doi.org/10.3201/eid1804.111102

References

1. Molia S, Lapeyre S, Sidibé MS, Sissoko K, N'Diaye MR, Diall M, et al. Semi-quantitative evaluation of the epidemiosurveillance network for highly pathogenic avian influenza in Mali [in French]. Epidémiologie et Santé Animale. 2010;57:91–103.
2. Servan de Almeida R, Maminiaina OF, Gil P, Hammoumi S, Molia S, Chevalier V, et al. Africa, a reservoir of new virulent strains of Newcastle disease virus? Vaccine. 2009;27:3127–9. http://dx.doi.org/10.1016/j.vaccine.2009.03.076

3. Musa OI, Aderibigbe SA, Salaudeen GA, Oluwole FA, Samuel SO. Community awareness of bird flu and the practice of backyard poultry in a north-central state of Nigeria. J Prev Med Hyg. 2010;51:146–51.
4. Pawitan JA. Averting avian influenza pandemic: SOS from a developing country. Lancet Infect Dis. 2006;6:756–7. http://dx.doi.org/10.1016/S1473-3099(06)70638-7
5. Barennes H, Martinez-Aussel B, Vongphrachanh P, Strobe M. Avian influenza risk perceptions, Laos. Emerg Infect Dis. 2007;13:1126–8.
6. Fasina FO, Bisschop SP, Ibironke AA, Meseko CA. Avian influenza risk perception among poultry workers, Nigeria. Emerg Infect Dis. 2009;15:616–7. http://dx.doi.org/10.3201/eid1504.070159
7. Awan MA, Otte MJ, James AD. The epidemiology of Newcastle disease in rural poultry: a review. Avian Pathol. 1994;23:405–23. http://dx.doi.org/10.1080/03079459408419012
8. Kanamori S, Jimba M. Compensation for avian influenza cleanup. Emerg Infect Dis. 2007;13:341–2. http://dx.doi.org/10.3201/eid1302.061391
9. Desvaux S, Figuié M. Formal and informal surveillance systems: how to build bridges? [in French]. Epidémiologie et Santé Animale. 2011;59–60:352–5.

Address for correspondence: Sophie Molia, Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Unité Propre de Recherche Animal et Gestion Intégrée des Risques, Campus International de Baillarguet, TA C-22/E, 34398 Montpellier Cedex 5, France; email: sophie.molia@cirad.fr

Myxozoan Parasite in Brain of Critically Endangered Frog

To the Editor: More than three quarters of critically endangered species of amphibians are threatened by infectious disease; several are already extinct (*1*). In 2010, the yellow-spotted bell frog (*Litoria castanea*), which was presumed to be extinct, was rediscovered in the Southern Tablelands of New South Wales, Australia. This species of frog had not been seen for 30 years, and a chytrid fungus, *Batrachochytrium dendrobatidis*, was thought to be the reason (*1*,*2*). The number of frogs in the rediscovered population is estimated to be 100; if numbers are that low, the yellow-spotted bell frog is the most critically endangered frog in Australia.

Several yellow-spotted bell frogs were collected for a captive breeding program at Taronga Zoo in Sydney, New South Wales, Australia. Generalized edema developed in a subadult male frog after 8 months of captivity in strict quarantine conditions. The frog subsequently died, and later an adult male frog was also found dead. Results of necropsy on both frogs at the Australian Registry of Wildlife Health revealed subcutaneous edema, intracoelomic fluid, and swollen kidneys with pale foci. Histopathologic examination demonstrated chronic severe tubulonephropathy and acute severe encephalomalacia. Coalescing foci of hemorrhage and malacia were observed in the caudal brainstem and were associated with small multinucleated (1 × 1 μm) parasites forming plasmodia-like structures 10–20 μm in diameter (Figure). Plasmodia were present in large numbers (1–5/40× field) in the spinal cord. Organisms that were morphologically consistent with myxozoan parasites detected in other frogs in Australia were found predominately within axons and were uncommonly present in vascular endothelial cells (*3*). Characteristic hepatic lesions, including lymphoplasmacytic hepatitis with biliary hyperplasia and loss of hepatocytes, were also present.

The cause of death was renal failure, a common problem in aged frogs; however, these frogs were young, and therefore the cause of the renal changes was perplexing. We considered whether toxins (e.g., improperly cured polyvinyl chloride glue) or an infectious process might be possible causes. Staff in the zoo's breeding program were questioned and indicated that the opportunity for introduction of a toxin was low. In addition, results for virus isolation and fungal and bacterial cultures were negative. We retrospectively reexamined histologic sections of an endangered booroolong frog (*Litoria booroolongensis*) that had similar brain lesions and intralesional myxozoan parasites (*3*). Tissue samples were submitted to the Faculty of Veterinary Science, The University of Sydney, for identification.

DNA was extracted from brain tissues (20 mg) by using the PureLink DNA Kit (Invitrogen, Mulgrave, Victoria, Australia). To test for myxozoans, we used a highly Myxozoa-specific PCR to amplify the complete internal transcribed spacer of the ribosomal DNA (*3*). Myxozoan-positive amplicons were directly sequenced at Macrogen Inc. (Seoul, South Korea), analyzed by using the CLC Main Workbench (CLC bio, Aahrus, Denmark), and deposited in GenBank (accession nos. JN977605–09).

PCR produced a 973-bp amplicon with DNA from brain and liver of the yellow-spotted bell frogs and the booroolong frog. DNA from the frogs showed 100% identity with each other, as did sequences from brain and liver. A BLASTN (*4*) search of

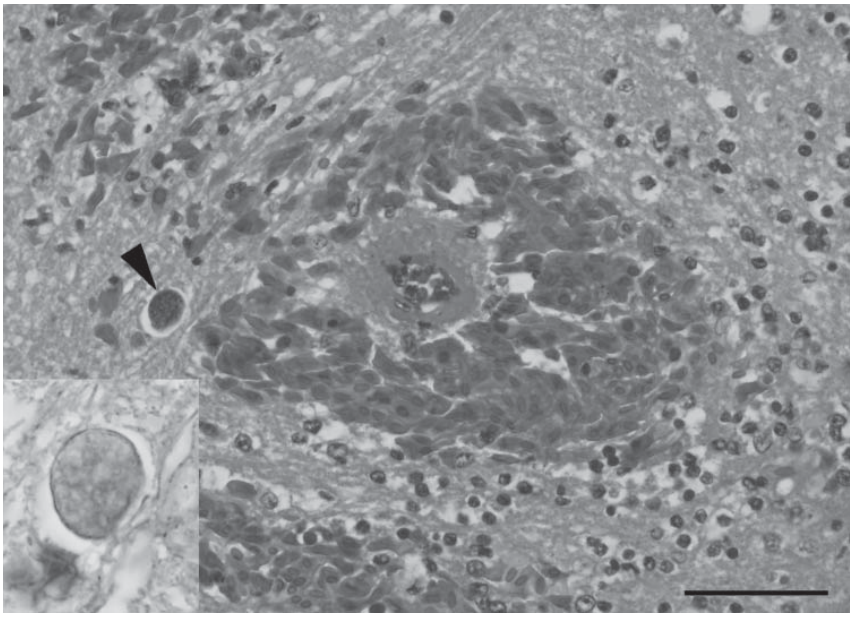


Figure. Acute severe encephalomalacia in the caudal brainstem of a captive yellow-spotted bell frog from Sydney, Australia. This lesion was characterized by hemorrhage, vascular necrosis, and parasites consistent with Myxozoa (arrowhead) (hematoxylin and eosin stain; scale bar = 50 μm). Staining for axons confirmed intraaxonal location of the myxozoan parasites (inset, Holmes silver nitrate with Loxul Fast Blue stain).

public DNA sequence repositories returned the internal transcribed spacer of the ribosomal DNA of a myxozoan parasite, *Cystodiscus axonis* (syn. *Myxidium* sp. 'brain'), as the most closely related sequence (*3*,*5*). Pairwise comparison revealed 100% identity with *C. axonis*. During the brain phase of infection, *C. axonis* parasites reside within the myelinated axons (*5*), and special staining for axons confirmed this location for the brain parasites in this study. Screening of tissue sections from frogs trapped in the same locality as the yellow-spotted bell frogs revealed the presence of the parasite in the central nervous system of 8 of 10 stony creek frogs (*Litoria wilcoxi*) and 1 of 5 Eastern banjo frogs (*Limnodynastes dumerilii*).

Little information exists about the pathologic significance of myxozoan parasite in frogs and tadpoles (*6*). In Australia, *Cystodiscus* spp. parasites of frogs are emerging and have spread widely along the eastern coast in the past 40 years; they were first detected in a frog collected in 1966 (*7*). Molecular characterization revealed 2 cryptic *Cystodiscus* parasites in frogs endemic to Australia and in the invasive cane toad (*3*). However, the cane toad did not introduce this parasite into Australia because cane toads from Hawaii, which are devoid of the parasite, were the source population for toads in Australia. The parasites seem to be native to Australia, and the invasive cane toad plays a spill-back role in their dissemination; however, it is not known how these parasites were disseminated outside the cane toad range (*3*).

Frog myxozoan parasites are yet to be documented as a cause of population decline; yet, the frequent presence of these parasites in moribund animals in captivity, including the yellow-spotted bell frog, demonstrates the need to monitor parasites in endangered frog populations worldwide. On the basis of our necropsy findings in the central nervous system of 2 yellow-spotted bell frogs, we encourage other investigators to consider the potential role that myxozoan parasites may play in wild and captive populations of declining frogs worldwide.

Acknowledgment

The Australian Registry of Wildlife Health, Taronga Conservation Society Australia, acknowledges the Australian Biosecurity Intelligence Network for information management and communication systems that contributed to this research. The Network is a project of the National Collaborative Research Infrastructure Strategy.

This study was funded through the Faculty of Veterinary Science, University of Sydney, diagnostic laboratory.

Ashlie Hartigan, Cheryl Sangster, Karrie Rose, David N. Phalen, and Jan Šlapeta

Author affiliations: The University of Sydney, Sydney, New South Wales, Australia (A. Hartigan, D. N. Phalen, J. Šlapeta); and Taronga Conservation Society Australia, Mosman, New South Wales, Australia (C. Sangster, K. Rose)

DOI: http://dx.doi.org/10.3201/eid1804.111606

References

1. Keesing F, Belden LK, Daszak P, Dobson A, Harvell CD, Holt RD, et al. Impacts of biodiversity on the emergence and transmission of infectious diseases. Nature. 2010;468:647–52. http://dx.doi.org/10.1038/nature09575
2. Mahony M. Review of the declines and disappearances within the bell frog species group (*Litoria aurea* species group) in Australia. In: Campbell A, editor. Declines and disappearances of Australian frogs. Canberra (Australia): Environment Australia; 1999. p. 81–93 [cited 2011 Nov 1]. http://www.environment.gov.au/biodiversity/threatened/publications/pubs/frogs.pdf
3. Hartigan A, Fiala I, Dyková I, Jirků M, Okimoto B, Rose K, et al. A suspected parasite spill-back of two novel *Myxidium* spp. (Myxosporea) causing disease in Australian endemic frogs found in the invasive Cane Toad. PLoS ONE. 2011;6:e18871. http://dx.doi.org/10.1371/journal.pone.0018871

4. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol. 2000;7:203–14. http://dx.doi.org/10.1089/10665270050081478
5. Hartigan A, Fiala I, Dyková I, Rose K, Phalen DN, Šlapeta J. New species of Myxosporea from frogs and resurrection of the genus *Cystodiscus* Lutz, 1889 for species with myxospores in gallbladders of amphibians. Parasitology. 2012; Epub ahead of print. http://dx.doi.org/10.1017/S0031182011002149
6. Sitjà-Bobadilla A. Can myxosporean parasites compromise fish and amphibian reproduction? Proc Biol Sci. 2009;276:2861–70. http://dx.doi.org/10.1098/rspb.2009.0368
7. Hartigan A, Phalen DN, Šlapeta J. Museum material reveals a frog parasite emergence after the invasion of the Cane Toad in Australia. Parasit Vectors. 2010;3:50. http://dx.doi.org/10.1186/1756-3305-3-50

Address for correspondence: Jan Šlapeta, Laboratory of Veterinary Parasitology, McMaster Building B14, Faculty of Veterinary Science, The University of Sydney, Sydney, New South Wales 2006, Australia; email: jan.slapeta@sydney.edu.au

Mayaro Virus Infection in Traveler Returning from Amazon Basin, Northern Peru

To the Editor: We report the case of a 27-year-old male Swiss tourist who spent 3.5 weeks (July 6–30, 2011) vacationing in the vicinity of Tarapoto, a small city located in the rainforests of the Amazon Basin in northern Peru. An acute febrile illness developed in the man during the second week of his stay. Signs and symptoms of illness were chills, malaise, frontal headache, generalized myalgia, a self-limiting painful cervical and inguinal lymphadenopathy (lasting ≈1 week), slowly progressive and pronounced polyarthralgic pains of the peripheral joints, and a transient nonpruritic maculopapular rash (starting at the forearms ≈1 week after onset of fever and spreading to the trunk and later to the neck and face before fading after 3 days).

The traveler sought care at the local hospital, where physicians diagnosed suspected dengue fever on the basis of the clinical signs and symptoms, and received symptomatic treatment with paracetamol. The fever and other signs subsided within 1 week, except the arthralgia, which did not improve. The polyarthritis initially was accompanied by swelling of the affected joints and showed a symmetric pattern, mainly affecting the small joints of the hands and feet as well as the wrists, ankles, and knees.

After returning home to Switzerland, the patient consulted his general practitioner (August 1, 2011) because of persisting, incapacitating joint pains. The patient reported stiffness of the affected joints, mainly in the morning and after immobility. Physical examination of the affected joints did not reveal visible clinical signs of inflammation (swelling, redness, effusion). Laboratory tests were performed for complete blood count, liver and kidney function, C-reactive protein, and serologic testing for dengue virus, chikungunya virus, *Borrelia burgdorferi*, *Chlamydia trachomatis*, Epstein-Barr virus, parvovirus B19, *Salmonella* Typhi, and *S.* Paratyphi, but none revealed a cause for the symptoms. Over almost 2 more months, the joint pains did not improve; thus, the patient was referred to a rheumatologic clinic and subsequently to the Swiss Tropical and Public Health Institute, Basel, Switzerland, for evaluation of a putative travel-related cause of the polyarthralgia.

Because of the patient's travel history, the course of the illness and clinical signs and symptoms experienced during the journey, and the evolution and characteristics of the persisting joint pains, we suspected an underlying Mayaro virus (MAYV) infection. Serologic testing (indirect immunofluorescence and virus neutralization assays) for several alphaviruses were performed as described (*1*), and the results (Table) confirmed our presumptive diagnosis.

Several viral infections (e.g., dengue, rubella, parvovirus B19, hepatitis B, hepatitis C, HIV, and human T-lymphotropic virus type 1) can be accompanied by arthralgia. However, the most prominent and long-lasting polyarthritic symptoms occur in patients infected by alphaviruses (family *Togaviridae*).

Alphaviruses are arthropod-borne viruses (arboviruses) that circulate among a wide variety of wild animals in relative mosquito vector–specific and host-specific enzootic cycles; infection in humans (dead-end hosts) is almost exclusively incidental. Clinical cases and virus isolation have been reported only from northern South America, where MAYV circulates in an enzootic sylvatic cycle (similar to that for yellow fever) involving forest-dwelling *Haemagogus* spp. mosquitoes as vectors and nonhuman primates as natural hosts (*2*). Infections in humans mostly occur sporadically, are strongly associated with occupational or recreational exposure in rainforest environments, and are assumed to represent spillover from the enzootic cycle (*2*).

MAYV was first isolated in Trinidad in 1954; since then, sporadic cases, clusters, outbreaks, and small epidemics of Mayaro fever have been reported from Brazil, Bolivia, Columbia, French Guiana, Guyana, Peru, Venezuela, and Surinam (*3*). In addition to the clinical cases and virus isolates reported from northern South America, serologic survey findings suggest the presence of MAYV in Costa Rica, Guatemala, and Panama (*4*) (online Appendix Figure, wwwnc.cdc.

Table. Results of serologic testing for a Mayaro virus–infected Swiss traveler returning from the Amazon Basin, northern Peru, 2011*

Virus	Antigenic complex	Indirect immunofluorescence assay				Virus neutralization assay	
		First serum sample†		Second serum sample‡		First serum sample†	Second serum sample‡
		IgG	IgM	IgG	IgM		
Mayaro virus	SF	2,560	1,280	2,560	40	40	160
Chikungunya virus	SF	160	<20	160	<20	ND	ND
O`Nyong-nyong virus	SF	20	<20	80	<20	ND	ND
Ross River virus	SF	160	<20	160	<20	ND	ND
Semliki Forest virus	SF	80	<20	80	<20	20	20
Barmah Forest virus	BF	20	<20	20	<20	ND	ND
Venezuelan equine encephalitis virus	VEE	80	<20	80	<20	ND	ND
Western equine encephalitis virus	WEE	80	<20	80	<20	ND	ND
Sindbis virus	WEE	160	<20	160	<20	<20	<20
Eastern equine encephalitis virus	EEE	<20	<20	<20	<20	ND	ND

*SF, Semliki Forest; ND, not done; BF, Barmah Forest; VEE, Venezuelan equine encephalitis; WEE, Western equine encephalitis; EEE, Eastern equine encephalitis.
†Sample obtained August 29, 2011.
‡Sample obtained September 12, 2011.

gov/EID/article/18/4/11-1717-FA1.htm).

Clinical signs of this acute, dengue-like, febrile illness last 3–7 days and typically include chills, headache, retro-orbital and epigastric pain, myalgia, arthralgia, nausea, vomiting, diarrhea, and a maculopapular rash (sometimes followed by desquamation) (*7*). However, as with other alphavirus infections (i.e., chikungunya [Africa, Asia], o'nyong-nyong [Africa], Ross River [Australia, Oceania], Barmah Forest [Australia], and Sindbis [Africa, Europe, Asia, Australia]), the hallmark of MAYV infection is the highly debilitating arthralgia. Permanent damage of the affected joints has not been reported. Hemorrhagic manifestations of MAYV infections are rare but have been described (*3*). Concerns over the potential emergence of urban transmission of MAYV were raised after a laboratory study showed vector competence of *Aedes aegypti* mosquitoes (*8*).

International travelers are rarely given a diagnosis of MAYV infection (*9*,*10*). This might be attributed to the overall low frequency of the infection; the dengue-like signs and symptoms, which may lead to a misdiagnosis; and the fact that the disease is not well known outside MAYV-endemic regions. Physicians treating patients with signs and symptoms of a dengue-like illness and a recent history of travel to MAYV-endemic areas should consider MAYV infection in the differential diagnosis, especially if arthralgia is prominent and prolonged and dominates the clinical picture.

Andreas Neumayr,[1] Martin Gabriel,[1] Jasmin Fritz, Stephan Günther, Christoph Hatz, Jonas Schmidt-Chanasit, and Johannes Blum

Author affiliations: Swiss Tropical and Public Health Institute, Basel, Switzerland (A. Neumayr, J. Fritz, C. Hatz, J. Blum); and Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany (M. Gabriel, S. Günther, J. Schmidt-Chanasit)

DOI: http://dx.doi.org/10.3201/eid1804.111717

References

1. Tappe D, Schmidt-Chanasit J, Ries A, Ziegler U, Müller A, Stich A. Ross River virus infection in a traveller returning from northern Australia. Med Microbiol Immunol (Berl). 2009;198:271–3. http://dx.doi.org/10.1007/s00430-009-0122-9
2. Weaver SC, Reisen WK. Present and future arboviral threats. Antiviral Res. 2010;85:328–45. http://dx.doi.org/10.1016/j.antiviral.2009.10.008
3. Mourão MP, Bastos MD, de Figueiredo RP, Gimaque JB, Dos Santos Galusso E, Kramer VM, et al. Mayaro fever in the city of Manaus, Brazil, 2007–2008. Vector Borne Zoonotic Dis. 2012;12:42–6. http://dx.doi.org/10.1089/vbz.2011.0669
4. Pinheiro FP, Travassos da Rosa APA, Freitas RB, Travassos da Rosa JFS, Vasconcelos PFC. Aspectos clínico-epidemiológicos das arboviroses. In: Instituto Evandro Chagas: 50 anos de contribuição às ciências biológicas e à medicina tropical, Belém, Brazil. Vol 1. Belém (Brazil): Fundação Serviços de Saúde Pública; 1986. p. 375–408.
5. Tesh RB, Watts DM, Russell KL, Damodaran C, Calampa C, Cabezas C, et al. Mayaro virus disease: an emerging mosquito-borne zoonosis in tropical South America. Clin Infect Dis. 1999;28:67–73. http://dx.doi.org/10.1086/515070
6. Coimbra TL, Santos CL, Suzuki A, Petrella SM, Bisordi I, Nagamori AH, et al. Mayaro virus: imported cases of human infection in São Paulo State, Brazil. Rev Inst Med Trop Sao Paulo. 2007;49:221–4. http://dx.doi.org/10.1590/S0036-46652007000400005
7. Pinheiro FP, LeDuc JW. Mayaro virus disease. In: Monath TP, editor. In: The arboviruses: epidemiology and ecology. Boca Raton (FL): CRC Press; 1988. p. 137–50.
8. Long KC, Ziegler SA, Thangamani S, Hausser NL, Kochel TJ, Higgs S, et al. Experimental transmission of Mayaro virus by *Aedes aegypti*. Am J Trop Med Hyg. 2011;85:750–7. http://dx.doi.org/10.4269/ajtmh.2011.11-0359
9. Hassing RJ, Leparc-Goffart I, Blank SN, Thevarayan S, Tolou H, van Doornum G, et al. Imported Mayaro virus infection in the Netherlands. J Infect. 2010;61:343–5. http://dx.doi.org/10.1016/j.jinf.2010.06.009
10. Receveur MC, Grandadam M, Pistone T, Malvy D. Infection with Mayaro virus in a French traveller returning from the Amazon region, Brazil, January, 2010. Euro Surveill. 2010;15:pii:19563.

[1]These authors contributed equally to this article.

Address for correspondence: Andreas Neumayr, Swiss Tropical and Public Health Institute, Socinstr 57, PO Box, 4002 Basel, Switzerland; email: andreas.neumayr@unibas.ch

Meningoencephalitis Complicating Relapsing Fever in Traveler Returning from Senegal

To the Editor: Although tick-borne relapsing fever (TBRF) may be caused by ≈20 *Borrelia* species (*1*), it is rarely diagnosed in travelers returning from the tropics (*2*). Approximately 20 travel-related cases have been published in the past 25 years, and most of them have been acquired in western Africa (mainly Senegal), where *Borrelia crocidurae* is the predominant species (*3*). All reported cases have been diagnosed by identification of spirochetes in blood smears or by quantitative buffy coat analysis. Neurologic involvement, which is frequent in TBRF (*4*), was reported for 3 confirmed travel-associated cases (*5*–*7*) and for 2 additional clustered cases not confirmed by microscopy (*8*). We report a case of acute meningoencephalitis in a returning traveler for whom TBRF was diagnosed by only PCR of serum and cerebrospinal fluid (CSF).

In December 2010, a 26-year-old Belgian woman was referred to the intensive care unit of the University Hospital of Antwerp, Belgium, because of fever and headache for 7 days and abrupt neurologic deterioration the day before admission. One month earlier, she had returned from a 3-week adventure trip to a rural area near Bambey, 120 km east of Dakar, Senegal. Immunization and malaria chemoprophylaxis had been appropriate. Diarrhea and fever developed the day she returned. She was empirically treated with ofloxacine for 5 days and recovered.

At admission, she reported high-grade fever, headache, and photophobia. She was somnolent, inadequate in her answers, and had neck stiffness. Laboratory investigations showed a leukocyte count of 20 × 10^9 cells/L (82% neutrophils) and a C-reactive protein level of 190 mg/L. Blood smears were repeatedly negative. Magnetic resonance imaging of the brain showed no abnormalities. CSF contained 350 leukocytes/mm^3 (95% lymphocytes) and had a protein level of 125 mg/dL.

Acyclovir, ceftriaxone, ampicillin, and doxycycline were empirically administered. A rash developed abruptly, and the patient became hypotensive and extremely agitated. Treatment with ampicillin was stopped because of a suspected allergic reaction. Blood and CSF cultures remained negative. Results of molecular testing of CSF for herpesvirus and enteroviruses were negative.

The patient recovered uneventfully after a 14-day course of ceftriaxone. Paired serologic samples did not show seroconversion for HIV, *Treponema pallidum*, cytomegalovirus, dengue virus, West Nile virus, *Toxoplasma gondii*, *Rickettsia* spp., *Coxiella burnetii*, *Leptospira* sp., and *B. burgdorferi*.

At the Université de la Méditerranée in Marseille, France, DNA samples from CSF and acute-phase and convalescent-phase serum samples were tested by using quantitative real-time PCR specific for a fragment of the 16S rRNA gene of *Borrelia* spp. (*9*). *Borrelia*-positive results were confirmed by using *Borrelia*-specific quantitative PCR specific for the internal transcribed spacer and primers Bor_ITS4_F: 5′GGCTT CGGGTCTACCACATCTA-3′ and Bor_ITS4_R: 5′-CCGGGAGGGGAG TGAAATAG-3′and probe Bor_ITS4_ P:5′-6FAM-TGCAAAAGGCACGCC ATCACC-TAMRA-3′.

An amplicon of 202 bp was obtained from a CSF DNA sample after amplification and sequencing of the flagellin B gene with primer set Bfpbu: 5′-GCTGAAGAGCTTGGAAT GCAACC-3′ and Bfpcr: 5′-TGATCA GTTATCATTCTAATAGCA-3′. This amplicon showed 100% similarity with sequences available in GenBank for *B. crocidurae* (accession no. GU357619). Indirect immunofluorescence with *B. crocidurae* antigen showed positive bands for IgM and IgG (Table).

B. crocidurae has emerged as a major zoonotic pathogen in rural western Africa and accounts for 5%–25% of febrile illnesses depending on year and location (*3*,*9*,*10*). Transmission occurs through nocturnal bites of soft ticks (*Ornithodoros sonrai*), which colonize rodent burrow openings in mud-built huts and houses with cement floors (*1*). Therefore, TBRF should be considered in any symptomatic traveler in disease-endemic areas.

We identified *B. crocidurae* DNA in the CSF of a patient with meningoencephalitis complicating relapsing fever. Meningitis and meningoencephalitis may develop in persons with travel-related TBRF (*5*–*8*). The neurologic outcome was favorable after treatment with ceftriaxone for 14 days (*4*), and relapse was not observed. Negative blood smears, even when repeated and read by laboratory experts, do not rule out TBRF. Recent studies have demonstrated that sensitivity of blood smear examination performed by trained microscopists does not exceed 50% compared with PCR methods (*9*) and is much lower in field settings (*9*,*10*). Abrupt deterioration (rash, hypotension, and increased encephalopathy) after treatment with antimicrobial drugs was probably related to a Jarish-Herxheimer reaction (*4*). This reaction was not immediately considered but was easily controlled by supportive treatment in the intensive care unit. The noninvestigated episode of fever upon return of the patient may have been the initial fever episode of TBRF, but it was lessened by the short course of ofloxacine (*4*). Absence of laboratory workup could have caused serious infections to be missed.

Table. Test results for 26-year-old woman who returned to Belgium from Senegal with meningoencephalitis complicating relapsing fever*

Characteristic	Date and test result	
	2010 Dec 21	2011 Jan 25
Borrelia DNA in serum	+ for 16S rRNA and ITS4 genes	–
Borrelia DNA in CSF	+ (100% similarity to *B. crocidurae flaB* gene; GenBank accession no. GU357619)	
B. crocidurae IgM titer	25	0
B. crocidurae IgG titer	400	400
B. dutonii IgM titer	0	0
B. dutonii IgG titer	200	200
B. recurrentis	–	–
B. burgdorferi	–	–

*+, positive; ITS, internal transcribed spacer; –, negative; CSF, cerebrospinal fluid; *fla*, flagellin.

In conclusion, this case indicates an unusual complication and condition in travel medicine with no straightforward diagnosis. However, it illustrates that TBRF should be systematically considered in the differential diagnosis of acute meningoencephalitis in travelers, even if microscopic results are negative, to prompt appropriate empirical treatment and molecular or serologic testing.

Emmanuel Bottieau, Elric Verbruggen, Camille Aubry, Cristina Socolovschi, and Erika Vlieghe

Author affiliations: Institute of Tropical Medicine, Antwerp, Belgium (E. Bottieau, E. Vlieghe); University Hospital of Antwerp, Antwerp (E. Verbruggen, E. Vlieghe); and Université de la Méditerranée, Marseilles, France (C. Aubry, C. Socolovschi)

DOI: http://dx.doi.org/10.3201/eid1804.111771

References

1. Cutler SJ. Possibilities for relapsing fever reemergence. Emerg Infect Dis. 2006;12:369–74. http://dx.doi.org/10.3201/eid1203.050899
2. Bottieau E, Clerinx J, Schrooten W, Van den Enden E, Wouters R, Van Esbroeck M, et al. Etiology and outcome of fever after a stay in the tropics. Arch Intern Med. 2006;166:1642–8. http://dx.doi.org/10.1001/archinte.166.15.1642
3. Vial L, Diatta G, Tall A, Ba el H, Bouganali H, Durand P, et al. Incidence of tick-borne relapsing fever in west Africa: longitudinal study. Lancet. 2006;368:37–43. http://dx.doi.org/10.1016/S0140-6736(06)68968-X
4. Cadavid D, Barbour AG. Neuroborreliosis during relapsing fever: review of the clinical manifestations, pathology, and treatment of infections in humans and experimental animals. Clin Infect Dis. 1998;26:151–64. http://dx.doi.org/10.1086/516276
5. Charmot G, Rodhain F, Dupont B, Sansonetti P, Lapresle C. Meningoencephalitis in a repatriate from Senegal. Think of borreliosis [in French]. Presse Med. 1986;15:979.
6. Colebunders R, De Serrano P, Van Gompel A, Wynants H, Blot K, Van den Enden E, et al. Imported relapsing fever in European tourists. Scand J Infect Dis. 1993;25:533–6. http://dx.doi.org/10.3109/00365549309008539
7. van Dam AP, Van Gool T, Wetsteyn JC, Dankert J. Tick-borne relapsing fever imported from west Africa: diagnosis by quantitative buffy coat analysis and in vitro culture of *Borrelia crocidurae.* J Clin Microbiol. 1999;37:2027–30.
8. Patrat-Delon S, Drogoul AS, Le Ho H, Biziraguzenyuka J, Rabier V, Arvieux C, et al. Recurrent tick-borne fever: a possible diagnosis in patients returning from Senegal [in French]. Med Mal Infect. 2008;38:396–9.
9. Parola P, Diatta G, Socolovschi C, Mediannikov O, Tall A, Bassene H, et al. Tick-borne relapsing fever borreliosis, rural Senegal. Emerg Infect Dis. 2011;17:883–5.
10. Nordstrand A, Bunikis I, Larsson C, Tsogbe K, Schwan TG, Nilsson M, et al. Tickborne relapsing fever diagnosis obscured by malaria, Togo. Emerg Infect Dis. 2007;13:117–23. http://dx.doi.org/10.3201/eid1301.060670

Address for correspondence: Emmanuel Bottieau, Department of Clinical Sciences, Institute of Tropical Medicine, Nationalestraat 155, Antwerp 2000, Belgium; email: ebottieau@itg.be

Serologic Evidence of Orthopoxvirus Infection in Buffaloes, Brazil

To the Editor: Since 1999, several exanthematous vaccinia virus (VACV) outbreaks affecting dairy cattle and rural workers have been reported in Brazil (*1*,*2*). VACV, the prototype of the genus *Orthopoxvirus* (OPV), exhibits serologic cross-reactivity with other OPV species and was used during the World Health Organization smallpox eradication campaign (*3*). The origin of VACV in Brazil is unknown, although some studies have suggested that VACV strains used during the campaign may be related to outbreaks of bovine vaccinia (BV) (*2*). In Brazil, BV affects the milk industry and public health services (*1*,*2*,*4*,*5*). During outbreaks, dairy cattle developed lesions on the teats and udders, causing a decrease in milk production (*1*,*2*,*4*,*5*).

Another VACV subspecies, buffalopox virus (BPXV), has been isolated from buffaloes (*Bubalus bubalis*) in rural areas in India and causes clinical signs that resemble those seen during BV outbreaks in Brazil (*6*). Recent genetic analysis of BPXV samples confirmed its close relationship to VACV-like viruses, although each virus has distinct genetic signatures (*1*,*2*,*6*). Until recently, buffalo herds have been almost exclusive to northern Brazil. However, the buffalo market has experienced great expansion in this country, and today, there are herds in all geographic regions of Brazil. These buffalo herds are hypothetically at risk for VACV infection, on the basis of the outbreaks caused by BPXV that have been described in India (*6*). To assess the risk for OPV infection in milk buffaloes in Brazil, we conducted a serosurvey of herds from southeastern Brazil, the region most affected by BV.

During October 2010, we screened milk buffalo herds in rural areas of Minas Gerais State, Brazil. Serum samples were collected from 48 female buffaloes used for milk production; these animals belonged to 3 neighboring properties in Carmo da Mata city (20°33′28″S, 44°52′15″W), which is in the same mesoregion where the VACV Passatempo virus strain was isolated during an outbreak in 2003 (*5*). Since then, several outbreaks have been reported in this area.

Serum samples were inactivated, and an OPV plaque-reduction neutralization test (PRNT) was performed (*7*). The serum titer was defined as the highest dilution that inhibited >70% of viral plaques relative to the level of inhibition of the negative controls. Samples also underwent ELISA for OPV IgG as described (*4*). Bovine serum samples were used as positive and negative controls (*1*,*4*). OPV-PRNT specificity (98.4%) and sensitivity (93.5%) were confirmed by using receiver-operating characteristic analysis as described (*8*). The tests were performed in duplicate.

Of the 48 buffalo serum samples, 15 (31.25%) contained neutralizing antibodies against OPV; of these, 6 (40%) had titers of 20, 5 (33.3%) had titers of 40, and 4 (26.6%) had titers ≥80 (Table). The ELISA yielded results similar to those of the PRNT; of the 48 serum samples, 17 (35.41) were IgG positive (Table). A total of 14 samples were coincident in the PRNT and the ELISA, including most of those with high titers by PRNT. To detect viral DNA, we conducted nested PCR to amplify the viral growth factor gene (*9*) and real-time PCR to amplify the A56R gene (*10*); results were negative for all 48 serum samples.

We detected antibodies against OPV in buffaloes in Brazil 10 years after the first reported VACV outbreak in cattle in southeastern Brazil (*1*). Because PRNT and ELISA indicate the presence of OPV antibodies in a nonspecific manner (OPV serologic cross-reaction), it was not possible to determine the species responsible for these results. However, seropositive buffaloes may have been exposed to VACV, the only OPV known to be circulating in Brazil (*1*,*2*,*4*,*5*,*8*).

The management of milk buffaloes in Brazil is similar to that of dairy cows, including manual milking (*1*,*4*,*5*). Cow milkers usually work on ≥2 farms, and the farm infrastructure commonly is unsophisticated (*1*,*4*,*5*). These conditions were shown to be favorable for the spread of VACV among cattle, which suggests that the same conditions could lead to the introduction of VACV into buffalo herds. Because some BV outbreaks are not reported by the farmers, it is not possible to know exactly how or when a buffalo herd in the study area was exposed to the virus. However, milkers who work with both cattle and buffalo may be a route of viral transmission, although other sources of exposure are possible (*8*). Although no exanthematous VACV outbreaks have been described in milk buffaloes in Brazil, our results suggest that buffalo herds may be exposed to VACV in BV-affected areas and therefore may be at risk for VACV infection. Further research is needed to determine routes of infection, including whether humans working as milkers contribute to virus transmission.

Table. Results of testing for orthopoxvirus seropositivity in milk buffalo herds, Minas Gerais State, Brazil, October 2010*

Test	No. (%) samples
PRNT	
Total positive	15 (31.2)
Titer	
20	6 (40.0)
40	5 (33.3)
80	2 (13.3)
160	2 (13.3)
Total negative	33 (68.7)
ELISA	
Total positive	17 (35.4)
Total negative	31 (64.6)
PRNT and ELISA positive	14 (29.2)

*Serum samples were collected from 48 female buffaloes used for milk production. A positive titer was defined as the highest dilution that inhibited >70% of viral plaques relative to the level of inhibition of the negative controls. Samples also underwent ELISA for orthopoxvirus IgG as described (*4*). PRNT, plaque-reduction neutralization test.

Acknowledgments

We thank João Rodrigues dos Santos, Ângela Sana Lopes, Ilda Gama, and colleagues from the Laboratório de Vírus for their excellent technical support.

Financial support was provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Fundação de Amparo à Pesquisa do Estado de Minas Gerais, and Ministério da Agricultura, Pecuária e Abastecimento. F.L.A. received fellowships from CNPq; E.G.K., C.A.B., G.S.T., and P.C.P.F. are researchers supported by CNPq.

Felipe Lopes de Assis,[1] Graziele Pereira,[1] Cairo Oliveira, Gisele Olinto Libânio Rodrigues, Marcela Menezas Gomes Cotta, Andre Tavares Silva-Fernandes, Paulo Cesar Peregrino Ferreira, Cláudio Antônio Bonjardim, Giliane de Souza Trindade, Erna Geessien Kroon, and Jônatas Santos Abrahão

Author affiliation: Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

DOI: http://dx.doi.org/10.3201/eid1804.111800

[1]These authors contributed equally to this article.

References

1. de Souza Trindade G, da Fonseca FG, Marques JT, Nogueira ML, Mendes LC, Borges AS, et al. Araçatuba virus: a vaccinialike virus associated with infection in humans and cattle. Emerg Infect Dis. 2003;9:155–60.
2. Damaso CR, Esposito JJ, Condit RC, Moussatche N. An emergent poxvirus from humans and cattle in Rio de Janeiro State: Cantagalo virus may derive from Brazilian smallpox vaccine. Virology. 2000;277:439–49. http://dx.doi.org/10.1006/viro.2000.0603
3. Damon IK. Poxviruses. In: Knipe DM, Howley PM, editors. Fields virology, 5th ed., vol. II. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 2947.
4. Silva-Fernandes AT, Travassos CE, Ferreira JM, Abrahão JS, Rocha ES, Viana-Ferreira F, et al. Natural human infections with vaccinia virus during bovine vaccinia outbreaks. J Clin Virol. 2009;44:308–13. http://dx.doi.org/10.1016/j.jcv.2009.01.007
5. Leite JA, Drumond BP, Trindade GS, Lobato ZI, da Fonseca FG, dos Santos JR, et al. Passatempo virus, a vaccinia virus strain, Brazil. Emerg Infect Dis. 2005;11:1935–8.
6. Bhanuprakash V, Venkatesan G, Balamurugan V, Hosamani M, Yogisharadhya R, Gandhale P, et al. Zoonotic infections of buffalopox in India. Zoonoses Public Health. 2010;57:e149–55. http://dx.doi.org/10.1111/j.1863-2378.2009.01314.x
7. Newman FK, Frey SE, Blevins TP, Mandava M, Bonifacio A Jr, Yan L, et al. Improved assay to detect neutralizing-antibody following vaccination with diluted or undiluted vaccinia (Dryvax) vaccine. J Clin Microbiol. 2003;41:3154–7. http://dx.doi.org/10.1128/JCM.41.7.3154-3157.2003
8. Abrahão JS, Silva-Fernandes AT, Lima LS, Campos RK, Guedes MI, Cota MM, et al. Vaccinia virus infection in monkeys, Brazilian Amazon. Emerg Infect Dis. 2010;16:976–9.
9. Abrahão JS, Lima LS, Assis FL, Alves PA, Silva-Fernandes AT, Cota MM, et al. Nested-multiplex PCR detection of *Orthopoxvirus* and *Parapoxvirus* directly from exanthematic clinical samples. Virol J. 2009;6:140. http://dx.doi.org/10.1186/1743-422X-6-140
10. de Souza Trindade G, Li Y, Olson VA, Emerson G, Regnery RL, da Fonseca FG, et al. Real-time PCR assay to identify variants of Vaccinia virus: implications for the diagnosis of bovine vaccinia in Brazil. J Virol Methods. 2008;152:63–71. http://dx.doi.org/10.1016/j.jviromet.2008.05.028

Address for correspondence: Jônatas Abrahão, Laboratório de Vírus, ICB, UFMG, Brazil; email: jonatas.abrahao@gmail.com

Methicillin-Susceptible *Staphylococcus aureus* ST398, New York and New Jersey, USA

To the Editor: Clinical infections with livestock-associated *Staphylococcus aureus* sequence type (ST) 398 have been reported in Europe, Canada, and the People's Republic of China (*1*), as well as the Caribbean (*2*,*3*), and Colombia (*4*). Most reports describe infection with methicillin-resistant *S. aureus*; relatively few describe infection with methicillin-susceptible *S. aureus* (MSSA). In the United States, colonization of healthy adults by ST398 has been reported in Iowa (*5*) and in New York, New York (*2*); MSSA infections have been anecdotally reported in St. Louis, Missouri (*6*), and The Bronx, New York (*7*). We describe 8 infections with MSSA ST398 in the New York City area during a 7-year period (2004–2010). Five infections with a related ST (ST291) from clonal complex (CC) 398 also were identified. These findings highlight the emergence of clinical infections with 2 distinct CC398 sequence types in the New York City area.

Retrospective typing of 4,167 clinical *S. aureus* isolates from various studies involving inpatients and outpatients in the New York City area identified 13 *mecA*-negative isolates with CC398-associated *spa* types (Table). Nine isolates were obtained from cultures of outpatients with skin and soft tissue infections; samples were submitted by physicians in the community. One isolate was associated with recurring skin and soft tissue infections in multiple body sites (BK21466); another was associated with genital infection (BK21732). Of the 4 ST398 isolates derived from bloodstream infections in hospitalized patients, 3 were recovered from intravenous drug users, 1 of whom died 1 day after admission for variceal bleeding (BK26722). Unlike the multidrug-resistant ST398 MSSA recently described in Colombia (*4*), most isolates in this study were susceptible to all antimicrobial drugs tested except penicillin, although several strains exhibited resistance to clindamycin and erythromycin. One isolate (BK23527) was submitted as oxacillin resistant (MIC ≈4 μg/mL) but lacked the *mecA* gene, which suggested that another mechanism was contributing to the resistance phenotype.

Multilocus sequence typing confirmed 8 isolates as ST398 (3–35–19–2–20–26–39); 5 isolates were assigned to ST291 (3–37–19–2–20–26–32), a double-locus variant of ST398 (online Appendix Figure, panel A, wwwnc.cdc.gov/EID/article/18/4/11-1419-FA1.htm). Most of the ST398 strains were *spa* type 109 (t571), described in MSSA carriage isolates from New York City (*2*) and MSSA infections from China (*1*), France (*8*), Martinique (*3*), the Dominican Republic (*2*,*3*), and Colombia (*4*). BURP (based upon repeat pattern) analysis clustered all of the *spa* types into *spa*-CC t571 (online Appendix Figure, panel B); ST398 isolates clustered with *spa* type 109 (t571), whereas ST291 isolates clustered with *spa* type 865 (t2313). Pulsed-field gel electrophoresis was also performed on the 11 available isolates. Although the ST291 isolates were sensitive to digestion with *Sma*I, pulsed-field gel electrophoresis was performed with *Cfr*9I to compare

Table. Characteristics of *Staphylococcus aureus* clonal complex 398 isolates, New York and New Jersey, USA, 2004–2010*

Isolate†	Year	Geographic location‡	Submitting institution§	Isolate source	Antimicrobial resistance	PFGE pattern	*spa* type	*spa* repeats	Ridom type	ST
BK13684	2004	Monmouth County, NJ	Laboratory A	Wound	PEN	–	539	XKAOAOBQO	t034	398
BK18505	2006	Manhattan	Laboratory A	Wound	–	A1	109	XKAOAOBO	t571	398
BK21466	2007	Staten Island	Laboratory A	Arm, face, leg, buttocks	–	A2	109	XKAOAOBO	t571	398
BK21732	2007	Manhattan	Laboratory A	Genital	PEN, CLI, ERY	A3	109	XKAOAOBO	t571	398
BK27037	2007	The Bronx	Hospital A	Blood, lung abscess	PEN, CLI, ERY	A4	109	XKAOAOBO	t571	398
BK23527	2008	Manhattan	Hospital B	Blood, buttocks	PEN, ERY, OXA	–	109	XKAOAOBO	t571	398
BK26722	2009	Manhattan	Hospital B	Blood	CLI, ERY	A1	109	XKAOAOBO	t571	398
BK31274	2010	Nassau County, NY	Hospital C	Blood, sternum	PEN, CLI, ERY	A1	1376	XKAOBO	t1451	398
BK13771	2004	Somerset County, NJ	Laboratory A	Wound	PEN, ERY	B4	716	XKBBM	t2993	291
BK13451	2004	Union County, NJ	Laboratory A	Wound	PEN	B2	718	XKBQBMM	t1149	291
BK19382	2006	Staten Island	Laboratory A	Right ear	PEN	B1	865	XKBQBBM	t2313	291
BK21746	2007	Manhattan	Laboratory A	Torso	PEN, ERY	B1	208	XKBQBBMM	t937	291
BK22183	2007	Manhattan	Laboratory A	Axilla	PEN	B3	208	XKBQBBMM	t937	291

*Antimicrobial drug susceptibilities were obtained from submitting institutions (unavailable for BK18505 and BK21466). PFGE was performed by using *Cfr*9I, with patterns assigned on the basis of 80% similarity cutoffs (BioNumerics version 6.5, Applied Maths, Austin, TX, USA); BK13684 and BK23527 were unavailable for PFGE analysis. *spa* typing was performed by using eGenomics software (www.egenomics.com), and Ridom *spa* types were assigned by using the SpaServer Web site (www.spaserver.ridom.de). Multilocus sequence typing was performed as described (http://saureus.mlst.net). PFGE, pulsed-field gel electrophoresis; *spa*, staphylococcal protein A; ST, sequence type; wound, skin and soft-tissue infections from unspecified body sites; PEN, penicillin; CLI, clindamycin; ERY, erythromycin; OXA, oxacillin (resistance ≥4 μg/mL was reported for BK23527, but *mecA* was not detected by real-time PCR).
†BK27037 has been described (*7*).
‡Manhattan, Staten Island, and the Bronx are boroughs of New York, New York.
§Laboratory A is a large outpatient commercial laboratory serving the metropolitan New York, New York, area.

all isolates simultaneously. As expected, the ST398 and ST291 isolates clustered separately (data not shown); 4 distinct patterns were observed within each cluster (Table). Only the ST398 isolates were positive for a CC398 lineage-specific PCR that targets the unique restriction-modification system *sau1-hsdS1* (*9*), further highlighting the differences between ST291 and ST398. None of the isolates harbored the genes coding for Panton-Valentine leukocidin.

Because of the retrospective nature of the findings, epidemiologic information for each isolate was limited. One patient (BK19382) reported travel to the Dominican Republic; Caribbean nationality was reported for BK27037 (Puerto Rico) and BK31274 (Trinidad). The cases described here occurred in urban and suburban settings, reflecting the likelihood that exposure to livestock was relatively low; however, travel history was unknown for most of the patients. Previous reports have linked ST398 transmission to other reservoirs, including companion animals, live animal food markets, and commercial meat products (*1*,*2*). However, data from a recent genome sequencing study suggest that MSSA ST398 is human in origin (*10*); other evidence suggests that certain lineages, particularly *spa* type 109 (t571), might circulate at low levels in humans in the absence of livestock exposure (*8*).

Our findings seem to support the hypothesis of low-level ST398 MSSA prevalence, and further surveillance might uncover additional cases of colonization or infection with ST398- and ST291-related strains in the New York City area. For example, active surveillance cultures performed at one of the 3 hospitals during January–March 2009 detected 7 additional ST398 and 3 additional ST291 isolates among 260 MSSA carriage strains (data not shown). In addition to the intrinsic virulence exhibited by ST398 MSSA in previous studies, the potential to acquire resistance to multiple classes of antimicrobial drugs (*1*,*4*,*10*), as well as virulence factors such as Panton-Valentine leukocidin (*8*), warrants continued surveillance in light of recent ST398 methicillin-resistant *S. aureus* outbreaks in health care settings (*1*).

Acknowledgments

We thank Bruce F. Farber for administrative support and clinical guidance and John Gattringer, Nancy Musa, and Michael Henry for gathering data related to this project.

This work was partially funded by the New York State Department of Health Hospital-Acquired Infection Reporting Program (D.A., M.E.S.) and Infection Prevention Project Program (K.S., B.K.) and by a grant from the New York Community Trust (B.N.K.).

José R. Mediavilla, Liang Chen, Anne-Catrin Uhlemann, Blake M. Hanson, Marnie Rosenthal, Kathryn Stanak, Brian Koll, Bettina C. Fries, Donna Armellino, Mary Ellen Schilling, Don Weiss, Tara C. Smith, Franklin D. Lowy, and Barry N. Kreiswirth

Author affiliations: University of Medicine and Dentistry of New Jersey, Newark, New Jersey, USA (J.R. Mediavilla, L. Chen, B.N. Kreiswirth); Columbia University, New York, New York, USA (A.-C. Uhlemann, F.D. Lowy); University of Iowa, Iowa City, Iowa, USA (B.M. Hanson, T.C. Smith); Jersey Shore University Medical Center, Neptune, New Jersey, USA (M. Rosenthal); Beth Israel Medical Center, New York (K. Stanak, B. Koll); Albert Einstein College of Medicine, Bronx, New York, USA (B.C. Fries); North Shore University Hospital, Manhasset, New York, USA (D. Armellino, M.E. Schilling); and New York City Department of Health and Mental Hygiene, New York (D. Weiss)

DOI: http://dx.doi.org/10.3201/eid1804.111419

References

1. Smith TC, Pearson N. The emergence of *Staphylococcus aureus* ST398. Vector Borne Zoonotic Dis. 2011;11:327–39. http://dx.doi.org/10.1089/vbz.2010.0072
2. Bhat M, Dumortier C, Taylor BS, Miller M, Vasquez G, Yunen J, et al. *Staphylococcus aureus* ST398, New York City and Dominican Republic. Emerg Infect Dis. 2009;15:285–7. http://dx.doi.org/10.3201/eid1502.080609
3. Uhlemann AC, Dumortier C, Hafer C, Taylor BS, Sanchez EJ, Rodriguez-Taveras C, et al. Molecular characterization of *Staphylococcus aureus* from outpatients in the Caribbean reveals the presence of pandemic clones. Eur J Clin Microbiol Infect Dis. 2011; Epub ahead of print. http://dx.doi.org/10.1007/s10096-011-1339-2
4. Jiménez JN, Vélez LA, Mediavilla JR, Ocampo AM, Vanegas JM, Rodríguez EA, et al. Livestock-associated methicillin-susceptible *Staphylococcus aureus* ST398 in woman, Colombia. Emerg Infect Dis. 2011;17:1970–1.
5. Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS ONE. 2009;4:e4258. http://dx.doi.org/10.1371/journal.pone.0004258
6. Orscheln RC, Hunstad DA, Fritz SA, Loughman JA, Mitchell K, Storch EK, et al. Contribution of genetically restricted, methicillin-susceptible strains to the ongoing epidemic of community-acquired *Staphylococcus aureus* infections. Clin Infect Dis. 2009;49:536–42. http://dx.doi.org/10.1086/600881
7. Varshney AK, Mediavilla JR, Robiou N, Guh A, Wang X, Gialanella P, et al. Diverse enterotoxin gene profiles among clonal complexes of *Staphylococcus aureus* isolates from the Bronx, New York. Appl Environ Microbiol. 2009;75:6839–49. http://dx.doi.org/10.1128/AEM.00272-09
8. Davies PR, Wagstrom EA, Bender JB. Lethal necrotizing pneumonia caused by an ST398 *Staphylococcus aureus* strain. Emerg Infect Dis. 2011;17:1152–3. http://dx.doi.org/10.3201/eid1706.101394
9. Stegger M, Lindsay JA, Moodley A, Skov R, Broens EM, Guardabassi L. Rapid PCR detection of *Staphylococcus aureus* clonal complex 398 by targeting the restriction-modification system carrying *sau1-hsdS1*. J Clin Microbiol. 2011;49:732–4. http://dx.doi.org/10.1128/JCM.01970-10
10. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. MBio. 2012;3:pii:e00305-11. http://dx.doi.org/10.1128/mBio.00305-11

Address for correspondence: Barry N. Kreiswirth, Public Health Research Institute Tuberculosis Center, University of Medicine and Dentistry of New Jersey, 225 Warren St, ICPH W210M, Newark, NJ 07103, USA; email: kreiswba@umdnj.edu

Rickettsia monacensis as Cause of Mediterranean Spotted Fever–like Illness, Italy

To the Editor: *Rickettsia conorii*, the etiologic agent of Mediterranean spotted fever (MSF), is transmitted to humans by the brown dog tick (*Rhipicephalus sanguineus*). MSF is endemic to Italy; incidence is highest in the south and on the islands of Sardinia and Sicily (*1*). Recently, the use of molecular methods has enabled identification of other rickettsiae of the spotted fever group (SFG) from *Ixodes ricinus* ticks in northeastern Italy and in other areas of Europe (*2*–*6*). *R. monacensis* was identified as an etiologic agent of MSF-like illness in Spain (*7*).

We report a case of MSF-like illness in a 28-year-old man from Sassari in northwestern Sardinia who was admitted to the Infectious Disease Unit of the University of Sassari Hospital in April 2011. At admission, he reported fever (38.2°C) and headache of 2 days' duration. At physical examination, he had a crusty skin lesion surrounded by edema and erythema, which was compatible with inoculation eschar, on the left calf. He had no rash. Laboratory results showed a slight leukocyte increase, hypocromic and microcytic anemia (hemoglobin 10.6 g/dL [reference range 13.1–17.1 g/dL], mean corpuscular volume 67.7 fL [reference range 81–88 fL], mean corpuscular hemoglobin concentration 29.6 g/dL [reference range 33–35 g/dL]), hyperbilirubinemia (total bilirubin 1.36 mg/dL [reference range 0.2–1.3 mg/dL], direct bilirubin 0.49 mg/dL [reference range 0.0–0.6 mg/dL]), and erythrocyte sedimentation rate 37 mm/h (reference range 0–25 mm/h). The remaining parameters

were within reference ranges. A small skin sample taken from the inoculation eschar and whole blood were stored at –30°C. The patient immediately started taking doxycycline 100 mg every 12 hours. Serologic tests were negative for *R. conorii* IgM and IgG (ELISA) and positive for SFG *Rickettsia* spp. IgG on indirect immunofluorescence with a titer of 128. After 24 hours of antimicrobial drug therapy, he was afebrile; he was discharged on day 3. He completed a 7-day course of doxycycline at home and recovered completely.

The skin biopsy sample, collected in phosphate-buffered saline, and whole blood were obtained before antimicrobial therapy began and were subjected to DNA extraction. Bacterial detection and identification were conducted by using molecular methods based on real-time PCR, classical PCR, and nucleotide sequencing (Table).

A set of primers for *glt*A gene that encodes the citrate synthase enzyme (*8*) was used to determine that the organism belonged to the genus *Rickettsia*, which includes the SFG and typhus group. Each real-time PCR reaction was performed by QuantiTect SYBR Green PCR kit (QIAGEN, Hilden, Germany) by using 20 ng of purified DNA. *R. conorii* and *R. typhii* were used as positive controls for SFG and typhus group, and *Anaplasma phagocytophilum*, *Bartonella henselae*, *Ehrlichia chaffeensis*, and *Coxiella burnetii* (*Bartonellaceae* and *Coxiellaceae* members) served as negative controls. Results were checked for the specific molecular length by electrophoresis on a 3% (wt/vol) agarose gel.

The skin biopsy specimen of the inoculation eschar was positive for *Rickettsia* spp. The whole blood sample was negative for *Rickettsia* spp.

These results were confirmed by amplification of the *ompA* gene by using the ompA–F and ompA–R primers (*9*) and by the sequencing of the PCR amplicon. The nucleotide sequence analyzed by using the BLAST search tool (www.ncbi.n/m.nib.gov/blast) showed 100% identity with the *R. monacensis* isolate N72 (GenBank accession no. FJ919650.1). We identified *R. monacensis* as cause of MSF-like illness in the patient reported here.

Our results have several clinical and microbiological implications. Although MSF-like illness is highly endemic to Sardinia, to our knowledge no pathogens other than *R. conorii* had ever been identified. Antibodies against *R. monacensis* were not detected by the *R. conorii* ELISA commonly used in hospital laboratories. In contrast, indirect immunofluorescence, which cannot distinguish between rickettsial species because of cross-reactivity, was positive. Therefore, the cocirculation of *R. monacensis* and, possibly, of other SFG rickettsiae, could lead to misdiagnosis and therapeutic delay. Furthermore, in consideration of the negative result in whole blood, a small skin sample from the eschar might improve the diagnostic sensitivity of PCR.

We did not perform entomologic studies. However, *I. ricinus* ticks, which are considered vectors of *R. monacensis*, are widely distributed in Italy and have been found in Sardinia, although less often than other tick species (*10*). Moreover, it is not excluded that other ticks might act as vectors for *R. monacensis* in Sardinia, where ticks of the genus *Rhipicephalus* are prominent. Molecular investigations of ticks could better clarify the extent of circulation of SFG rickettsiae in Sardinia.

Identification of *R. monacensis* as a cause of MSF-like illness in Sardinia expands the list of pathogenic rickettsiae circulating in Italy. It also highlights the need for further investigation in humans and vectors to understand infection dynamics and improve diagnosis and treatment of this potentially life-threatening disease.

This study was supported by a Centro Nazionale per il Controllo e la Prevenzione delle Malattie Project of the Italian Health Ministry.

Giordano Madeddu, Fabiola Mancini, Antonello Caddeo, Alessandra Ciervo, Sergio Babudieri, Ivana Maida, Maria Laura Fiori, Giovanni Rezza, and Maria Stella Mura

Author affiliations: University of Sassari, Sassari, Italy (G. Madeddu, A. Caddeo, S. Babudieri, I. Maida, M.L. Fiori, M.S. Mura); and Istituto Superiore di Sanità, Rome, Italy (F. Mancini, A. Ciervo, G. Rezza)

DOI: http://dx.doi.org/10.3201/eid1804.111583

References

1. Ciceroni L, Pinto A, Ciarrocchi S, Ciervo A. Current knowledge of rickettsial diseases in Italy. Ann N Y Acad Sci. 2006;1078:143–9. http://dx.doi.org/10.1196/annals.1374.024
2. Márquez FJ, Muniain MA, Soriguer RC, Izquierdo G, Rodríguez-Bano J, Borobio MV. Genotypic identification of an undescribed spotted fever group rickettsia in *Ixodes ricinus* from southwestern Spain. Am J Trop Med Hyg. 1998;58:570–7.
3. Beninati T, Lo N, Noda H, Esposito F, Rizzoli A, Favia G, et al. First detection of spotted fever group rickettsiae in *Ixodes ricinus* from Italy. Emerg Infect Dis. 2002;8:983–6.

Table. Selected inner primers used to amplify rickettsial *glt*A and *omp*A genes*

Rickettsial groups	Gene	Primer	Nucleotide sequence, 5′ → 3′	Product size, bp	Reference
Rickettsiae spotted fever group plus typhus group	*gtlA*	gltA–F	TCGCAAATGTTCACGGTACTTT	74	(*8*)
		gltA–R	TCGTGCATTTCTTTCCATTGTG		
Rickettsiae ompA	*ompA*	ompA–F	ATGGCGAATATTTCTCCAAAA	632	(*9*)
		ompA–R	GTTCCGTTAATGGCAGCATCT		

**gltA*, citrate synthase; *ompA*, outer membrane protein A.

4. Simser JA, Palmer AT, Fingerle V, Wilske B, Kurtti TJ, Munderloh UG. *Rickettsia monacensis* sp. nov., a spotted fever group *Rickettsia*, from ticks (*Ixodes ricinus*) collected in a European city park. Appl Environ Microbiol. 2002;68:4559–66. http://dx.doi.org/10.1128/AEM.68.9.4559-4566.2002
5. Sréter-Lancz Z, Sréter T, Széll Z, Egyed L. Molecular evidence of *Rickettsia helvetica* and *R. monacensis* infections in *Ixodes ricinus* from Hungary. Ann Trop Med Parasitol. 2005;99:325–30. http://dx.doi.org/10.1179/136485905X28027
6. Chmielewski T, Podsiadly E, Karbowiak G, Tylewska-Wierzbanowska S. *Rickettsia* spp. in ticks, Poland. Emerg Infect Dis. 2009;15:486–8. http://dx.doi.org/10.3201/eid1503.080711
7. Jado I, Oteo JA, Aldámiz M, Gil H, Escudero R, Ibarra V, et al. *Rickettsia monacensis* and human disease, Spain. Emerg Infect Dis. 2007;13:1405–7.
8. Paris DH, Blacksell SD, Stenos J, Graves SR, Unsworth NB, Phetsouvanh R, et al. Real-time multiplex PCR assay for detection and differentiation of rickettsiae and orientiae. Trans R Soc Trop Med Hyg. 2008;102:186–93. http://dx.doi.org/10.1016/j.trstmh.2007.11.001
9. Zhang L, Jin J, Fu X, Raoult D, Fournier PE. Genetic differentiation of Chinese isolates of *Rickettsia sibirica* by partial *ompA* gene sequencing and multispacer typing. J Clin Microbiol. 2006;44:2465–7. http://dx.doi.org/10.1128/JCM.02272-05
10. Di Todaro N, Piazza C, Otranto D, Giangaspero A. Ticks infesting domestic animals in Italy: current acarological studies carried out in Sardinia and Basilicata regions. Parassitologia. 1999;41(Suppl 1): 39–40.

Address for correspondence: Giordano Madeddu, Dipartimento di Medicina Clinica, Sperimentale e Oncologica, Università degli Studi di Sassari, Via de Nicola 1, 07100 Sassari, Italy; email: giordano.madeddu@uniss.it

Leishmania Resistance to Miltefosine Associated with Genetic Marker

To the Editor: During 2000–2010, serial *Leishmania* isolates obtained from an HIV-infected patient who was not responding to treatment showed a gradual decrease in in vitro miltefosine susceptibility. We performed *L. donovani* miltefosine transporter (*Ldmt*) gene analysis to identify an association between miltefosine resistance of reference *L. donovani* lines and variability in miltefosine response of *L. infantum* isolates. A new single-nucleotide polymorphism (SNP), L832F, was identified, which might be a marker of miltefosine resistance in leishmaniasis.

The patient, a 46-year-old woman, had lived in France since 1994 but regularly returned to Algeria, her country of birth. HIV-1 infection was diagnosed in 1991. Antiretroviral therapy was initiated in 1993, leading to undetectable viral load and a CD4+ T-cell count of 185 cells/mm^3 (reference >450/mm^3). Concurrent conditions were thoracic herpes zoster in 1996, hairy leukoplakia of tongue, oropharyngeal candidiasis, and chronic renal failure of unknown cause since 2000.

Visceral leishmaniasis was diagnosed in 1998 by culture of a bone marrow smear, which showed intracellular amastigotes. Use of meglumine antimonate (Glucantime; Sanofi, Paris, France), a drug of choice for the treatment of leishmaniasis, was contraindicated because of pancreatitis in the patient and in vitro isolate susceptibility variation; therefore, induction therapy consisted of liposomal amphotericin B (AmpB [AmBisome; Astellas Pharma US, Deerfield, IL, USA]) at a dose of 3 mg/kg/d for 5 consecutive days, then 1× week for 5 weeks (total dose 30 mg/kg) during 1998–2000 (Table). The same medication was administered for relapses at 4 mg/kg/d for 5 days, then 4 mg/kg 1× week for 5 weeks (total dose 40 mg/kg) during 2001–2010. Given the adverse effects of AmpB and the availability of oral miltefosine (Impavido; AEterna Zentaris Inc., Quebec City, Quebec, Canada), the latter drug was used for maintenance treatment during 2001–2007 at 50 mg 2×/d. Leishmaniasis was monitored by leukocytoconcentration and culture of blood samples on Novy-Nicolle-McNeal medium.

When signs of biological and clinical relapse appeared, bone marrow was aspirated for parasite detection. After culture of the aspirate and isoenzyme determination, the strain was identified as *L. infantum*, zymodeme MON-24. Eleven relapses were documented; all were confirmed by positive direct examination of bone marrow or blood, but cultures of only 7 samples yielded positive results (Table).

The susceptibility of 4 cryopreserved isolates (S_1, S_3, S_4, and S_6; Table) to AmpB and to miltefosine was studied in the in vitro promastigote and axenic amastigote form by determining the concentrations inhibiting parasite growth by 50% (*1*,*2*). The 50% inhibitory concentration (IC_{50}) was determined in parallel for the following reference *L. donovani* lines: a wild-type *L. donovani* LV9 (MHOM/ET/67/HU3) line (LV9 WT), a wild-type *L. donovani* DD8 (MHOM/IN/80/DD8) line (DD8 WT), a laboratory miltefosine-resistant line obtained from LV9 WT (LV9 miltefosine-R, resistant to 90 μmol/L miltefosine), and the laboratory AmB-resistant line obtained from DD8 WT (DD8 AmB-R, resistant to 1.4 μmol/L AmB) on promastigote and axenic amastigote forms (*3*,*4*).

The AmB susceptibility of the isolates did not change notably over time; IC_{50} values ranged from 0.09

Table. Comparions of IC_{50} for AmpB and miltefosine against promastigotes and axenic amastigotes and distribution of *LdMT* SNPs in *Leishmania infantum* isolates and reference strains*

Isolate	Year	AmpB	Miltefosine	IC_{50}, µmol/L ± SEM				*Ldmt* SNP
				AmpB		Miltefosine		
				Promastigotes	Axenic amastigotes	Promastigotes	Axenic amastigotes	
–	1998	3 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	–	–	–	–	–
S_1	2000	3 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	0.09 ± 0.04†	0.10 ± 0.03	7.14 ± 0.56†	5.00± ± 0.7†	L832
–	2001	4 mg/kg/d × 5 d; then 1×/wk × 5 wk	50 mg 2×/d	–	–	–	–	–
S_3	2005	4 mg/kg/d × 5 d; then		0.13 ± 0.03	0.20 ± 0.03	25.93 ± 1.46†	21.00 ± 1.50†	832_L/F
S_4		1×/wk × 5 wk‡		0.24 ± 0.01†	0.15 ± 0.02	27.89 ± 1.76†	31.90 ± 1.60†	
–	2007	4 mg/kg/d × 5 d; then 1×/wk × 5 wk		–	–	–	–	–
S_6	2008	4 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	0.16 ± 0.03	0.11 ± 0.03	44.30 ± 3.70†	50.10 ± 1.00†	832F
S_7	2010	4 mg/kg/d × 5 d; then 1×/wk × 5 wk	–	–	–	–	–	L832
Reference strain								
LV9 WT				0.03 ± 0.02	0.02 ± 0.05	4.46 ± 0.29†	6.20 ± 0.3	L832
LV9 Miltefosine-R				0.22 ± 0.04	0.70 ± 0.09	45.84 ± 2.40†	54.20 ± 2.20†	832F
DD8 WT				0.06 ± 0.02†	0.05 ± 0.03†	17.40 ± 1.70	12.40 ± 1.50	L832
DD8 AmpB-R				1.42 ± 0.06†	1.00 ± 0.07†	15.20 ± 1.00	10.30 ± 1.20	L832

*IC_{50}, 50% inhibitory concentration; AmpB, amphotericin B; *Ldmt*, *Leishmania donovani* miltefosine transporter gene; SNP, single-nucleotide polymorphism; –, assay not performed because sample unavailable or not culturable; WT, wild type; R, resistant.
†Significance was analyzed by using the nonparametric Mann-Whitney U test to compare the IC_{50} of the isolates with the IC_{50} of reference strains; $p<0.01$ was considered significant. IC_{50} of AmpB and miltefosine was compared with IC_{50} of reference strains and S_1/S_3, S_1/S_4, and S_1/S_6. Miltefosine: S_1/S_3, S_1/S_4, S_1/S_6, S_4/S_6; $p<0.01$. AmpB: S_1/S_4 significant $p<0.01$; S_1/S_3, S_1/S_6 not significant.
‡For each relapse.

µmol/L to 0.24 µmol/L, regardless of parasite form, similar to those of wild-type reference strains (Table). In contrast, the IC_{50} values of miltefosine increased greatly over time, from 5.00 µmol/L to 50.10 µmol/L. During the 6 years of follow-up with miltefosine maintenance therapy, the susceptibility of the isolate (S_6) obtained 6 months after miltefosine treatment withdrawal in 2008 was 6-fold higher than that of the first isolate (S_1) obtained in 2000.

The *L. donovani* miltefosine transporter protein (LdMT) promotes miltefosine translocation (*5*), and LdMT inactivation in *L. donovani* promastigotes leads to miltefosine resistance at the promastigote and amastigote stages (*6*). In 2003 and 2006 studies, several mutations were linked to the inability of parasites to take up miltefosine and to miltefosine resistance (*5*,*7*). In a 2009 study, the weak expression of LdMT and its β subunit LdROS3 in *L. braziliensis* isolates was linked to diminished sensitivity (*8*). We sequenced the entire *Ldmt* gene (3,294 bp) in the reference strains and the clinical isolates for SNP analysis (*5*,*7*). Only 1 new SNP, L832F, was found in the miltefosine-resistant reference strain (LV9 miltefosine-R) and in clinical isolate S_6. The L832 wild-type allele was found in isolate S_1 and in the miltefosine-sensitive reference lines (LV9, DD8, and DD8 AmpB-R), whereas both alleles were found in isolates S_3 and S_4, with a decrease in the wild-type allele (Table). The last isolate, which was obtained 3 years after miltefosine withdrawal and could not be subcultured, had reverted to the wild-type allele (L832).

These results point to a relation between the 832F allele and diminished susceptibility to miltefosine. Analysis of this case of miltefosine resistance in a patient co-infected with *Leishmania* sp. and HIV strongly suggests that an SNP (L832F) in the *Ldmt* gene could represent a molecular marker of miltefosine resistance in *L. infantum* and *L. donovani*.

Sandrine Cojean, Sandrine Houzé, Djamel Haouchine, Françoise Huteau, Sylvie Lariven, Véronique Hubert, Florence Michard, Christian Bories, Francine Pratlong, Jacques Le Bras, Philippe Marie Loiseau, and Sophie Matheron

Author affiliations: University of Paris-Sud, Châtenay-Malabry, France (S. Cojean, F. Huteau, C. Bories, P.M. Loiseau); Paris Descartes University, Paris, France (S. Houzé, D. Haouchine, V. Hubert, J. Le Bras); Paris Diderot University, Paris (S. Lariven, F. Michard, S. Matheron); and Montpellier University, Montpellier, France (F. Pratlong)

DOI: http://dx.doi.org/10.3201/eid1804.110841

References

1. Vieira NC, Herrenknecht C, Vacus J, Fournet A, Bories C, Figadère B, et al. Selection of the most promising 2-substituted quinoline as antileishmanial can-

didate for clinical trials. Biomed Pharmacother. 2008;62:684–9. http://dx.doi.org/10.1016/j.biopha.2008.09.002
2. Vermeersch M, da Luz RI, Toté K, Timmermans JP, Cos P, Maes L. In vitro susceptibilities of *Leishmania donovani* promastigote and amastigote stages to antileishmanial reference drugs: practical relevance of stage-specific differences. Antimicrob Agents Chemother. 2009;53:3855–9. http://dx.doi.org/10.1128/AAC.00548-09
3. Mbongo N, Loiseau PM, Billion MA, Robert-Gero M. Mechanism of amphotericin B resistance in *Leishmania donovani* promastigotes. Antimicrob Agents Chemother. 1998;42:352–7.
4. Seifert K, Matu S, Perez-Victoria J, Castanys S, Gamarro F, Croft SL. Characterisation of *Leishmania donovani* promastigotes resistant to hexadecylphosphocholine (miltefosine). Int J Antimicrob Agents. 2003;22:380–7. http://dx.doi.org/10.1016/S0924-8579(03)00125-0
5. Perez-Victoria FJ, Gamarro F, Ouellette M, Castanys S. Functional cloning of the miltefosine transporter. A novel P-type phospholipid translocase from *Leishmania* involved in drug resistance. J Biol Chem. 2003;278:49965–71. http://dx.doi.org/10.1074/jbc.M308352200
6. Seifert K, Pérez-Victoria FJ, Stettler M, Sánchez-Cañete MP, Castanys S, Gamarro F, et al. Inactivation of the miltefosine transporter, LdMT, causes miltefosine resistance that is conferred to the amastigote stage *of Leishmania donovani* and persists in vivo. Int J Antimicrob Agents. 2007;30:229–35. http://dx.doi.org/10.1016/j.ijantimicag.2007.05.007
7. Pérez-Victoria FJ, Sánchez-Cañete MP, Seifert K, Croft SL, Sundar S, Castanys S, et al. Mechanisms of experimental resistance of *Leishmania* to miltefosine: implications for clinical use. Drug Resist Updat. 2006;9:26–39. http://dx.doi.org/10.1016/j.drup.2006.04.001
8. Sánchez-Cañete MP, Carvalho L, Pérez-Victoria FJ, Gamarro F, Castanys S. Low plasma membrane expression of the miltefosine transport complex renders *Leishmania braziliensis* refractory to the drug. Antimicrob Agents Chemother. 2009;53:1305–13. http://dx.doi.org/10.1128/AAC.01694-08

Address for correspondence: Sandrine Cojean, UMR 8076 CNRS BioCIS; Groupe Chimiothérapie Antiparasitaire; Univ Paris-Sud; 5 Rue Jean-Baptiste Clément, 92290 Châtenay-Malabry, France; email: sandrine.cojean@u-psud.fr

Prolonged KI Polyomavirus Infection in Immunodeficient Child

To the Editor: Two novel polyomaviruses (PyVs), KIPyV and WUPyV, were identified in respiratory and fecal specimens from children with signs and symptoms of respiratory tract infection (*1*,*2*). A review of literature on emerging viruses in transplant recipients indicated that up to 80% of patients harboring these PyVs are co-infected with another respiratory virus, complicating interpretation of positive findings (*3*). Seroprevalence of KIPyV and WUPyV in healthy blood donors in Germany have been reported to be 67% and 89%, respectively (*4*).

The effect of these viruses in immunocompromised patients is unknown. Some studies report a higher frequency of KIPyV DNA detection in hematopoietic stem cell transplant (HSCT) recipients (*5*–*7*) than in immunocompetent patients. In fact, HCST recipients might be more prone to productive infection with KIPyV and WUPyV than to infection with PyVs JC and BK (BKPyV) (*5*).

We report prolonged detection of KIPyV DNA in the respiratory tract of an immunocompromised child. A 12-year-old girl with severe combined immunodeficiency was admitted to the Freiburg University Medical Center, Germany, in November 2009 for treatment of progressive respiratory problems and cytomegalovirus (CMV) disease. Although the molecular basis of the immune disorder was unknown, HSCT was indicated because of uncontrolled CMV infection and progressive clinical deterioration.

Allogenic HSCT was performed in February 2010. Pretransplant treatments included thyothepa (day –7; 8 mg/kg), fludarabine (days –6 to –3; 120 mg/m^2), treosulfan (days –6 to – 4; 42 g/m^2), and antithymocyte globulin (days –4 to –2; 45 mg/kg). The patient received bone marrow cells (4.2 × 10^6 CD34-positive cells/kg) from an 8/10 human leukocyte antigen-matched, CMV-positive, unrelated donor. Graft-versus-host disease prophylaxis consisted of cyclosporine A (from day –1) and methotrexate (days +1, +3, +6; 10 mg/m^2). Leukocyte, granulocyte, and platelet engraftment occurred on days +18, +19, and +32, respectively. Full donor chimera was detected by day +62 (Figure, panel A).

Before hospitalization, the child had had several pulmonary infections. At admission, chest radiograph showed middle lobe atelectasis but no visible infiltrates. On day –83, human bocavirus was detected. On day –27, the occurrence of bilateral infiltrates was assessed, and pneumonia was diagnosed. On day +55, fever and hypoxia were monitored; chest radiograph revealed regressive infiltrates in the lower lobes but central infiltrates in the upper lobes. Rhinovirus RNA was detected at this time and persisted in the respiratory tract until day +98 (Figure, panel A). Retrospectively, KIPyV DNA was detected in 6 nasopharyngeal aspirate specimens, 4 throat swab specimens, and 1 bronchoalveolar lavage specimen collected between days –103 and +98 (Figure, panel B). No KIPyV was detected in EDTA-treated blood samples at any time. Stool samples were not available. The highest level of KIPyV DNA (10^9 copies/mL) was detected on day +16. Starting from day +43, a steady decrease in KIPyV viral load was observed. Phenotypical analysis of blood leukocytes on day +55 showed normal CD56+/16+ natural killer cells and good T-cell engraftment but no B cells. On day +108, viral clearance had occurred. Sequencing of the small t antigen amplified from all available samples was performed (*8*) and showed 100% nucleotide identity (GenBank accession no. JN874415).

A central indication for performing HSCT was uncontrolled CMV infection. Before transplantation, while the patient was receiving gancyclovir treatment, she had a high CMV DNA load (Figure, panel B). A typical mutation in the UL97 gene, conferring resistance to gancyclovir, was confirmed. Antiviral therapy was switched to foscarnet (day −30 to day +70), and CMV DNA drastically decreased. After T-cell engraftment, further decrease was followed by decrease of Epstein-Barr virus and BKPyV DNA load, which had considerably increased until day +50 (Figure, panel B).

In this immunocompromised child, KIPyV DNA was in the respiratory tract for 7 months. High prevalence of KIPyV in HSCT patients has been reported, suggesting that T-cell impairment might be a factor in facilitating KIPyV replication (*5*,*7*). Cellular immunity is crucial for containing CMV and BKPyV replication (*9*). Our observations also support a central role for cell-mediated immunity in controlling KIPyV. In fact, the peak of KIPyV DNA replication occurred during the aplastic phase. Moreover, a concomitant decrease in KIPyV, CMV, Epstein-Barr virus, and BKPyV DNA load observed after noting normal CD56+/16+ natural killer cells and T-cell engraftment further supports this hypothesis. Therefore, we theorize that early infection in childhood with KIPyV probably results in latency in the presence of a functional immune system. Immune impairment might result in reactivation. Sequence identity of KIPyV DNA from sequentially collected respiratory samples in this case further supports the conclusion that reactivation, rather than reinfection by heterologous strains, occurred.

The contribution of KIPyV to respiratory disease remains ambiguous: in the posttransplantation period, rhinovirus detection correlates with increasing pulmonary infiltrates. However, before transplantation, KIPyV was identified as the sole agent in the respiratory tract; increasing viral loads seem to correlate with the development of bilateral infiltrates. The relevance of human bocavirus co-detection in 1 sample before transplantation remains unclear. Future prospective studies are needed to establish a correlation between immunosuppression, KIPyV shedding, and the occurrence of respiratory symptoms in immunocompromised patients.

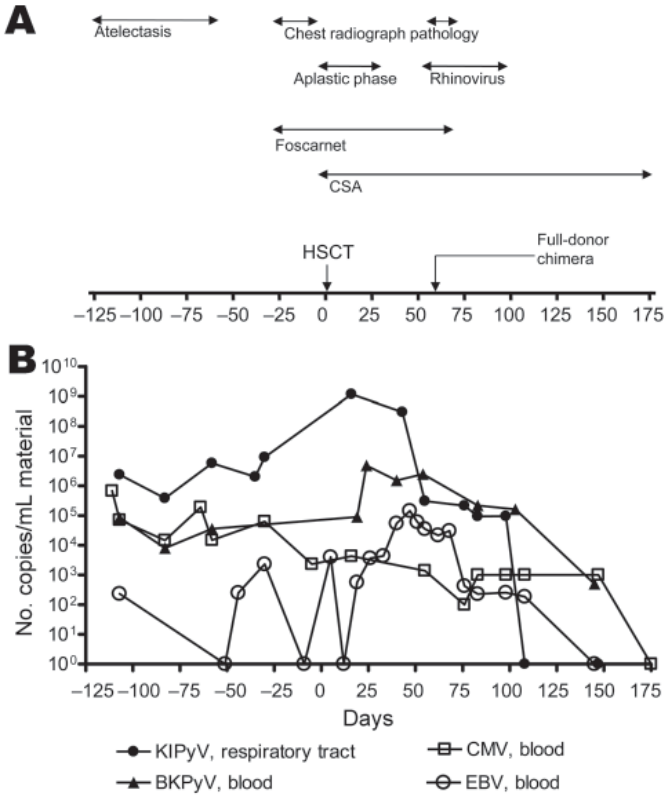


Figure. Timeline of clinical and virologic features for a 12-year-old immunocompromised child, before and after HSCT, Germany, 2009. A) Main clinical events and therapeutic measures. B) Viral DNA load measured by real-time PCR. CSA, cyclosporin A; HSCT, hematopoietic stem cell transplant; KIPyV, KI polyomavirus; BKPyV, BK polyomavirus; CMV, cytomegalovirus; EBV, Epstein-Barr virus.

Acknowledgments

We thank B. Weissbrich for kindly providing the WUPyV and KIPyV VP1 plasmids as positive controls for PCR and Gudrun Woywodt for excellent technical assistance.

This work was supported by Bundesministerium für Bildung und Forschung (contract #01ES0830).

Valeria Falcone, Marcus Panning, Brigitte Strahm, Thomas Vraetz, Sibylle Bierbaum, Dieter Neumann-Haefelin, and Daniela Huzly

Author affiliation: Freiburg University Medical Center, Freiburg, Germany

DOI: http://dx.doi.org/10.3201/eid1804.111588

References

1. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, et al. Identification of a third human polyomavirus. J Virol. 2007;81:4130–6. http://dx.doi.org/10.1128/JVI.00028-07

2. Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, et al. Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog. 2007;3:e64. http://dx.doi.org/10.1371/journal.ppat.0030064
3. Kumar D. Emerging viruses in transplantation. Curr Opin Infect Dis. 2010;23:374–8.
4. Neske F, Prifert C, Scheiner B, Ewald M, Schubert J, Opitz A, et al. High prevalence of antibodies against polyomavirus WU, polyomavirus KI, and human bocavirus in German blood donors. BMC Infect Dis. 2010;10:215. http://dx.doi.org/10.1186/1471-2334-10-215
5. Mourez T, Bergeron A, Ribaud P, Scieux C, de Latour RP, Tazi A, et al. Polyomaviruses KI and WU in immunocompromised patients with respiratory disease. Emerg Infect Dis. 2009;15:107–9. http://dx.doi.org/10.3201/1501.080758
6. Rao S, Garcea RL, Robinson CC, Simoes EA. WU and KI polyomavirus infections in pediatric hematology/oncology patients with acute respiratory tract illness. J Clin Virol. 2011;52:28–32. http://dx.doi.org/10.1016/j.jcv.2011.05.024
7. Mueller A, Simon A, Gillen J, Schildgen V, Tillmann RL, Reiter K, et al. Polyomaviruses KI and WU in children with respiratory tract infection. Arch Virol. 2009;154:1605–8. http://dx.doi.org/10.1007/s00705-009-0498-2
8. Babakir-Mina M, Ciccozzi M, Alteri C, Polchi P, Picardi A, Greco F, et al. Excretion of the novel polyomaviruses KI and WU in the stool of patients with hematological disorders. J Med Virol. 2009;81:1668–73. http://dx.doi.org/10.1002/jmv.21559
9. Egli A, Binggeli S, Bodaghi S, Dumoulin A, Funk GA, Khanna N, et al. Cytomegalovirus and polyomavirus BK posttransplant. Nephrol Dial Transplant. 2007;22 Suppl 8:viii72-viii82.

Address for correspondence: Valeria Falcone, Department of Virology, Freiburg University Medical Center, Hermann-Herder-Strasse 11, 79104 Freiburg, Germany; email: valeria.kapper-falcone@uniklinik-freiburg.de

High Virulence of African Swine Fever Virus Caucasus Isolate in European Wild Boars of All Ages

To the Editor: African swine fever (ASF) is a serious disease that is currently affecting domestic pigs and wild boars in the Russian Federation. The disease is caused by *African swine fever virus* (ASFV; family *Asfarviridae*), and its continuing spread imposes a growing risk for introduction to disease-free areas with a high density of pigs and/or wild boars. We recently reported on the experimental characterization of ASFV Caucasus isolates in European wild boar piglets and juveniles (*1*), age classes that were deemed to be the most susceptible to ASFV. The extreme virulence of the virus strain led to an almost peracute disease and 100% mortality. On the basis of these data, a scenario of endemicity driven by chronically diseased animals or ASFV carriers seems unlikely. Nevertheless, ASF continues to occur in wild boars.

The clinical course of some infectious diseases is age dependent; thus, we supplemented our previous study (*1*) with a limited study among adult wild boars to help clarify their role in the epidemiology of ASFV. To achieve this goal, we orally inoculated 1 boar (10 years of age), 2 sows (4 and 5 years, respectively), and 1 boar piglet with a 3×10^6 50% tissue culture infectious dose of the ASFV Caucasus isolate.

Severe, unspecific clinical signs (fever, depression, anorexia, dyspnea, ataxia) developed in all animals. Infection was confirmed by PCR of blood samples and fecal and oral swab samples obtained 6 days after inoculation. All animals died or were euthanized in a moribund state 8–9 days after inoculation, confirming that ASFV causes severe, acute disease and is fatal for 100% of infected adult European wild boars. No antibodies were detected in serum samples throughout the experiment.

The available data show no indication of chronic ASF disease or ASFV carrier states among adult wild boars, conditions that could potentially contribute to long-term persistence of disease in an affected region. In terms of risk assessment, the most likely routes for the introduction of ASFV into wild boar populations are spillover from domestic pigs, exposure to ASFV-contaminated carcasses under climate conditions favoring the persistence of infectious virus, contact with fomites, and consumption of ASFV-contaminated animal feed.

Sandra Blome, Claudia Gabriel, Klaas Dietze, Angele Breithaupt, and Martin Beer

Author affiliations: Friedrich-Loeffler-Institut, Greifswald–Insel Riems, Germany (S. Blome, C. Gabriel, A. Breithaupt, M. Beer); and Food and Agriculture Organization of the United Nations, Rome, Italy (K. Dietze)

DOI: http://dx.doi.org/10.3201/eid1804.111813

Reference

1. Gabriel C, Blome S, Malogolovkin A, Parilov S, Kolbasov D, Teifke JP, et al. Characterization of African swine fever virus Caucasus isolate in European wild boars. Emerg Infect Dis. 2011;17:2342–5. http://dx.doi.org/10.3201/eid1712.110430

Address for correspondence: Sandra Blome, Institute of Diagnostic Virology, Friedrich-Loeffler-Institut, Suedufer 10, 17493 Greifswald–Insel Riems, Germany; email: sandra.blome@fli.bund.de

Charles E. Burchfield (1893–1967) ***Camouflage design*** **(1918)** Watercolor and graphite on paper mounted on black paper (27.3 cm x 32.1 cm) The Charles E. Burchfield Foundation Archives at the Burchfield Penney Art Center at Buffalo State College, Buffalo, New York**,** Gift of the Charles E. Burchfield Foundation, 2006

Military Magic or Nature's Fool

Polyxeni Potter

"If this world lasts for a million years or two million years, or more, never can you exhaust the subject matter of humanity and nature," said Charles Burchfield regarding his source of inspiration. "I don't know how much time I've got left, but I'd like to have at least another lifetime… to say what I want to say about nature." This fascination began during the artist's childhood in Salem, Ohio, where he "formed the habit of wandering off to the woods and fields… in search of wild flowers in the spring or colored leaves in the fall." These, along with blossoming trees and all manner of plants, made up his earliest drawings, also described in copious journals, more than 10,000 pages. "I hereby dedicate my life and soul to the study and love of nature, with the purpose to bring it before the mass of uninterested public."

Burchfield's art education began at the Cleveland School of Art, where he became familiar with the teachings of artist and philosopher Arthur Wesley Dow who, ahead of his time, believed that nature should be depicted not in realistic terms but in a harmonious presentation of compositional elements (line, color, light and dark). During these years, Burchfield absorbed such diverse influences as Chinese scroll paintings; the works of Hiroshige, Hokusai, and Aubrey Beardsley; and Russian ballet designs by Léon Bakst. But "The greatest inspiration to me was Henry G. Keller… not only a good painter but also a good teacher…. He made you feel as though art was the most important thing in the world, and you couldn't do better than to be an artist if you had the aptitude for it." Keller said that his student's "inability to see form" and his "virtually complete concentration on two-dimensional pattern amounted almost to genius."

A scholarship in 1916 to the national Academy of Design in New York City did not appeal, as Burchfield dropped out of the program after 1 day. But during his brief stay in the city, he met art critic and instructor Mary Mowbray-Clarke, who showed his works at her gallery, the Sunwise Turn Book shop, launching his career as a leading artist. Later, he was to describe 1917 as his "golden year." This period and up to 1918 was also his most prolific. This was too a time of war, so he joined the army. "I was made a sergeant and I had a half a dozen men under me, and we were doing camouflage. And I was happy making the designs for camouflage."

After this brief career in the military, he returned to Salem, where he continued to paint around his work as accountant in an auto parts company. When a better opportunity came up, he moved to Buffalo, New York, to work as designer for H. M. Birge and Sons, "the finest wallpaper firm in the country." "I had to make a living somehow…. I knew it would be years before I could hope to make any money out of painting." It was 1929 and his work was shown in the prestigious Montross Gallery when he made the decision to devote all his time to painting.

Burchfield felt most comfortable with the medium of watercolor. "I use a dry paper and what is called a dry brush, which isn't dry, of course, in that it has the minimum amount of water in it, and I stand them up… just as a man painting an oil painting, except that you are using different materials." Of his technique, he said, "I like to advance and retreat, just like a man writing a book. I doubt that very few of them ever sit down and leave a paragraph as it first

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

DOI: http://dx.doi.org/10.3201/eid1804.AC1804

comes into their head. They work over it, delete things and add things…. A picture has a life and direction of its own, and the artist has to find out which way the picture wants to go and follow …. It sounds like doubletalk, but it's true."

Camouflage design, on this month's cover, one of two surviving studies from Burchfield's military days, is a mix of abstract and realistic elements and like all his work reaches for more than meets the eye. At home with modern camouflage, he built on art invented by those like him, who studied nature. American naturalist and artist Abbott Handerson Thayer (1849–1921), sometimes referred to as the father of camouflage because of his pioneering research on protective coloration in nature, laid out basic principles: the color of a camouflaged object must match its background. Strong patterns work better than delicate ones because they interfere with how we read the outline of a form. A dark strip at the top and a white splash underneath disrupt our expectations of how an object will appear (countershading).

Crude forms have been used in warfare since antiquity. Shakespeare knew camouflage. In Macbeth, at the wood of Birnam, Malcolm said, "Let every soldier hew him down a bough / And bear't before him: thereby shall we shadow / The numbers of our host and make discovery /Err in report of us." And then, "As I did stand my watch upon the hill, / I look'd toward Birnam, and anon, methought, / The wood began to move."

Thayer's ideas were quickly put to military and artistic use. And when the United States entered World War I, several artists, Burchfield among them, became involved, producing various patterns, initially representational then boldly abstract. When cubism swept the art scene, obliterating the distinction between an object and its background, it influenced military camouflage designs. In The Autobiography of Alice B. Toklas, Gertrude Stein described an evening in 1915, when she and Toklas were strolling with Picasso and his mistress Eva Gouel. "All of a sudden down the street came some big cannon, the first any of us had seen painted, that is, camouflaged. Pablo stopped, he was spell-bound. '*C'est nous qui avons fait ça*,' he said."

Near the end of his life, Burchfield went back to the paintings of his golden year, his time most inspired by nature. He pasted them into new works, seamlessly expanding and absorbing them into larger creations incorporating the most profound discoveries of his later artistic career. These masterpieces, the culmination of his love for nature, seem to complete his desire to become one with it.

The merger between art and nature sensed by camouflage artists has broad applicability in disease emergence, where the borders, geographic and biologic, blend as smoothly and seamlessly as in art. Burchfield's *Camouflage design* with its graceful curves, flat patterns, and strong colors over linear designs is a perfect portrait of natural elements, overlapping and filled with movement, light, or heat, the underpinnings as well as the surface of nature, and most of all, its mystery and intrigue.

A look at the ecologic dynamics, meteorologic variables, and transmission cycles, along with human travel and vector control of malaria alone as it reemerges around the world, attests to Burchfield's acute understanding of complexity underneath the surface. In Ecuador, the railway inadvertently provided transportation of the vector and parasite to higher elevations. In other parts of the world, dengue virus activity, a major cause of illness in travelers, is increasing and, until an effective vaccine is licensed, will likely remain a threat to military troops operating where the disease is endemic. Recent outbreaks in Hawaii, Texas, and Florida resulted in recommendations on public health response, including such vector control activities as spraying for adult mosquitoes and eliminating standing water around homes. In Veneto Italy, surveillance to identify cases of West Nile fever, imported dengue and chikungunya infections in travelers also highlighted, among other measures, the need for vector control. But, while artists and the military have managed to fool human enemies by camouflage, we have yet to adequately fool mosquito and other vectors of disease.

Bibliography

1. Adalja AA, Sell TK, Bouri N, Franco C. Lessons learned during dengue outbreaks in the United States, 2001–2011. Emerg Infect Dis. 2012;18:608–14. http://dx.doi.org/10.3201/eid1804.110968
2. Behrens RR. False colors: art, design and modern camouflage. Dysart (IA): Bobolink Books; 2002.
3. Gibbons RV, Streitz M, Babina T, Fried JR. Dengue and US military operations from the Spanish–American War through today. Emerg Infect Dis. 2012;18:623–30. http://dx.doi.org/10.3201/eid1804.110134
4. Gobbi F, Barzon L, Capelli G, Angheben A, Pacenti M, Napoletano G, et al. Surveillance for West Nile, dengue, and chikungunya virus infections, Veneto Region, Italy, 2010. Emerg Infect Dis. 2012;18:671–3. http://dx.doi.org/10.3201/eid1804.110753
5. Gober R, Burlingham C. Heat waves in a swamp: the paintings of Charles Burchfield. Los Angeles: Hammer Museum; 2009.
6. Oral history interview with Charles Burchfield, 1959 Aug 19, Archives of American Art, Smithsonian Institution [cited 2012 Feb 24]. http://www.aaa.si.edu/collections/interviews/oral-history-interview-charles-burchfield-12702
7. Penrose R. Picasso: his life and work. New York: Harper and Row; 1973.
8. Burchfield C. Charles Burchfield's journals: the poetry of place. Townsend B, editor. Albany (NY): SUNY Press; 1993.
9. Pinault LL, Hunter FF. Malaria in highlands of Ecuador since 1900. Emerg Infect Dis. 2012;18:615–22. http://dx.doi.org/10.3201/eid1804.111267

Address for correspondence: Polyxeni Potter, EID Journal, Centers for Disease Control and Prevention, 1600 Clifton Rd NE, Mailstop D61, Atlanta, GA 30333, USA; email: pmp1@cdc.gov

EMERGING INFECTIOUS DISEASES

Upcoming Issue

Transmission Dynamics, Border Entry Screening, and School Holidays during Pandemic (H1N1) 2009, China

Spatial Analysis of Determinants of Malaria Incidence among Adults, Ontario, Canada

Temporal Trends in *Bordetella pertussis* Populations, 1949–2010, Denmark

No Association between 2008–09 Seasonal Influenza Vaccine and Pandemic (H1N1) 2009 Infections, Manitoba, Canada

Virulent West Nile Virus Kunjin Strain, Australia

Streptococcus pneumoniae Serotype 5 Invasive Disease, Western Canada, 2005–2009

Bartonella spp. Bacteremia in Rheumatic Fever Patients from a Lyme Disease–endemic Region

Invasive *Haemophilus influenzae* Serotype e and f Disease, England and Wales

Novel Strain of Andes Virus and Fatal Human Infection, Central Bolivia

Antimicrobial Drug Resistance in *Escherichia coli* from Humans and Food Animals, United States, 1950–2002

Risk Factors for Intestinal Invasive Amebiasis, Japan, 2003–2009

Rhabdomyolysis Associated with Antimicrobial Drug–Resistant *Mycoplasma pneumoniae*

Origin of Human T-Lymphotropic Virus Type 1 in Rural West Africa

Unsuspected Rickettsioses in Patients with Acute Febrile Illness, Sri Lanka, 2007

Hepatitis E Virus Infection in Solid Organ Transplant Recipients, the Netherlands

Sapovirus Outbreaks in Adult Institutions, Oregon and Minnesota, 2002–2009

Adenovirus Type 7 Outbreak in Police Training Center, Malaysia, March 2011

Complete list of articles in the May issue at
http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

April 13–16, 2012
SHEA Spring 2012 Conference
Jacksonville, FL, USA
http://www.shea2012.org

May 6–9, 2012
8th International Symposium on Shiga Toxin (Verocytotoxin) Producing *Escherichia coli* Infections
Amsterdam, the Netherlands
http://www.vtec2012.org

May 9–13, 2012
8th International Congress on Autoimmunity 2012
Granada, Spain
http://www2.kenes.com/autoimmunity/pages/home.aspx

June 13–16, 2012
15th International Congress on Infectious Diseases (ICID)
Bangkok, Thailand
http://www.isid.org/15th_icid

July 22–27, 2012
XIX International AIDS Conference (AIDS 2012)
Washington, DC, USA
http://www.aids2012.org/

August 25–29, 2012
2012 Infectious Disease Board Review Course
Ritz-Carlton, Tysons Corner
McLean, VA, USA
http://www.IDBoardReview.com

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). Include the date of the event, the location, the sponsoring organization(s), and a website that readers may visit or a telephone number or email address that readers may contact for more information.

Announcements may be posted on the journal Web page only, depending on the event date.

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to **www.medscape.org/journal/eid**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Determinants for Autopsy after Unexplained Deaths Possibly Resulting from Infectious Causes, United States

CME Questions

1. You are part of a county task force charged with developing means to monitor emerging infections, and you are preparing for a discussion of unexplained deaths possibly resulting from infectious causes (UDPIC).

In the current study, what was a significant characteristic of cases of UDPIC?

A. Most cases occurred among male patients
B. Most cases occurred among children
C. Most cases occurred among persons of black race
D. Most cases occurred among persons of races other than black or white

2. Which of the following was the most common category of UDPIC from the national sample in the current study?

A. Sepsis/shock
B. Gastrointestinal disease
C. Neurologic disease
D. Unknown/other syndrome

3. Which of the following age groups was most likely to have received an autopsy in the current study of UDPIC?

A. Children and adolescents under age 18 years
B. Adults age 18–39 years
C. Adults age 40–49 years
D. Adults age 50 or older

4. What other characteristic was most associated with receiving an autopsy in the current study?

A. UDPIC due to sepsis/shock
B. Living in an urban center
C. White race
D. Race other than white or black

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

Earning CME Credit

To obtain credit, you should first read the journal article. After reading the article, you should be able to answer the following, related, multiple-choice questions. To complete the questions (with a minimum 70% passing score) and earn continuing medical education (CME) credit, please go to **www.medscape.org/journal/eid**. Credit cannot be obtained for tests completed on paper, although you may use the worksheet below to keep a record of your answers. You must be a registered user on Medscape.org. If you are not registered on Medscape.org, please click on the New Users: Free Registration link on the left hand side of the website to register. Only one answer is correct for each question. Once you successfully answer all post-test questions you will be able to view and/or print your certificate. For questions regarding the content of this activity, contact the accredited provider, CME@medscape.net. For technical assistance, contact CME@webmd.net. American Medical Association's Physician's Recognition Award (AMA PRA) credits are accepted in the US as evidence of participation in CME activities. For further information on this award, please refer to http://www.ama-assn.org/ama/pub/category/2922.html. The AMA has determined that physicians not licensed in the US who participate in this CME activity are eligible for *AMA PRA Category 1 Credits*™. Through agreements that the AMA has made with agencies in some countries, AMA PRA credit may be acceptable as evidence of participation in CME activities. If you are not licensed in the US, please complete the questions online, print the certificate and present it to your national medical association for review.

Article Title

Influenza-associated Hospitalizations by Industry, 2009–10 Influenza Season, United States

CME Questions

1. Which of the following statements regarding employment and the risk for hospitalization for influenza in the current study is most accurate?

A. There was a broad increase in the risk for hospitalization for influenza among unemployed vs. employed individuals
B. Employment was a risk factor for hospitalization for influenza only among older adults
C. Employment was a risk factor for hospitalization for influenza only among retail workers
D. There was no relationship between employment status and the risk for hospitalization for influenza

2. All of the following industry sectors experienced overrepresentation of hospitalized influenza cases EXCEPT:

A. Transportation and warehousing
B. Public administration
C. Healthcare
D. Accommodation and food service

3. Which of the following statements regarding the interaction between underlying medical conditions and the risk for hospitalization for influenza in the current study is most accurate?

A. Underlying medical conditions were associated with an increased risk for hospitalization in all industry sectors
B. Educational services carried the highest risk for hospitalization due to its high rate of underlying medical conditions
C. Arts and entertainment carried the highest risk for hospitalization due to its high rate of underlying medical conditions
D. Underlying medical conditions were a risk factor for hospitalization only among unemployed individuals

4. What other factors might have most influenced the rate of hospitalization for influenza in the current study?

A. Access to healthcare
B. Female sex
C. Location of employment
D. Race other than white or black

Activity Evaluation

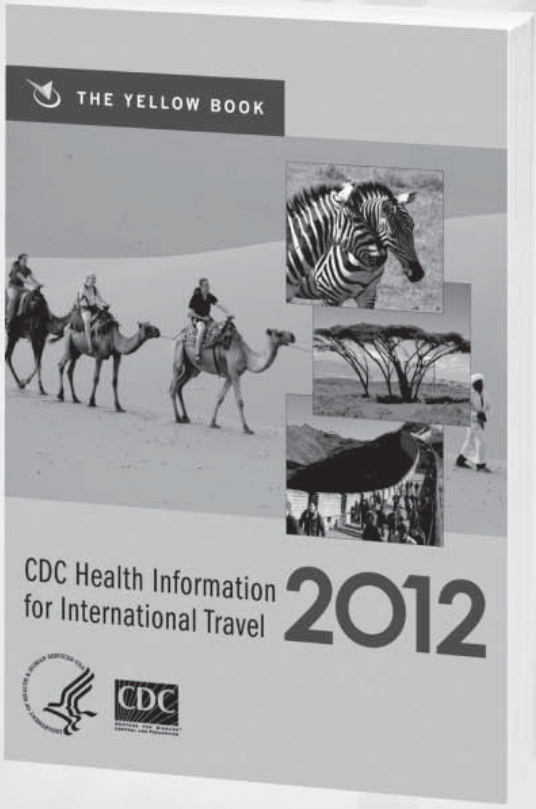
1. The activity supported the learning objectives.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
2. The material was organized clearly for learning to occur.				
Strongly Disagree				Strongly Agree
1	2	3	4	5
3. The content learned from this activity will impact my practice.				
Strongly Disagree				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

CDC Health Information for International Travel 2012

CDC

Health risks are dynamic and ever-changing, both at home and while traveling abroad. To stay abreast of the most up-to-date health recommendations, for decades health care professionals and travelers have relied on the Centers for Disease Control and Prevention's user-friendly Health Information for International Travel (commonly referred to as the The Yellow Book) as a trusted reference. Updated biennially by a team of experts, this book is the only publication for all official government recommendations for international travel.

The book's features include clear and easy-to-read disease risk maps, information on where to find health care during travel, specific health information and itineraries for popular tourist destinations, detailed country-specific information for yellow fever and malaria, advice for those traveling with infants and children, and a comprehensive catalog of diseases, their clinical pictures, and their epidemiologies. The Yellow Book addresses the pre-travel consult and provides post-travel clinical guidance on ways to approach common syndromes of returned travelers who are ill.



May 2011 640 pp.
9780199769018 Paperback $45.00

FEATURES

> Authoritative and complete information on precautions that the traveler should take for nearly all forseeable risks

> The definitive resource for health care professionals who see patients for pre-travel consultation

> The only publication for the US Government's most up-to-date recommendations for traveler safety

4 EASY WAYS TO ORDER!

Phone: 800-451-7556
Fax: 919-677-1303
Web: www.oup.com/us
Mail: Oxford University Press, Order Dept.
2001 Evans Road, Cary, NC 27513

OXFORD
UNIVERSITY PRESS

EMERGING INFECTIOUS DISEASES

www.cdc.gov/eid

JOURNAL BACKGROUND AND GOALS

What are "emerging" infectious diseases?

Infectious diseases whose incidence in humans has increased in the past 2 decades or threatens to increase in the near future have been defined as "emerging." These diseases, which respect no national boundaries, include

- New infections resulting from changes or evolution of existing organisms.
- Known infections spreading to new geographic areas or populations.
- Previously unrecognized infections appearing in areas undergoing ecologic transformation.
- Old infections reemerging as a result of antimicrobial resistance in known agents or breakdowns in public health measures.

Why an "Emerging" Infectious Diseases journal?

The Centers for Disease Control and Prevention (CDC), the agency of the U.S. Public Health Service charged with disease prevention and health promotion, leads efforts against emerging infections, from AIDS, hantavirus pulmonary syndrome, and avian flu, to tuberculosis and West Nile virus infection. CDC's efforts encompass improvements in disease surveillance, the public health infrastructure, and epidemiologic and laboratory training.

Emerging Infectious Diseases represents the scientific communications component of CDC's efforts against the threat of emerging infections. However, even as it addresses CDC's interest in the elusive, continuous, evolving, and global nature of these infections, the journal relies on a broad international authorship base and is rigorously peer-reviewed by independent reviewers from all over the world.

What are the goals of Emerging Infectious Diseases?

1) Recognition of new and reemerging infections and understanding of factors involved in disease emergence, prevention, and elimination. Toward this end, the journal

   - Investigates factors known to influence emergence: 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.
   - Reports laboratory and epidemiologic findings within a broader public health perspective.
   - Provides swift updates of infectious disease trends and research: new methods of detecting, characterizing, or subtyping pathogens; developments in antimicrobial drugs, vaccines, and prevention or elimination programs; case reports.

2) Fast and broad dissemination of reliable information on emerging infectious diseases. Toward this end, the journal

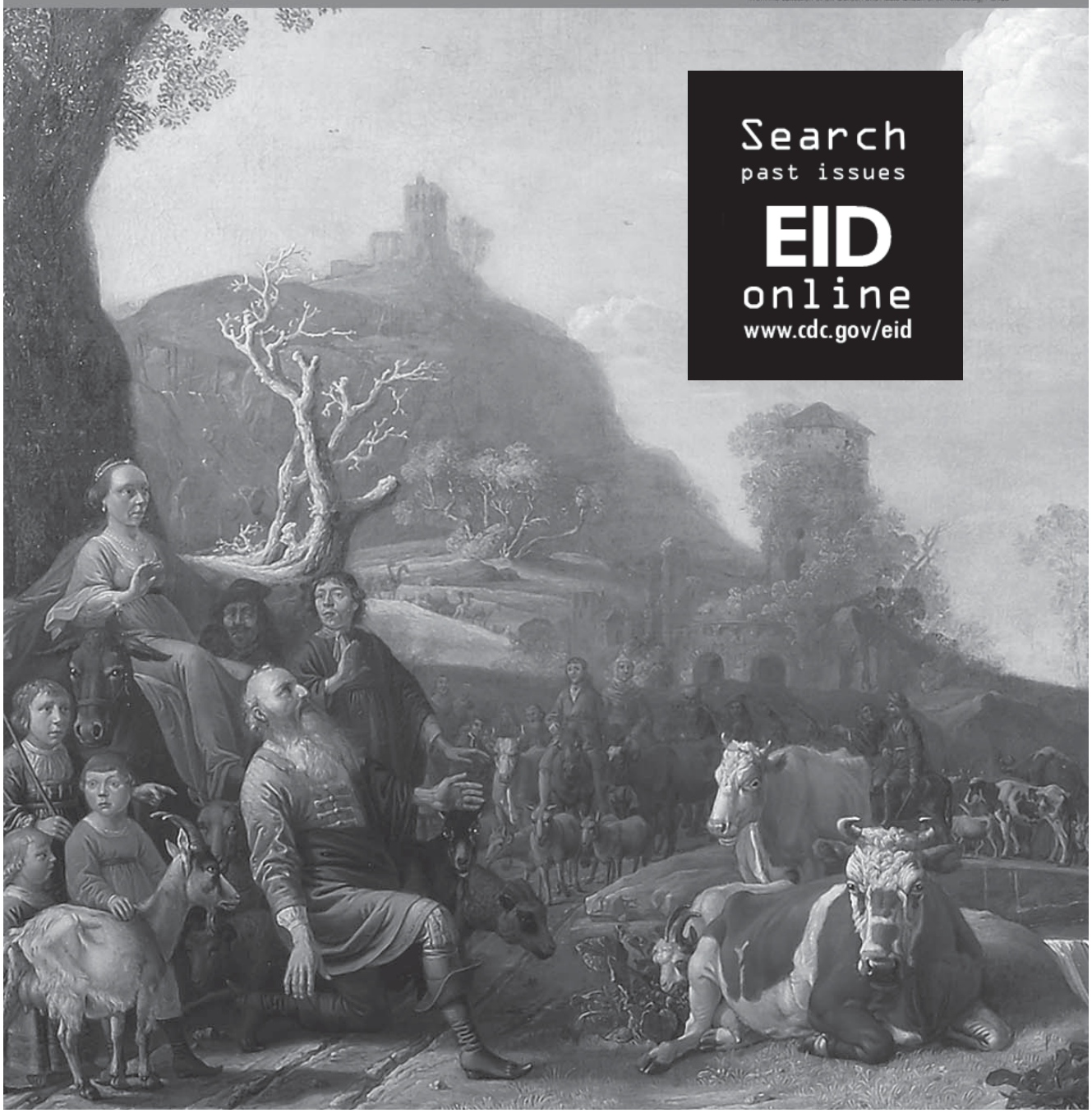
   - Publishes reports of interest to researchers in infectious diseases and related sciences, as well as to public health generalists learning the scientific basis for prevention programs.
   - Encourages insightful analysis and commentary, stimulating global interest in and discussion of emerging infectious disease issues.
   - Harnesses electronic technology to expedite and enhance global dissemination of emerging infectious disease information.

EMERGING INFECTIOUS DISEASES®

March 2012

Food-Producing Animals

From the collection of Dr. Gordon and Adele Gilbert of St. Petersburg, Florida



EMERGING INFECTIOUS DISEASES®

Emerging Infectious Diseases is published monthly by the Centers for Disease Control and Prevention, 1600 Clifton Road, Mailstop D61, Atlanta, GA 30333, USA. Telephone 404-639-1960, fax 404-639-1954, email eideditor@cdc.gov.

∞ Emerging Infectious Diseases is printed on acid-free paper that meets the requirements of ANSI/NISO 239.48-1992 (Permanence of Paper)