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January 2011

Foodborne Infections

Detail of John Reading, 1934. Collection of The John and Mable Ringling Museum of Art, the State Art Museum of Florida, a division of Florida State University.

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EMERGING INFECTIOUS DISEASES

January 2011

On the Cover

Peter Paul Rubens (1577–1640)
The Gathering of the Manna (c. 1625)
Oil on canvas (487.68 cm × 411.48 cm)

Bequest of John Ringling, 1936, Collection of The John and Mable Ringling Museum of Art, the State Art Museum of Florida, a division of Florida State University

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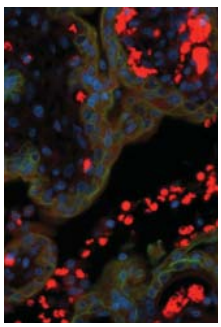

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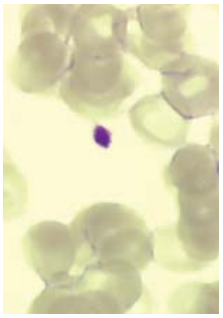

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Etymologia

Public Health Implications of Cysticercosis Acquired in the United States

Frank Sorvillo, Patricia Wilkins, Shira Shafir, and Mark Eberhard

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Release date: December 22, 2010; Expiration date: December 22, 2011

Learning Objectives

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

- Describe the presentation and prevalence of cysticercosis acquired within the United States
- Describe how cysticercosis appears to be transmitted within the United States
- Describe public health strategies that could be useful in controlling cysticercosis transmission within the United States.

Editor

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Cysticercosis has emerged as a cause of severe neurologic disease in the United States that primarily affects immigrants from Latin America. Moreover, the relevance of cysticercosis as a public health problem has been highlighted by local transmission. We searched the biomedical literature for reports documenting cases of cysticercosis acquired in the United States. A total of 78 cases, principally neurocysticercosis, were reported from 12 states during 1954–2005. A confirmed or presumptive source of infection was identified among household members or close personal contacts of 16 (21%) case-patients. Several factors, including the severe, potentially fatal, nature of cysticercosis; its fecal–oral route of transmission; the considerable economic effect; the availability of a sensitive and specific serologic test for infection by adult *Taenia solium* tapeworms; and the demonstrated ability to find a probable source of infection among contacts, all provide a compelling rationale for implementation of public health control efforts.

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Cysticercosis, infection with the larval stage of the pork tapeworm, *Taenia solium*, is a known cause of illness and death in humans (*1*,*2*). Neurocysticercosis, the most serious form of the disease, occurs when larvae invade the central nervous system. The disease is dependent on

a 2-host life cycle in which humans serve as the definitive host and pigs as the intermediate host. Eggs, which are directly infectious, are shed in the feces of humans infected with the adult tapeworm. When these eggs are ingested by pigs, larvae emerge from the eggs, penetrate the intestinal mucosa, and disseminate through the bloodstream to various tissues where the larval stage, or cysticercus, develops. The cycle is completed when humans, the only naturally infected definitive host, consume raw or undercooked pork containing cysticerci, which attach to the small intestine and develop into the adult tapeworm. However, humans may also become infected with the larval stage when they ingest *T. solium* tapeworm eggs, typically in contaminated food or water. Cysticercosis is therefore a fecal–oral-transmitted disease acquired by ingestion of eggs excreted in the feces of a human tapeworm carrier.

Cysticercosis is widely prevalent in Latin America, Asia, and parts of Africa; ≈50 million persons are infected globally (*2*,*3*). The disease is routinely seen in the United States in immigrants from disease-endemic regions, particularly Latin America (*4*,*5*). Recent attention has focused on cysticercosis as one of the neglected infections associated with poverty in the United States and also as a major cause of preventable epilepsy (*6*). The relevance of this parasitic infection as a public health problem in the United States has been highlighted by reports of autochthonous cases (*7*). This phenomenon was underscored by Schantz et al. in their report of an outbreak of cysticercosis in an Orthodox Jewish community in New York City in 1990–1991 (*8*). Several other publications, particularly over the past 25 years, have documented cysticercosis acquired in the United States (*5*,*7*–*25*). We present an aggregation of these published reports and discuss the attendant public health issues, including policy implications and approaches to prevention.

A search of PubMed was conducted by using the terms cysticercosis, neurocysticercosis, *Taenia solium*, taeniasis, and United States. Web-based searches were also conducted by using the same terms. Articles were reviewed for cases of locally acquired cysticercosis or reference to other publications documenting cysticercosis in persons without a history of travel outside the United States. Chapters in books dealing exclusively with cysticercosis and selected infectious disease texts were also reviewed.

We defined a case of cysticercosis as confirmed if there were clinical manifestations consistent with cysticercosis and a positive serologic test result or evidence of infection based on biopsy findings. A presumptive case was defined by clinical and imaging studies indicative of cysticercosis but lacking serologic or biopsy confirmation. An autochthonous case was defined as cysticercosis in a US-born person with no history of travel to a disease-endemic area before onset of symptoms indicated in the publication. Case-patients with any such travel history, independent of duration, or those for which information on travel was not reported, were excluded from consideration as locally acquired.

Twenty publications from 1954 through 2004 that documented 78 total cases of US-acquired cysticercosis from 12 states were identified (Table 1). Five (25%) reports detailing 44 (56.4%) cases were from California, all from Los Angeles County. Neurocysticercosis was most commonly reported (97.4%), although ocular (*1*) and subcutaneous (*1*) infections were also observed. No deaths were reported, but long-term follow-up of clinical status was rarely reported. Demographic information was typically not provided. However, among studies reporting such data, the mean age of case-patients with locally acquired infection was 21.3 years (range 14 months–80 years). Ages for several pediatric patients were not reported; therefore, the available data on age must be viewed with caution. Among those few reports providing information on gender, 10 cases were in female patients and 6 were in male patients. Information on race or ethnicity was rarely included.

Nine reports documented multiple cases of US-acquired cysticercosis (*5*,*7*,*9*–*11*,*15*,*17*,*19*,*23*). In the New York City cluster of neurocysticercosis, 4 initial cases in which patients experienced seizures were identified, and an additional 7 persons were found to be seropositive. Investigation determined that the probable sources of infection were domestic workers with tapeworm infection who had emigrated from areas endemic for taeniasis/cysticercosis.

In a pilot cysticercosis surveillance system conducted in Los Angeles County from 1988 through 1990, ten locally acquired cases were identified and represented 7% of 138 total incident cases (*15*). The mean age of these patients was 13.7 years (range 4–33 years), and 7 (70%) patients were female. Most patients (70%) were Hispanic, 2 (20%) were white, and 1 (10%) was black. It is noteworthy that 7 additional cases of cysticercosis were identified in immigrants from disease-endemic areas who were long-term residents (mean length of residency 15.4 years) and had no history of additional travel or exposure outside the United States since their immigration (Table 2). This phenomenon has also been recognized by McCormick (*20*), who reported 20 cases of cysticercosis among foreign-born persons with >10 years residence in the United States and no history of subsequent travel, and by Earnest et al. (*19*), who documented 5 such cases with ≥7 years residence (Table 2). Given that the median incubation period for cysticercosis has been estimated to be 3.5 years (*26*), some of these cases may reflect additional local transmission and thus may indicate a risk for exposure to visitors (family and/or friends) from areas endemic for *T. solium* tapeworm infection or exposure at a social event where visitors or recent immigrants may have helped prepare food. Similarly, 32

Table 1. Published reports of cysticercosis cases that were acquired in the United States, 1954–2005

Location	Year of onset or report	No. cases	Form	Probable or suspected source	Reference
Minnesota	2005	1	Neurologic	NR	(*9*)
Chicago, IL	1996	2	Neurologic	NR	(*10*)
Oregon	1995–2000	5	Neurologic	1 had a household visitor from a disease-endemic area	(*11*)
Chicago, IL	1986–1994	8	Neurologic	4 had recent visitors from disease-endemic areas	(*12*)
Houston, TX	1985–1991	1	Neurologic	NR	(*13*)
South Carolina	1990	1	Neurologic	Neighbor's friends from a disease-endemic area	(*14*)
Boston, MA	1990	1	Neurologic	Father with tapeworm	(*14*,*15*)
Los Angeles, CA	1988–1990	10	Neurologic	Household contact with tapeworm in 2 cases	(*16*)
New York	1989	4†	Neurologic	Domestic employees, 1 who had tapeworm	(*8*)
North Carolina	1989	1	Neurologic	Seasonal workers from disease-endemic area	(*14*)
Boston, MA	1986†	1	Neurologic	NR	(*17*)
Los Angeles, CA	1980–1986	14	Neurologic	NR	(*18*)
Denver, CO	1976–1985	1	Neurologic	NR	(*19*)
Los Angeles, CA	1981–1982	7	Neurologic	NR	(*20*)
Los Angeles, CA	1973–1983	12	Neurologic	NR	(*5*)
Los Angeles, CA	1979	1	Subcutaneous	Domestic employee who had tapeworm	(*21*)
Pennsylvania	1979†	1	Neurologic	Father who had tapeworm	(*22*)
Hershey, PA	1975	1	Ocular	NR	(*23*)
Louisiana	1954	3	1 case neurologic and subcutaneous	NR	(*24*)
Louisiana	1954	1	Neurologic	NR	(*25*)

*Some reports did not name specific cities within the state. NR, not reported.
†There were 7 additional seropositive cases detected in the community from which the initial cases were identified.

cases of cysticercosis in US-born residents who had traveled to disease-endemic areas were noted in the articles reviewed. Given our strict definition, these were considered as travel-related cases but may in fact include cases of authochthonous cysticercosis.

Among this series of US-acquired cysticercosis a probable source (*T. solium* tapeworm carrier) was identified for 6 cases and presumptive source (close contacts from disease-endemic areas) for 10. This finding represents 21% of the cases despite the fact that information on a source of infection was not reported, and perhaps not sought, for most cases. In the Los Angeles County pilot surveillance system, follow-up, which included examination of stool samples from close contacts, was conducted for 72 patients. A tapeworm carrier(s) was found among contacts for 5 (7%) of these patients. Although the numbers were small, a probable source was identified more commonly among US-born patients (22%) than among those who were foreign born (5%).

Discussion

Cysticercosis is generally viewed as a disease of developing countries or immigrants from areas where the disease is endemic. However, our review underscores that cysticercosis acquired in the United States can occur in many geographic regions of the country. Moreover, when looked for, a likely source of infection can frequently be found, principally among household members who are major sources of eggs and therefore infection. Like other fecal–oral-transmitted diseases, cysticercosis can be spread either directly or through contaminated food. Persons infected with the adult *T. solium* tapeworms are typically asymptomatic and may not be aware of their infection or of the potential risk to themselves and others. If hygiene is poor, transmission of eggs may occur, particularly within households where repeated opportunities for exposure exist. Even in areas where cysticercosis is endemic, the disease is recognized as a focal disease with clustering of cases identified around tapeworm carriers (*27*). This focal nature makes cysticercosis particularly amenable to public health follow-up and directed control efforts. The ability to find a probable source of infection among contacts to patients with cysticercosis shows that public health follow-up can be successfully conducted. Treatment of tapeworm carriers can eliminate them as possible sources of continuing infection. Such follow-up is routinely conducted by the Los Angeles County Department of Public Health.

The number of cases of cysticercosis acquired in the United States reported in the biomedical literature is clearly a minimum estimate. Because cysticercosis is not a notifiable condition in most jurisdictions and surveillance systems are rarely implemented, reliable information on US transmission is unavailable, and the true prevalence of locally acquired disease is largely unknown. Therefore, it is uncertain what proportion of actually occurring autochthonous cases our review represents. We are aware of

Table 2. Reports of cysticercosis in immigrants with long-term residency and no history of foreign travel after arrival in the United States

Location	No. cases	Mean years of residency	Reference
Los Angeles, CA	7	15.4	(*16*)
Denver, CO	5	≥7	(*19*)
Los Angeles, CA	20	>10	(*20*)

several recognized cases of cysticercosis acquired in the United States, including cases in which a source has been determined, but not reported in the literature (M. Tormey, pers. comm.). Moreover, as our review indicates, several publications have documented cysticercosis in immigrants with long-term continuous residence in the United States; some of these infections may have been locally acquired. It is also possible that cysticercosis cases assumed to have been travel related may, in fact, have been autochthonous. In addition, analysis of US mortality data identified 33 cysticercosis deaths among US-born residents over a 13-year period (1990–2002), which represented 15% of all cysticercosis deaths (*28*). However, it was not possible to ascertain from mortality records how many, if any, of these cases may have been acquired in the United States.

Our findings must be viewed with caution. Given that more than half of the case-patients were from Los Angeles County, where travel back and forth to Mexico may be more frequent than it is elsewhere, our findings may be skewed and possibly overestimate the probability of finding a source of infection. In addition, it is uncertain how many studies involved chart review versus patient interview for determining history of exposure in a disease-endemic area, and it is possible some cases reported as locally acquired may have been imported.

Emigration from taeniasis/cysticercosis-endemic areas to the United States is common. In 2008, ≈3.4 million immigrants from Mexico, >700,000 from Central and South American countries, and >1 million from areas of Asia were legal permanent residents of the United States (*29*). Moreover, undocumented immigration from such areas continues to occur in considerable numbers. The US Immigration and Naturalization Service estimates that 11.8 million unauthorized immigrants, nearly 7 million of them from Mexico, resided in the United States in January 2007, and an average of 470,000 persons emigrate from foreign countries each year (*30*). Cysticercosis and taeniasis are widely prevalent in Latin America. Although data are limited, a high prevalence of tapeworm carriers has been observed in these populations. In a study of migrant farmworkers in southern California, DeGiorgio et al., using a sensitive and specific serologic test developed by the Centers for Disease Control and Prevention (Atlanta, GA, USA), documented a *T. solium* tapeworm prevalence of 1.1% (*31*). This level is comparable to that observed in disease-endemic areas. A survey of intestinal parasites among farmworkers in North Carolina found that 3% of workers from Central American countries had *Taenia* spp. tapeworm eggs in their stools (*32*). Because *T. solium* tapeworm eggs are morphologically indistinguishable from *T. saginata* tapeworm eggs (beef tapeworm), it was not possible to determine how many of these represented *T. solium* tapeworm infection; however, *T. saginata* tapeworms, which do not cause human cysticercosis, are less common than *T. solium* tapeworms in Central America. Cardenas et al. reported finding *Taenia* spp. tapeworm in 3.3% of selected residents tested from the Ciudad Juarez, Mexico, and El Paso, Texas, border communities (*33*).

Cysticercosis infection acquired in the United States may also occur through consumption of food contaminated by a *T. solium* tapeworm–infected commercial food handler or, theoretically, from contaminated produce. Under favorable conditions, *Taenia* spp. tapeworm eggs can survive for relatively long periods in the environment (*34*), and human feces used as fertilizer or contaminated water used for irrigation can contaminate crops before importation. Transmission of several infectious agents, including hepatitis A (linked to imported green onions from Mexico) and *Cyclospora cayatenensis* (associated with raspberries imported from Guatemala), has been reported (*35*,*36*). Although the report must be viewed with caution, *Taenia* spp. tapeworm eggs were recovered from several varieties of vegetables obtained in local markets in the northeastern Mexican state of Tamaulipas (which borders the United States), suggesting the possibility of transmission from contaminated produce (*37*). Whether these eggs represented *T. solium* tapeworms and were viable is unknown, and this finding has not been verified by other studies. US transmission of cysticercosis linked to contaminated food products has never been documented, and the small number of reported cases of autochthonous cysticercosis may indicate that the risk is low. However, underreporting of the disease and its long incubation period make study of this possible phenomenon difficult.

Several aspects of cysticercosis provide compelling rationales for implementation of public health efforts for the control of this disease. Cysticercosis is a preventable severe infection that can include long-term neurologic sequelae and death. Moreover, it is a fecal–oral-transmitted disease, and a probable source of infection among contacts can frequently be found. In addition, a sensitive (95%) and specific (100%) immunoblot serologic test that uses adult worm antigens is available for identification of tapeworm carriers; however, the duration of antibody response is unknown and could therefore reduce the specificity of identifying active infection in field use (*38*). Nonetheless, this test can be performed on a blood specimen obtained from a finger stick and is more sensitive than stool examination for

diagnosis. In addition, stool specimens may be difficult to obtain, multiple specimens are recommended, a skilled microscopist is required, and *Taenia* spp. tapeworms cannot be differentiated from each other on the basis of egg morphologic appearance. Given these factors, the availability of this serologic test substantially improves the ease and potential for follow-up. Adding to the rationale for public health action is the recognition that the economic effect of cysticercosis is considerable. A review of hospital discharge data in Los Angeles County estimated hospitalization costs >$100 million for a 17-year period (*39*).

We believe there is strong justification for routine public health response to a case of cysticercosis. Such a response should include establishing surveillance for the disease and required reporting of cases. When cases are identified, follow-up and testing of household members and other close contacts should be initiated in an attempt to find tapeworm carriers. Such carriers can then be treated and removed as sources of continuing transmission. Investigation of locally acquired cysticercosis cases should be standard public health practice. Although data are limited, tapeworm carriers can also be found among contacts to foreign-born patients (≈5% of the time), and therefore investigation should be considered for all cysticercosis cases; however, imported cases with inactive infection (calcified lesions, indicating probable remote infection) should be a low priority for such follow-up (*16*). Decisions of prioritizing surveillance and control activities must be made on the basis of existing resources and competing needs. Given that a substantial (>20%) proportion of persons with cysticercosis may also be infected with the adult tapeworm, it is also advisable to screen cysticercosis patients if the diagnosing physician has not performed this screening (*26*). Public health authorities should also be aware that a single tapeworm carrier may be a source of infection for multiple cases of cysticercosis; therefore, the possibility of a common exposure among cases should be evaluated. As part of the public health control efforts for cysticercosis, any *Taenia* spp. tapeworm carriers who work as food handlers should be removed from work until successfully treated or confirmation is obtained that the infection is not *T. solium* tapeworm. Screening of domestic workers from areas where the disease is endemic has also been recommended (*3*).

Consideration should be given to making cysticercosis a nationally notifiable disease. Currently, only 2 states, California and Oregon, require reporting of cysticercosis. In collaboration with the Centers for Disease Control and Prevention, the Department of Public Health in Los Angeles County is conducting modified active surveillance that includes education of local providers and routine contact with key health care facilities that treat large numbers of cysticercosis cases. When a case is reported, public health nurses initiate an investigation and follow-up that includes obtaining a finger-stick specimen for serologic testing on all close contacts to identify a possible source of infection. When found, persons harboring a *T. solium* tapeworm are treated to prevent possible ongoing transmission. With heightened awareness, improved surveillance, reporting, and follow-up, cysticercosis transmission in the United States can be prevented and the infection's effects on public health can be reduced.

Dr Sorvillo is professor-in-residence in the Department of Epidemiology at the School of Public Health, University of California, Los Angeles, and professor of health sciences in the College of Health Sciences at Touro University International. His research interests include the epidemiology and control of infectious diseases, particularly parasitic agents.

References

1. Schantz PM, Wilkins PP, Tsang VC. Immigrants, imaging, and immunoblots: the emergence of neurocysticercosis as a significant public health problem. In: Scheld WM, Craig WA, Hughes JM, editors. Emerging infections. Washington: ASM Press; 1998. p. 213–42.
2. Garcia HH, Del Brutto OH; Cysticercosis Working Group in Peru. Neurocysticercosis: updated concepts about an old disease. Lancet Neurol. 2005;4:653–61. DOI: 10.1016/S1474-4422(05)70194-0
3. Phiri IK, Ngowi H, Afonso S, Matenga E, Boa M, Mukaratirwa S, et al. The emergence of *Taenia solium* cysticercosis in eastern and southern Africa as a serious agricultural problem and public health risk. Acta Trop. 2003;87:13–23.
4. Schantz PM, Wikins PP, Tsang VC. *Taenia solium* cysticercosis as an imported disease. In: Garcia HH, Martinez SM, eds. *Taenia solium*, taeniasis/cysticercosis Lima, Peru: Editorial Universo; 1999. p. 263–72.
5. Richards FO, Schantz PM, Ruiz-Tiben E, Sorvillo FJ. Cysticerosis in Los Angeles County. JAMA. 1985;254:3444–8. DOI: 10.1001/jama.254.24.3444
6. Hotez PJ. Neglected infections of poverty in the United States of America. PLoS Negl Trop Dis. 2008;2:e256. DOI: 10.1371/journal.pntd.0000256
7. Wallin MT, Kurtzke JF. Neurocysticerosis in the United States: review of an important emerging infection. Neurology. 2004;63:1559–64.
8. Schantz PM, Moore AC, Munoz JL, Hartman BJ, Schaefer JA, Aron AM, et al. Neurocysticercosis in an Orthodox Jewish community in New York City. N Engl J Med. 1992;327:692–5. DOI: 10.1056/NEJM199209033271004
9. Mody R, Nield LS, Stauffer W, Kamat D. Seizures in a 20-month-old native of Minnesota. Pediatr Emerg Care. 2005;21:860–2. DOI: 10.1097/01.pec.0000190232.20233.45
10. Stamos JK, Rowley AH, Hahn YS, Chadwick EG, Schabtz PM, Wilson M. Neurocysticercosis: report of unusual pediatric cases. Pediatrics. 1996;98:974–7.
11. Townes JM, Hoffman CJ, Kohn MA. Neurocysticerccosis in Oregon, 1995–2000. Emerg Infect Dis. 2004;10:508–10.
12. Rosenfeld EA, Byrd SE, Shulman ST. Neurocysticercosis among children in Chicago. Clin Infect Dis. 1996;23:262–8.
13. Shandera WX, White AC, Chen JC, Diaz P, Armstrong R. Neurocysticercosis in Houston, Texas: a report of 112 cases. Medicine (Baltimore). 1994;73:37–52.
14. Centers for Disease Control and Prevention. Locally acquired neurocysticercosis—North Carolina, Massachusetts, and South Carolina, 1989–1991. MMWR Morb Mortal Wkly Rep. 1992;41:1–4.

15. Kruskal BA, Moths L, Teele DW. Neurocysticercosis in a child with no history of travel outside the continental United States. Clin Infect Dis. 1993;16:290–2.
16. Sorvillo FJ, Waterman SH, Richards FO, Schantz PM. Cysticercosis surveillance: locally acquired and travel-related infections and detection of intestinal tapeworm carriers in Los Angeles County. Am J Trop Med Hyg. 1992;47:365–71.
17. Grossman EA. Cysticercosis acquired in the United States, without compatible travel history. Neurology. 1986;36:305.
18. Mitchell WG, Crawford TO. Intraparenchymal cerebral cysticercosis in children: diagnosis and treatment. Pediatrics. 1988;82:76–82.
19. Earnest MP, Reller LB, Filllely CM, Grek AJ. Neurocysticercosis in the United States: 35 cases and a review. Rev Infect Dis. 1987;9(5):961–79.
20. McCormick GF. Cysticercosis—review of 230 patients. Bull Clin Neurosci. 1985;50:76–101.
21. Sorvillo FJ, Brecht C, Chambers P, Waterman SH. Cysticercosis transmission in a non-endemic area. Journal of Border Health. 1988;4:2–5.
22. Tasker WG, Plotkin SA. Cerebral cysticercosis. Pediatrics. 1979;63:761–3.
23. Messner KH, Kammerer WS. Intraocular cysticercosis. Arch Ophthalmol. 1979;97:1103–5.
24. Campana M, Swartzwelder C, Comer O. Human cysticercosis in the United States. J Parasitol. 1954;40(Oct Suppl):46.
25. Dent JH. Cysticercosis cerebri–cestode infestation of human brain; report of a case occurring in Louisiana. J Am Med Assoc. 1957;164:401–5.
26. Dixon HB, Lipscomb FM. Cysticercosis: an analysis and follow-up of 450 cases. Spec Rep Ser Med Res Counc (G B). 1961;299:1–58.
27. Lescano AG, Garcia HH, Gilman RH, Gavida CM, Tsang VC, Rodriguez S, et al. *Taenia solium* cysticercosis hotspots surrounding tapeworm carriers: clustering on seroprevalence but not on seizures. PLoS Negl Trop Dis. 2009;3:e371. DOI: 10.1371/journal.pntd.0000371
28. Sorvillo FJ, DeGiorgio C, Waterman SH. Deaths from cysticercosis in the United States. Emerg Infect Dis. 2007;13:230–5. DOI: 10.3201/eid1302.060527
29. Department of Homeland Security. Washington: estimates of the legal permanent resident population in 2008; updated 2009 October [cited 2009 Dec 2]. http://www.dhs.gov/files/statistics/immigration.shtm
30. Department of Homeland Security. Washington: estimates of the unauthorized immigrant population residing in the United States: January 2007 [cited 2010 Oct 26]. http://www.dhs.gov/xlibrary/assets/statistics/publications/ois_ill_pe_2007.pdf
31. DeGiorgio C, Pietsch-Escueta S, Tsang V, Corral-Leyva G, Ng L, Medina MT, et al. Sero-prevalence of *Taenia solium* cysticercosis and *Taenia solium* taeniasis in California, USA. Acta Neurol Scand. 2005;111:84–8. DOI: 10.1111/j.1600-0404.2005.00373.x
32. Ciesielski SD, Seed JR, Oriz JC, Metts J. Intestinal parasites among North Carolina migrant farmworkers. Am J Public Health. 1992;82:1258–62. DOI: 10.2105/AJPH.82.9.1258
33. Cardenas VM, Mena KD, Otiz M, Karri S, Variyam E, Behravesh CB, et al. Hyperendemic *H. pylori* and tapeworm infections in a U.S.–Mexico border population. Public Health Rep. 2010;125:441–7.
34. Gemmell MA. Taeniidae: modification to the lifespan of the egg and the regulation of tapeworm populations. Exp Parasitol. 1977;41:314–28. DOI: 10.1016/0014-4894(77)90104-7
35. Wheeler C, Vogt TM, Armstrong GL, Vaughan G, Weltman A, Nainan OV, et al. An outbreak of hepatitis A associated with green onions. N Engl J Med. 2005;353:890–7. DOI: 10.1056/NEJMoa050855
36. Herwaldt BL, Ackers ML; The Cyclospora Working Group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. N Engl J Med. 1997;336:1548–56. DOI: 10.1056/NEJM199705293362202
37. Rivero LB, Navarro A. Prevalence of *Taenia solium* eggs in vegetables for human consumption in Cd. Victoria, Tamaulipas. Border Epidemiological Bulletin. 1986;14:1–4.
38. Wilkins PP, Allan JC, Verastegui M, Acosta M, Eason AG, Garcia HH, et al. Development of a serologic assay to detect *Taenia solium* taeniasis. Am J Trop Med Hyg. 1999;60:199–204.
39. Croker C, Reporter R, Mascola L. Use of statewide hospital discharge data to evaluate the economic burden of neurocysticerocis in Los Angeles County (1991–2008). Am J Trop Med Hyg. 2010;83:106–10. DOI: 10.4269/ajtmh.2010.09-0494

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Foodborne Illness Acquired in the United States—Major Pathogens

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Estimates of foodborne illness can be used to direct food safety policy and interventions. We used data from active and passive surveillance and other sources to estimate that each year 31 major pathogens acquired in the United States caused 9.4 million episodes of foodborne illness (90% credible interval [CrI] 6.6–12.7 million), 55,961 hospitalizations (90% CrI 39,534–75,741), and 1,351 deaths (90% CrI 712–2,268). Most (58%) illnesses were caused by norovirus, followed by nontyphoidal *Salmonella* spp. (11%), *Clostridium perfringens* (10%), and *Campylobacter* spp. (9%). Leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *Toxoplasma gondii* (8%). Leading causes of death were nontyphoidal *Salmonella* spp. (28%), *T. gondii* (24%), *Listeria monocytogenes* (19%), and norovirus (11%). These estimates cannot be compared with prior (1999) estimates to assess trends because different methods were used. Additional data and more refined methods can improve future estimates.

Estimates of the overall number of episodes of foodborne illness are helpful for allocating resources and prioritizing interventions. However, arriving at these estimates is challenging because food may become contaminated by many agents (e.g., a variety of bacteria, viruses, parasites, and chemicals), transmission can occur by nonfood mechanisms (e.g., contact with animals or consumption of contaminated water), the proportion of disease transmitted by food differs by pathogen and by host factors (e.g. age and immunity), and only a small proportion of illnesses are confirmed by laboratory testing and reported to public health agencies.

Laboratory-based surveillance provides crucial information for assessing foodborne disease trends. However, because only a small proportion of illnesses are diagnosed and reported, periodic assessments of total episodes of illness are also needed. (Hereafter, episodes of illness are referred to as illnesses.) Several countries have conducted prospective population-based or cross-sectional studies to supplement surveillance and estimate the overall number of foodborne illnesses (*1*). In 2007, the World Health Organization launched an initiative to estimate the global burden of foodborne diseases (*2*).

In 1999, the Centers for Disease Control and Prevention provided comprehensive estimates of foodborne illnesses, hospitalizations, and deaths in the United States caused by known and unknown agents (*3*). This effort identified many data gaps and methodologic limitations. Since then, new data and methods have become available. This article is 1 of 2 reporting new estimates of foodborne diseases acquired in the United States (hereafter referred to as domestically acquired). This article provides estimates of major known pathogens; the other provides estimates for agents of acute gastroenteritis not specified in this article (*4*).

Methods

Adequate data for preparing national estimates were available for 31 pathogens. We estimated the number of foodborne illnesses, hospitalizations, and deaths caused by these 31 domestically acquired pathogens by using data shown in the online Appendix Table (www.cdc.gov/EID/content/17/1/7-appT.htm) and online Technical Appendix 1 (www.cdc.gov/EID/content/17/1/7-Techapp1.pdf).

Data were mostly from 2000–2008, and all estimates were based on the US population in 2006 (299 million persons). Estimates were derived from statistical models with many inputs, each with some measure of uncertainty (*5*). To reflect this uncertainty, we used probability distributions to describe a range of plausible values for all model

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inputs. We expressed model outputs as probability distributions summarized by a mean point estimate with 90% credible intervals (CrIs). We used 2 types of modeling approaches for different types of data: 1) models that began with counts of laboratory-confirmed illnesses and were adjusted for undercounts (because of underreporting and underdiagnosis) and thus scaled up to the estimated number of illnesses and 2) models that began with a US population and used incidence data to scale down to the estimated number of illnesses (Table 1). The modeling approaches used and parameters of these probability distributions are detailed in online Technical Appendixes 2 and 3 (www.cdc.gov/EID/content/17/1/7-Techapp2.pdf and www.cdc.gov/EID/content/17/1/7-Techapp3.pdf, respectively); the proportions cited are modal values.

Illnesses

Laboratory-based surveillance data were available for 25 pathogens (online Appendix Table). The following events must occur for an illness to be ascertained and included in laboratory-based surveillance: the ill person must seek medical care, a specimen must be submitted for laboratory testing, the laboratory must test for and identify the causative agent, and the illness must be reported to public health authorities. If a break occurs in any of the first 3 steps of this surveillance chain, the causative agent will not be laboratory confirmed (underdiagnosis). Furthermore, although all laboratory-confirmed illnesses are reported by active surveillance, some will not be reported by passive surveillance (underreporting). Therefore, to estimate the number of illnesses caused by pathogens under public health surveillance, we determined the number of laboratory-confirmed illnesses and adjusted for underdiagnosis and, if necessary, for underreporting by using a series of component multipliers.

Laboratory-confirmed illnesses for these 25 pathogens were reported through 5 surveillance programs: the Foodborne Diseases Active Surveillance Network (FoodNet) for *Campylobacter* spp., *Cryptosporidium* spp., *Cyclospora cayetanensis*, Shiga toxin–producing *Escherichia coli* (STEC) O157, STEC non-O157, *Listeria monocytogenes*, nontyphoidal *Salmonella* spp., *Salmonella enterica* serotype Typhi, *Shigella* spp., and *Yersinia enterocolitica*; the National Notifiable Diseases Surveillance System (NNDSS) for *Brucella* spp., *Clostridium botulinum*, *Trichinella* spp., hepatitis A virus, and *Giardia intestinalis*; the Cholera and Other *Vibrio* Illness Surveillance (COVIS) system for toxigenic *Vibrio cholerae*, *V. vulnificus*, *V. parahemolyticus*, and other *Vibrio* spp.; the National Tuberculosis Surveillance System (NTSS) for *Mycobacterium bovis*; and the Foodborne Disease Outbreak Surveillance System (FDOSS) for *Bacillus cereus, Clostridium perfringens,* enterotoxigenic *E. coli* (ETEC), *Staphylococcus aureus*, and *Streptococcus* spp. group A (online Appendix Table; online Technical Appendix 1). When data were available from >1 surveillance system, we used active surveillance data from FoodNet, except for *Vibrio* spp., for which we used COVIS because of geographic clustering of *Vibrio* spp. infections outside FoodNet sites. We used data on outbreak-associated illnesses from FDOSS only for pathogens for which no data were available from other systems.

Because FoodNet conducts surveillance at 10 sites (*6*), we estimated the number of laboratory-confirmed illnesses in the United States by applying incidence from FoodNet to the estimated US population for 2006 (*7*). We constructed a probability distribution based on extrapolation of rates by year (2005–2008) in each FoodNet site (online Technical Appendix 3). We used data from 2005–2008 because the FoodNet surveillance area was constant during that period and because FoodNet began collecting information on foreign travel in 2004. We used data from 2000–2007 for NNDSS, COVIS, and FDOSS and annual counts of reported illnesses for our probability distributions. Some evidence of trend was found for illness caused by hepatitis A virus, *S. aureus,* and *Vibrio* spp.; therefore, recent years were weighted more heavily (online Technical Appendixes

Table 1. Modeling approaches used to estimate the total number of illnesses for different types of data, United States*

Pathogens for which laboratory-confirmed illnesses were scaled up			Pathogens for which US population was scaled down
Active surveillance data	Passive surveillance data	Outbreak surveillance data	
Campylobacter spp.	*Brucella* spp.	*Bacillus cereus*	Astrovirus
Cryptosporidium spp.	*Clostridium botulinum*	*Clostridium perfringens*	Norovirus
Cyclospora cayetanensis	*Giardia intestinalis*	ETEC†	Rotavirus
STEC O157	Hepatitis A virus	*Staphylococcus aureus*	Sapovirus
STEC non-O157	*Mycobacterium bovis*	*Streptococcus* spp. group A	*Toxoplasma gondii*
Listeria monocytogenes	*Trichinella* spp.		
Salmonella spp., nontyphoidal‡	*Vibrio cholera*, toxigenic		
S. enterica serotype Typhi	*Vibrio parahaemolyticus*		
Shigella spp.	*Vibrio vulnificus*		
Yersinia enterocolitica	*Vibrio* spp., other		

*ETEC, enterotoxigenic *Escherichi coli*; STEC, Shiga toxin–producing *E. coli.*
†Numbers of *E. coli* other than STEC or ETEC assumed to be same as for ETEC.
‡Includes all serotypes other than Typhi.

2, 3). NTSS was used to determine the number of reported illnesses caused by *M. bovis* during 2004–2007.

We assumed that all laboratory-confirmed illnesses were reported to FoodNet active surveillance in the relevant catchment areas. Because COVIS and NNDSS conduct passive surveillance, we applied an underreporting multiplier (1.1 for bacteria and 1.3 for parasites) derived by comparing incidence of all nationally notifiable illnesses ascertained through FoodNet with that reported to NNDSS (online Technical Appendix 4, www.cdc.gov/EID/content/17/1/7-Techapp4.pdf). For the 5 bacteria for which only outbreak data were available, we estimated the number of laboratory-confirmed illnesses by creating an underreporting multiplier as follows. We determined the proportion of illnesses ascertained through FoodNet that were caused by *Campylobacter* spp., *Cryptosporidium* spp., *C. cayatanensis*, *L. monocytogenes*, *Salmonella* spp., *Shigella* spp., STEC, *Vibrio* spp., and *Y. enterocolitica* that were also reported to FDOSS as outbreak associated and applied the inverse of this proportion, 25.5, to those pathogens (online Technical Appendix 4). We assumed that all illnesses caused by *M. bovis* were reported to NTSS.

To adjust for underdiagnosis resulting from variations in medical care seeking, specimen submission, laboratory testing, and test sensitivity, we created pathogen-specific multipliers. To adjust for medical care seeking and specimen submission, we pooled data from FoodNet Population Surveys in 2000–2001, 2002–2003 (*8*), and 2006–2007 (Centers for Disease Control and Prevention, unpub. data) from which we estimated the proportion of persons who in the past month reported an acute diarrheal illness (≥3 loose stools in 24 hours lasting >1 day or resulting in restricted daily activities) and sought medical care and submitted a stool sample for that illness. Because persons with more severe illness are more likely to seek care (*9*), we estimated pathogen-specific proportions of persons with laboratory-confirmed infections who had severe illness (e.g., bloody diarrhea) and used medical care seeking and stool sample submission rates for bloody (35% and 36%, respectively) and nonbloody (18% and 19%, respectively) diarrhea as surrogates for severe and mild cases of most illnesses (online Technical Appendix 3). However, for infections with *L. monocytogenes*, *M. bovis*, and *V. vulnificus* and severe infections with hepatitis A virus, we assumed high rates of medical care seeking (i.e., we assumed that 100% of persons with *M. bovis* infection and 90% with *L. monocytogenes*, *V. vulnificus*, or severe hepatitis A virus infections sought care) and specimen submission (100% for hepatitis A virus and *M. bovis*, 80% for others). We accounted for percentage of laboratories that routinely tested for specific pathogens (25%–100%) and test sensitivity (28%–100%) by using data from FoodNet (*10*,*11*) and other surveys of clinical diagnostic laboratory practices (online Technical Appendix 3). For the 5 pathogens for which data were from outbreaks only, we used the nontyphoidal *Salmonella* spp. underdiagnosis multiplier.

Alternative approaches were used for infections not routinely reported by any surveillance system (i.e., diarrheagenic *E. coli* other than STEC and ETEC, *T. gondii*, astrovirus, rotavirus, sapovirus, and norovirus) (online Technical Appendixes 1–3). We assumed diarrheagenic *E. coli* other than STEC and ETEC to be as common as ETEC. Illnesses caused by *T. gondii* were estimated by using nationally representative serologic data from the 1999–2004 National Health and Nutrition Examination Survey (*12*) and an estimate that clinical illness develops in 15% of persons who seroconvert (*13*). We assumed that 75% of children experience an episode of clinical rotavirus illness by 5 years of age, consistent with findings from other studies (*14*), and used this estimate for astrovirus and sapovirus. We estimated norovirus illnesses by applying mean proportion of all acute gastroenteritis caused by norovirus (11%) according to studies in other industrialized countries (*15*–*18*) to estimates of acute gastroenteritis from FoodNet Population Surveys (online Appendix Table; online Technical Appendixes 1–3) (*4*).

Hospitalizations and Deaths

For most pathogens, numbers of hospitalizations and deaths were estimated by determining (from surveillance data) the proportion of persons who were hospitalized and the proportion who died and applying these proportions to the estimated number of laboratory-confirmed illnesses (online Appendix Table; online Technical Appendixes 1, 3). Rates of hospitalization and death caused by *G. intestinalis* and *T. gondii* were based on the 2000–2006 Nationwide Inpatient Sample. Because some persons with illnesses that were not laboratory confirmed would also have been hospitalized and died, we doubled the number of hospitalizations and deaths to adjust for underdiagnosis, similar to the method used by Mead et al. (*3*) but applied an uncertainty distribution (online Technical Appendix 3). For diarrheagenic *E. coli* other than STEC and ETEC, total numbers of hospitalizations and deaths were assumed to be the same as those for ETEC. For rotavirus, we used previous estimates (*14*). For astrovirus and sapovirus, we assumed that the number was 25% that of rotavirus (*19*,*20*). Numbers of norovirus hospitalizations and deaths were determined by multiplying the estimated number of hospitalizations and deaths caused by acute gastroenteritis, estimated by using national data on outpatient visits resulting in hospitalization, hospital discharge surveys, and death certificates (online Appendix Table; online Technical Appendixes 1–3)

(*4*), by the same norovirus proportion (11%) used to estimate illnesses (*15*–*18*).

Domestically Acquired Foodborne Illnesses

Data from published studies and surveillance were used to determine, for each pathogen, the proportion of illnesses acquired while the person had been traveling outside the United States (online Technical Appendixes 1, 3). The remaining proportion was considered domestically acquired. We based our estimates of the proportion of domestically acquired foodborne illnesses caused by each pathogen on data from surveillance, risk factor studies, and a literature review (online Technical Appendixes 1, 3).

Uncertainty Analysis

We used empirical data, when available, to define entire distributions or parameters of distributions (online Technical Appendix 3). When data were sparse, we made reasoned judgments based on context, plausibility, and previously published estimates. The parametric distribution used for almost all multipliers was a 4-parameter beta (modified PERT) distribution (*21*). The first 3 parameters are low, modal, and high. The fourth parameter is related to the variability of the distribution. We typically fixed this last parameter at 4, which yields the simple PERT distribution (*21*). However, when describing the outbreak reporting multiplier, we used a value of 20 (online Technical Appendix 4).

Uncertainty in the estimates is the cumulative effect of uncertainty of each of the model inputs. We iteratively generated sets of independent pathogen-specific adjustment factors and used these multipliers to estimate illnesses, hospitalizations, and deaths (Figure; online Technical Appendix 2). On the basis of 100,000 iterations, we obtained empirical distributions of counts corresponding to Bayesian posterior distributions and used these posterior distributions to generate a point estimate (posterior mean) and upper and lower 5% limits for 90% CrIs. Because incidence of illnesses differed by location and over time, we included these variations in the models, which led to wider CrIs than if we had assumed that inputs represented independent random samples of a fixed US population. We used SAS version 9.2 (SAS Institute, Cary, NC, USA) for these analyses.

Results

Foodborne Illnesses

We estimate that each year in the United States, 31 pathogens caused 37.2 million (90% CrI 28.4–47.6 million) illnesses, of which 36.4 million (90% CrI 27.7–46.7 million) were domestically acquired; of these, 9.4 million (90% CrI 6.6–12.7 million) were foodborne (Table 2; expanded version available online, www.cdc.gov/EID/content/17/1/7-T2.htm). We estimate that 5.5 million (59%) foodborne illnesses were caused by viruses, 3.6 million (39%) by bacteria, and 0.2 million (2%) by parasites. The pathogens that caused the most illnesses were norovirus (5.5 million, 58%), nontyphoidal *Salmonella* spp. (1.0 million, 11%), *C. perfringens* (1.0 million, 10%), and *Campylobacter* spp. (0.8 million, 9%).

Hospitalizations

We estimate that these 31 pathogens caused 228,744 (90% CrI 188,326–275,601) hospitalizations annually, of which 55,961 (90% CrI 39,534–75,741) were caused by contaminated food eaten in the United States (Table 3; expanded version available online, www.cdc.gov/EID/content/17/1/7-T3.htm). Of these, 64% were caused by bacteria, 27% by viruses, and 9% by parasites. The leading causes of hospitalization were nontyphoidal *Salmonella* spp. (35%), norovirus (26%), *Campylobacter* spp. (15%), and *T. gondii* (8%).

Deaths

We estimate that these 31 pathogens caused 2,612 deaths (90% CrI 1,723–3,819), of which 1,351 (90% CrI

$$Count \times Year \times Sub \times Ob \times \left\{ \begin{array}{c} CS(Severe) \times SS(Severe) \times PS \\ + \\ CS(Mild) \times SS(Mild) \times (1 - PS) \end{array} \right\} \times LT \times LS \times \left[\begin{array}{c} 1 \\ \text{or} \\ H \\ \text{or} \\ D \end{array} \right] \times [1 \text{ or } F] \Rightarrow$$

Illnesses	Illnesses, foodborne
Hospitalizations	Hospitalizations, foodborne
Deaths	Deaths, foodborne

Figure. Example schematic diagram of the estimation and uncertainty model used to estimate episodes of illness, hospitalizations, and deaths in the United States. *Count*, data (empirical distribution); *Year,* factor to standardize non-2006 counts to 2006 (constant); *Sub*, expansive factor to scale area surveillance to the entire US population (constant); *Ob,* expansive factor to scale outbreak counts up to outbreak plus sporadic counts (beta distribution); *CS*, expansive factor to scale care seekers to all ill, with severe and mild illness versions (PERT distribution); *SS,* expansive factor to scale submitted samples to all visits, with severe and mild illness versions (PERT distribution); *PS*, estimated proportion of illnesses that are severe (PERT distribution); *LT,* expansive factor to scale tests performed up to samples submitted (PERT distribution); *LS,* expansive factor to scale positive test results up to true positive specimens (PERT distribution); *H,* contractive factor to scale illnesses down to hospitalized illnesses (PERT distribution); *D*, contractive factor to scale illnesses down to deaths (PERT distribution); *F*, contractive factor to scale illnesses down to foodborne illnesses (PERT distribution).

712–2,268) were caused by contaminated food eaten in the United States (Table 3). Of these, 64% were caused by bacteria, 25% by parasites, and 12% by viruses. The leading causes of death were nontyphoidal *Salmonella* spp. (28%), *T. gondii* (24%), *L. monocytogenes* (19%), and norovirus (11%).

Table 2. Estimated annual number of episodes of domestically acquired foodborne illnesses caused by 31 pathogens, United States*

Pathogen	Laboratory confirmed	Multipliers: Under-reporting	Multipliers: Under-diagnosis	Travel related, %	Foodborne, %†	Domestically acquired foodborne, mean (90% credible interval)
Bacteria						
Bacillus cereus, foodborne	85‡	25.5	29.3	<1	100	63,400 (15,719–147,354)
Brucella spp.	120§	1.1	15.2	16	50	839 (533–1,262)
Campylobacter spp.	43,696¶	1.0	30.3	20	80	845,024 (337,031–1,611,083)
Clostridium botulinum, foodborne	25§	1.1	2.0	<1	100	55 (34–91)
Clostridium perfringens, foodborne	1,295‡	25.5	29.3	<1	100	965,958 (192,316–2,483,309)
STEC O157	3,704¶	1.0	26.1	4	68	63,153 (17,587–149,631)
STEC non-O157	1,579¶	1.0	106.8	18	82	112,752 (11,467–287,321)
ETEC, foodborne	53‡	25.5	29.3	55	100	17,894 (24–46,212)
Diarrheagenic *E. coli* other than STEC and ETEC	53	25.5	29.3	<1	30	11,982 (16–30,913)
Listeria monocytogenes	808¶	1.0	2.1	3	99	1,591 (557–3,161)
Mycobacterium bovis	195¶	1.0	1.1	70	95	60 (46–74)
Salmonella spp., nontyphoidal	41,930¶	1.0	29.3	11	94	1,027,561 (644,786–1,679,667)
S. enterica serotype Typhi	433¶	1.0	13.3	67	96	1,821 (87–5,522)
Shigella spp.	14,864¶	1.0	33.3	15	31	131,254 (24,511–374,789)
Staphylococcus aureus, foodborne	323‡	25.5	29.3	<1	100	241,148 (72,341–529,417)
Streptococcus spp. group A, foodborne	15‡	25.5	29.3	<1	100	11,217 (15–77,875)
Vibrio cholerae, toxigenic	8§	1.1	33.1	70	100	84 (19–213)
V. vulnificus	111§	1.1	1.7	2	47	96 (60–139)
V. parahaemolyticus	287§	1.1	142.4	10	86	34,664 (18,260–58,027)
Vibrio spp., other	220§	1.1	142.7	11	57	17,564 (10,848–26,475)
Yersinia enterocolitica	950¶	1.0	122.8	7	90	97,656 (30,388–172,734)
Subtotal						3,645,773 (2,321,468–5,581,290)
Parasites						
Cryptosporidium spp.	7,594¶	1.0	98.6	9	8	57,616 (12,060–166,771)
Cyclospora cayetanensis	239¶	1.0	83.1	42	99	11,407 (137–37,673)
Giardia intestinalis	20,305§	1.3	46.3	8	7	76,840 (51,148–109,739)
Toxoplasma gondii		1.0	0.0	<1	50	86,686 (64,861–111,912)
Trichinella spp.	13§	1.3	9.8	4	100	156 (42–341)
Subtotal						232,705 (161,923–369,893)
Viruses						
Astrovirus	NA	NA	NA	0	<1	15,433 (5,569–26,643)
Hepatitis A virus	3,576§	1.1	9.1	41	7	1,566 (702–3,024)
Norovirus	NA	NA	NA	<1	26	5,461,731 (3,227,078–8,309,480)
Rotavirus	NA	NA	NA	0	<1	15,433 (5,569–26,643)
Sapovirus	NA	NA	NA	0	<1	15,433 (5,569–26,643)
Subtotal						5,509,597 (3,273,623–8,355,568)
Total						9,388,075 (6,641,440–12,745,709)

*All estimates based on US population in 2006. Modal or mean value shown unless otherwise stated; see online Technical Appendix 3 (www.cdc.gov/EID/content/17/1/7-Techapp3.pdf) for the parameters of these distributions. STEC, Shiga toxin–producing *Escherichia coli*; ETEC, enterotoxigenic *E. coli*; NA, not applicable. An expanded version of this table is available online (www.cdc.gov/EID/content/17/1/7-T2.htm).
†Percentage foodborne among domestically acquired illnesses.
‡Passive surveillance data on outbreak-associated illnesses from the Foodborne Disease Outbreak Surveillance System. Estimates based on the number of foodborne illnesses ascertained in surveillance and therefore assumed to reflect only foodborne transmission.
§Passive surveillance data from Cholera and Other *Vibrio* Illness Surveillance or the National Notifiable Disease Surveillance System.
¶Active surveillance data from Foodborne Diseases Active Surveillance Network, adjusted for geographic coverage; data from the National Tuberculosis Surveillance System for *M. bovis*.

Discussion

We estimate that foods consumed in the United States that were contaminated with 31 known agents of foodborne disease caused 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths each year. Norovirus caused the most illnesses; nontyphoidal *Salmonella* spp., norovirus, *Campylobacter* spp., and *T. gondii* caused the most hospitalizations; and nontyphoidal *Salmonella* spp., *T. gondii*, *L. monocytogenes*, and norovirus caused the most deaths. Scarce data precluded estimates for other known infectious and noninfectious agents, such as chemicals. Foodborne diseases are also caused by agents not yet recognized as being transmitted in food and by unknown agents (*22*). The numbers of illnesses caused by these unspecified agents are estimated elsewhere (*4*).

Studies estimating the overall number of foodborne illnesses have been conducted in England and Wales and in Australia (*23*,*24*). Similar to our findings, in Australia norovirus was the leading cause of foodborne illness, accounting for 30% of illnesses caused by known pathogens.

Table 3. Estimated annual number of domestically acquired foodborne hospitalizations and deaths caused by 31 pathogens, United States*

Pathogen	Hospitalization rate, %†	Hospitalizations, mean (90% credible interval)	Death rate, %†	Deaths, mean (90% credible interval)
Bacteria				
Bacillus cereus, foodborne‡	0.4	20 (0–85)	0	0
Brucella spp.	55.0	55 (33–84)	0.9	1 (0–2)
Campylobacter spp.	17.1	8,463 (4,300–15,227)	0.1	76 (0–332)
Clostridium botulinum, foodborne‡	82.6	42 (19–77)	17.3	9 (0–51)
Clostridium perfringens, foodborne‡	0.6	438 (44–2,008)	<0.1	26 (0–163)
STEC O157	46.2	2,138 (549–4,614)	0.5	20 (0–113)
STEC non-O157	12.8	271 (0–971)	0.3	0 (0–0)§
ETEC, foodborne	0.8	12 (0–53)	0	0
Diarrheagenic *E. coli* other than STEC and ETEC	0.8	8 (0–36)	0	0
Listeria monocytogenes	94.0	1,455 (521–3,018)	15.9	255 (0–733)
Mycobacterium bovis	55.0	31 (21–42)	4.7	3 (2–3)
Salmonella spp., nontyphoidal	27.2	19,336 (8,545–37,490)	0.5	378 (0–1,011)
S. enterica serotype Typhi	75.7	197 (0–583)	0	0
Shigella spp.	20.2	1,456 (287–3,695)	0.1	10 (0–67)
Staphylococcus aureus, foodborne‡	6.4	1,064 (173–2,997)	<0.1	6 (0–48)
Streptococcus spp. group A, foodborne‡	0.2	1 (0–6)	0	0
Vibrio cholerae, toxigenic	43.1	2 (0–5)	0	0
V. vulnificus	91.3	93 (53–145)	34.8	36 (19–57)
V. parahaemolyticus	22.5	100 (50–169)	0.9	4 (0–17)
Vibrio spp., other	37.1	83 (51–124)	3.7	8 (3–19)
Yersinia enterocolitica	34.4	533 (0–1,173)	2.0	29 (0–173)
Subtotal		35,796 (21,519–53,414)		861 (260–1,761)
Parasites				
Cryptosporidium spp.	25.0	210 (58–518)	0.3	4 (0–19)
Cyclospora cayetanensis	6.5	11 (0–109)	0.0	0
Giardia intestinalis	8.8	225 (141–325)	0.1	2 (1–3)
Toxoplasma gondii	2.6	4,428 (2,634–6,674)	0.2	327 (200–482)
Trichinella spp.	24.3	6 (0–17)	0.2	0 (0–0)
Subtotal		4,881 (3,060–7,146)		333 (205–488)
Viruses				
Astrovirus	0.4	87 (32–147)	<0.1	0
Hepatitis A virus	31.5	99 (42–193)	2.4	7 (3–15)
Norovirus	0.03	14,663 (8,097–23,323)	<0.1	149 (84–237)
Rotavirus	1.7	348 (128–586)	<0.1	0
Sapovirus	0.4	87 (32–147)	<0.1	0
Subtotal		15,284 (8,719–23,962)		157 (91–245)
Total		55,961 (39,534–75,741)		1,351 (712–2,268)

*All estimates were based on US population in 2006. STEC, Shiga toxin–producing *Escherichia coli*; ETEC, enterotoxigenic *E. coli*. An expanded version of this table is available online (www.cdc.gov/EID/content/17/1/7-T3.htm).
†For laboratory-confirmed illnesses. Unadjusted hospitalization and death rates are presented here. These rates were doubled to adjust for underdiagnosis before being applied to the number of laboratory-confirmed cases to estimate the total number of hospitalizations and deaths. The hospitalization and death rates for astrovirus, norovirus, rotavirus, and sapovirus presented here are the percentage of total estimated illness and were not subject to further adjustment.
‡Estimates based on the number of foodborne illnesses ascertained in surveillance, therefore assumed to reflect only foodborne transmission.
§We report median values instead of means for the distributions of deaths caused by STEC non-O157 because of extremely skewed data.

In England and Wales, norovirus accounted for only 8% of known foodborne illnesses; however, stool sample reexamination using molecular techniques documented higher rates (*18*). Nontyphoidal *Salmonella* spp. and *Campylobacter* spp. were leading causes of foodborne illnesses in all 3 countries (England and Wales, Australia, and the United States), although nontyphoidal *Salmonella* spp. accounted for a greater proportion of illness in the United States. Recent serologic data from Europe suggest that *Salmonella* spp. infections are more common than estimated by our methods; however, many infections may be asymptomatic (*25*). Our estimates did not capture mild illnesses associated with some pathogens. For example, mild cases of botulism are often recognized as part of outbreaks, but affected persons seldom seek medical care and are not captured by surveillance except during outbreaks (*26*,*27*). Likewise, *L. monocytogenes* is rarely diagnosed as the cause of gastroenteritis and fever, partly because this organism is not detected by routine stool culture (*28*). Early spontaneous abortion or miscarriage associated with listeriosis may also be underdiagnosed.

Accurately estimating hospitalizations and deaths caused by foodborne pathogens is particularly challenging. National data on outpatient visits resulting in hospitalization, hospital discharges, and death certificates probably substantially underestimate pathogen-specific cases because for pathogen-specific diagnoses to be recorded, health care providers must order the appropriate diagnostic tests and coding must be accurate. Particularly in vulnerable populations, dehydration or electrolyte imbalance from a gastrointestinal illness may exacerbate a chronic illness, resulting in hospitalization or death well after resolution of the gastrointestinal illness; thus, the gastrointestinal illness may not be coded as a contributing factor. Moreover, if a pathogen is not detected, infections may be coded as noninfectious illnesses (*29*). For norovirus, we estimated the number of hospitalizations and deaths by applying the estimated proportion of acute gastroenteritis illnesses caused by norovirus to overall estimates of hospitalizations and deaths from acute gastroenteritis; this choice is supported by studies of hospitalizations for norovirus (*30*,*31*). For most other pathogens, we used data from surveillance to estimate pathogen-specific hospitalizations and deaths and doubled the numbers to adjust for underdiagnosis. More precise information about the degree of undercounting of hospitalizations and deaths for each pathogen would improve these estimates.

Our methods and data differed from those used for the 1999 estimates (*3*). Our estimate of medical care seeking among persons with a diarrheal illness, derived from the 3 most recent FoodNet Population Surveys conducted during 2000–2007, was higher than that estimated from the 1996–1997 FoodNet Population Survey used for the 1999 estimates (35% and 18% among persons reporting bloody and nonbloody diarrhea, respectively, compared with 15% and 12% in the earlier [1999] study) (*8*). These data resulted in lower underdiagnosis multipliers, which contributed to lower estimates of number of illnesses. The biggest change from the earlier estimate was the estimated number of norovirus illnesses, which decreased for 2 reasons. First, the number of acute gastrointestinal illnesses estimated from the FoodNet Population Survey and used in the current study was lower than the estimated number of acute gastrointestinal illnesses used in the 1999 assessment. The earlier study used data from 1996–1997; the sample size was one fifth as large as ours and incorporated data from US studies conducted before 1980 (*32*,*33*). Both estimates excluded persons reporting concurrent cough or sore throat, but the proportion of persons reporting these signs and symptoms was higher in the FoodNet Population Surveys we used than that in the older US studies (38% vs. 25%), contributing to a lower estimated prevalence of acute gastroenteritis (0.60 vs. 0.79 episodes/person/year) (*4*,*32*,*33*). Additionally, the current study excluded persons with vomiting who were ill for <1 day or whose illness did not result in restricted daily activities, whereas the earlier study included all vomiting episodes. These factors contributed to the new estimate of acute gastroenteritis being 24% lower than the earlier estimate, more likely the result of increased accuracy than a true decrease in illnesses (*4*). Second, the lower current estimate for norovirus illnesses resulted from a lower proportion of norovirus estimated to be foodborne (decreased from 40% to 26%); this lower proportion is similar to that estimated in recent studies from other countries (*23*,*24*). Because of these reasons and use of other data sources and methods, our estimate cannot be compared with the 1999 estimate for the purpose of assessing trends. FoodNet provides the best data on trends over time (*34*).

Data used in the current study came from a variety of sources and were of variable quality and representativeness. FoodNet sites, from which we used data for 10 pathogens, are not completely representative of the US population, but 1 study indicated that demographic data from FoodNet and from the 2005 US census did not differ much (*6*). For 5 pathogens, only data on foodborne outbreak–related cases were available. No routine surveillance data were available for most viruses, forcing us to use a different modeling approach for viruses than for most other pathogens. Given the large number of norovirus illnesses in these estimates, the paucity of supporting data is a major limitation. Moreover, combining different methods is not optimal because methods themselves may affect the estimates. We chose our modeling approach and used the PERT distribution for many inputs because data were sometimes limited and subjective decisions were required. Other investigators could

have chosen other distributions, for good reasons, and arrived at different estimates.

Our assumptions about the proportion of illnesses transmitted by food profoundly affect our estimates, but data on which to base these estimates were often lacking. We used data from surveillance, risk factor studies, and the current literature to estimate the proportion of pathogen-specific illnesses caused by consumption of contaminated food (*35*), but it is not known how representative these data are of total illnesses and whether the foodborne proportion is similar across age groups. For example, the proportion of some illnesses acquired from animals (e.g., STEC O157) may be higher among children than adults (*36*), and the proportions that spread person-to-person (e.g., norovirus) may be higher among institutionalized elderly persons (*37*). Because a higher proportion of cases are reportedly associated with hospitalization or death in these vulnerable groups, we may have overestimated the total contribution of foodborne transmission for these outcomes.

The methods used for this study could be adapted to estimate the proportion of illnesses attributable to other modes of transmission, such as waterborne and direct animal contact. The estimates from this study can be used to help direct policy and interventions; to conduct other analyses (e.g., evaluation of economic cost of these diseases and attribution to various food commodities); and as a platform for developing estimates of effects of disease caused by sequelae of foodborne infections.

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References

1. Flint JA, Van Duynhoven YT, Angulo FJ, DeLong SM, Braun P, Kirk M, et al. Estimating the burden of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food: an international review. Clin Infect Dis. 2005;41:698–704. DOI: 10.1086/432064
2. Kuchenmuller T, Hird S, Stein C, Kramarz P, Nanda A, Havelaar AH. Estimating the global burden of foodborne diseases—a collaborative effort. Euro Surveill. 2009;14:pii:19195.
3. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5:607–25. DOI: 10.3201/eid0505.990502
4. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis. 2011;17:16–22.
5. Powell M, Ebel E, Schlosser W. Considering uncertainty in comparing the burden of illness due to foodborne microbial pathogens. Int J Food Microbiol. 2001;69:209–15. DOI: 10.1016/S0168-1605(01)00495-0
6. Scallan E. Activities, achievements, and lessons learned during the first 10 years of the Foodborne Diseases Active Surveillance Network: 1996–2005. Clin Infect Dis. 2007;44:718–25.
7. US Census Bureau. Population estimates [cited 2010 March 9]. http://www.census.gov/popest/states/NST-ann-est.html
8. Jones TF, McMillian MB, Scallan E, Frenzen PD, Cronquist AB, Thomas S, et al. A population-based estimate of the substantial burden of diarrhoeal disease in the United States; FoodNet, 1996–2003. Epidemiol Infect. 2007;135:293–301.
9. Scallan E, Jones TF, Cronquist A, Thomas S, Frenzen P, Hoefer D, et al. Factors associated with seeking medical care and submitting a stool sample in estimating the burden of foodborne illness. Foodborne Pathog Dis. 2006;3:432–8. DOI: 10.1089/fpd.2006.3.432
10. Voetsch AC, Angulo FJ, Rabatsky-Ehr T, Shallow S, Cassidy M, Thomas SM, et al. Laboratory practices for stool-specimen culture for bacterial pathogens, including *Escherichia coli* O157:H7, in the FoodNet sites, 1995–2000. Clin Infect Dis. 2004;38(Suppl 3):S190–7. DOI: 10.1086/381586
11. Jones JL, Lopez A, Wahlquist SP, Nadle J, Wilson M. Survey of clinical laboratory practices for parasitic diseases. Clin Infect Dis. 2004;38(Suppl 3):S198–202. DOI: 10.1086/381587
12. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. *Toxoplasma gondii* infection in the United States, 1999–2004, decline from the prior decade. Am J Trop Med Hyg. 2007;77:405–10.
13. World Health Organization. Toxoplasmosis. Technical report series no. 431. Geneva: The Organization; 1969.
14. Widdowson MA, Meltzer MI, Zhang X, Bresee JS, Parashar UD, Glass RI. Cost-effectiveness and potential impact of rotavirus vaccination in the United States. Pediatrics. 2007;119:684–97.
15. Wheeler JG, Sethi D, Cowden JM, Wall PG, Rodrigues LC, Tompkins DS, et al. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. BMJ. 1999;318:1046–50.
16. de Wit MA, Koopmans MP, Kortbeek LM, Wannet WJ, Vinje J, van Leusden F, et al. Sensor, a population-based cohort study on gastroenteritis in the Netherlands: incidence and etiology. Am J Epidemiol. 2001;154:666–74. DOI: 10.1093/aje/154.7.666
17. Marshall JA, Hellard ME, Sinclair MI, Fairley CK, Cox BJ, Catton MG, et al. Incidence and characteristics of endemic Norwalk-like virus–associated gastroenteritis. J Med Virol. 2003;69:568–78. DOI: 10.1002/jmv.10346
18. Amar CF, East CL, Gray J, Iturriza-Gomara M, Maclure EA, McLauchlin J. Detection by PCR of eight groups of enteric pathogens in 4,627 faecal samples: re-examination of the English case–control Infectious Intestinal Disease Study (1993–1996). Eur J Clin Microbiol Infect Dis. 2007;26:311–23.
19. Dennehy PH, Nelson SM, Spangenberger S, Noel JS, Monroe SS, Glass RI. A prospective case–control study of the role of astrovirus in acute diarrhea among hospitalized young children. J Infect Dis. 2001;184:10–5.
20. Klein EJ, Boster DR, Stapp JR, Wells JG, Qin X, Clausen CR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. Clin Infect Dis. 2006;43:807–13. DOI: 10.1086/507335

21. Vose D. Risk analysis. A quantitative guide. 2nd ed. Chichester (UK): John Wiley & Sons; 2000.
22. Tauxe RV. Emerging foodborne pathogens. Int J Food Microbiol. 2002;78:31–41. DOI: 10.1016/S0168-1605(02)00232-5
23. Adak GK, Meakins SM, Yip H, Lopman BA, O'Brien SJ. Disease risks from foods, England and Wales, 1996–2000. Emerg Infect Dis. 2005;11:365–72.
24. Hall G, Kirk MD, Becker N, Gregory JE, Unicomb L, Millard G, et al. Estimating foodborne gastroenteritis, Australia. Emerg Infect Dis. 2005;11:1257–64.
25. Simonsen J, Teunis P, Van Pelt W, Van Duynhoven Y, Krogfelt KA, Sadkowska-Todys M, et al. Usefulness of seroconversion rates for comparing infection pressures between countries. Epidemiol Infect. 2010; Apr 12:1–8 [Epub ahead of print].
26. St Louis ME, Peck SH, Bowering D, Morgan GB, Blatherwick J, Banerjee S, et al. Botulism from chopped garlic: delayed recognition of a major outbreak. Ann Intern Med. 1988;108:363–8.
27. Sobel J, Malavet M, John S. Outbreak of clinically mild botulism type E illness from home-salted fish in patients presenting with predominantly gastrointestinal symptoms. Clin Infect Dis. 2007;45:e14–6. DOI: 10.1086/518993
28. Dalton CB, Austin CC, Sobel J, Hayes PS, Bibb WF, Graves LM, et al. An outbreak of gastroenteritis and fever due to *Listeria monocytogenes* in milk. N Engl J Med. 1997;336:100–5. DOI: 10.1056/NEJM199701093360204
29. Pinner RW, Teutsch SM, Simonsen L, Klug LA, Graber JM, Clarke MJ, et al. Trends in infectious diseases mortality in the United States. JAMA. 1996;275:189–93.
30. Patel MM, Widdowson MA, Glass RI, Akazawa K, Vinje J, Parashar UD. Systematic literature review of role of noroviruses in sporadic gastroenteritis. Emerg Infect Dis. 2008;14:1224–31.
31. Jansen A, Stark K, Kunkel J, Schreier E, Ignatius R, Liesenfeld O, et al. Aetiology of community-acquired, acute gastroenteritis in hospitalised adults: a prospective cohort study. BMC Infect Dis. 2008;8:143. DOI: 10.1186/1471-2334-8-143
32. Badger GF, Curtiss C, Dingle JH, Hodges RG, Jordan WS Jr, McCorkle LP. A study of illness in a group of Cleveland families. XI. The occurrence of gastrointestinal symptoms. Am J Hyg. 1956;64:349–56.
33. Monto AS, Koopman JS. The Tecumseh Study. XI. Occurrence of acute enteric illness in the community. Am J Epidemiol. 1980;112:323–33.
34. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. MMWR Morb Mortal Wkly Rep. 2010;59:418–22.
35. Pires SM, Evers EG, van Pelt W, Ayers T, Scallan E, Angulo FJ, et al. Attributing the human disease burden of foodborne infections to specific sources. Foodborne Pathog Dis. 2009;6:417–24.
36. Crump JA, Sulka AC, Langer AJ, Schaben C, Crielly AS, Gage R, et al. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. N Engl J Med. 2002;347:555–60. DOI: 10.1056/NEJMoa020524
37. Blanton LH, Adams SM, Beard RS, Wei G, Bulens SN, Widdowson MA, et al. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000–2004. J Infect Dis. 2006;193:413–21. DOI: 10.1086/499315

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Foodborne Illness Acquired in the United States—Unspecified Agents

Elaine Scallan,[1] Patricia M. Griffin, Frederick J. Angulo, Robert V. Tauxe, and Robert M. Hoekstra

Each year, 31 major known pathogens acquired in the United States caused an estimated 9.4 million episodes of foodborne illness. Additional episodes of illness were caused by unspecified agents, including known agents with insufficient data to estimate agent-specific illness, known agents not yet recognized as causing foodborne illness, substances known to be in food but of unproven pathogenicity, and unknown agents. To estimate these additional illnesses, we used data from surveys, hospital records, and death certificates to estimate illnesses, hospitalizations, and deaths from acute gastroenteritis and subtracted illnesses caused by known gastroenteritis pathogens. If the proportions acquired by domestic foodborne transmission were similar to those for known gastroenteritis pathogens, then an estimated 38.4 million (90% credible interval [CrI] 19.8–61.2 million) episodes of domestically acquired foodborne illness were caused by unspecified agents, resulting in 71,878 hospitalizations (90% CrI 9,924–157,340) and 1,686 deaths (90% CrI 369–3,338).

Foodborne diseases are a major cause of illness and death in the United States. In another article, we estimated that each year, major known pathogens acquired in the United States caused 9.4 million episodes of foodborne illness, resulting in 55,961 hospitalizations and 1,351 deaths (*1*). (Hereafter, episodes of illness are referred to as illnesses.) Although the number of illnesses caused by these pathogens is substantial, these illnesses represent only a subset of the total illnesses.

An additional proportion of foodborne illness is probably caused by a heterogeneous group of less understood agents. First, many agents that cause acute gastroenteritis are recognized as known or possible causes of foodborne illness, but because of a paucity of data, the number of agent-specific illnesses cannot be estimated. This category includes infectious agents (e.g., *Aeromonas* spp., *Edwardsiella* spp., and *Plesiomonas* spp.) and noninfectious agents (e.g., mushroom and marine biotoxins, metals, and other inorganic toxins). Second, some known agents may not be recognized as being transmitted in food. Detection of *Clostridium difficile* in retail meat products suggests that it may sometimes be transmitted by that route (*2*), and foodborne transmission of *Trypanosoma* spp. has recently been recognized in Brazil but not in the United States (*3*). Third, microbes, chemicals, and other substances known to be in food could at some time be shown to cause acute illness. Fourth, agents of foodborne illness continue to be discovered. Many major foodborne pathogens, e.g., *Campylobacter* spp. and *Escherichia coli* O157, were recognized only in recent decades (*4*,*5*). For some outbreaks (e.g., Brainerd diarrhea), even when specimens are obtained quickly, no causative agent can be identified (*6*,*7*). Additional agents of foodborne illness probably remain undescribed (*8*). This article provides estimates of foodborne gastroenteritis illnesses, hospitalizations, and deaths in the United States, other than those caused by the 31 major known pathogens considered in our companion article (*1*).

Methods

We defined unspecified agents as agents that cause acute gastroenteritis but that were not included in our estimate of foodborne illness caused by 31 major known pathogens (*1*). They include known agents with insufficient data for estimating agent-specific episodes of illness; known agents not yet recognized as causing foodborne ill-

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ness; microbes, chemicals, or other substances known to be in food but for which pathogenicity is unproven; and agents not yet described. To estimate the extent of foodborne illness caused by unspecified agents, we estimated the number of acute gastroenteritis illnesses, hospitalizations, and deaths and subtracted the estimated number of acute gastroenteritis illnesses, hospitalizations, and deaths caused by 24 major known pathogens that typically or often cause diarrhea or vomiting (Figure 1). We refer to them as the 24 known gastroenteritis pathogens, although for a few, diarrhea or vomiting was not the main clinical sign. Estimates of illness were not made for unspecified agents that do not typically result in acute gastroenteritis.

We used data from the 24 known gastroenteritis pathogens to estimate the proportion of unspecified agents that were acquired in the United States (hereafter referred to as domestically acquired) and transmitted in food. Most of our data were from 2000 through 2007, and all estimates were based on the US population in 2006 (299 million persons) (*9*). To account for uncertainty, we used probability distributions to describe a range of plausible values for all model inputs. The modeling approach used and parameters of these probability distributions are detailed in the online Technical Appendix (www.cdc.gov/EID/content/17/1/16-Techapp.pdf). Our model outputs are in the form of probability distributions summarized by a mean point estimate with 90% credible intervals (CrIs).

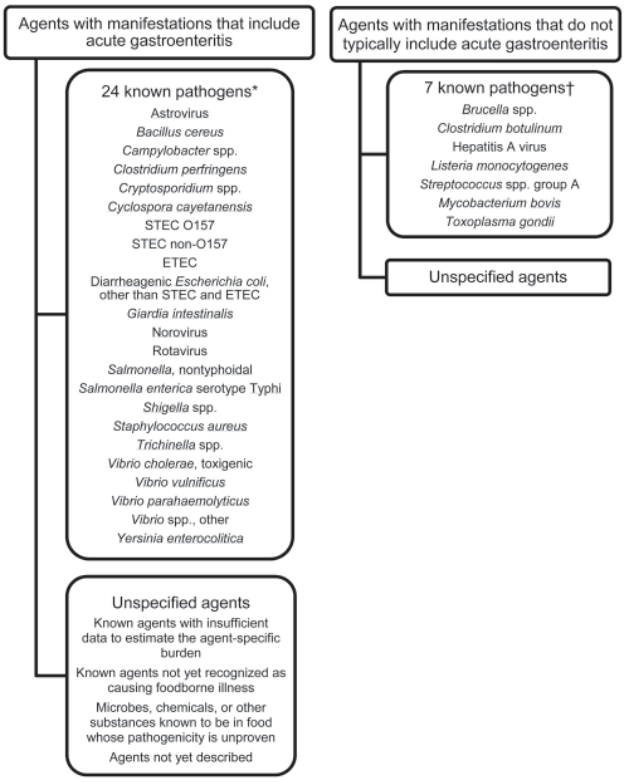

Figure 1. Agents that cause foodborne illness. STEC, Shiga toxin–producing *Escherichia coli*; ETEC, enterotoxigenic *E. coli*. *For most of these pathogens, the major manifestation is gastroenteritis. For some, i.e., *Salmonella enterica* serotype Typhi, *Trichinella* spp., and *Vibrio vulnificus*, some persons have diarrhea or vomiting, and the signs may initially look like those of gastroenteritis. †Most of these agents have major manifestations that do not typically include gastroenteritis. Diarrhea and vomiting can occur with some of these pathogens, e.g., *Clostridium botulinum* and hepatitis A virus, but are relatively uncommon. Only invasive *Listeria monocytogenes* infection, not diarrheal illness, is included in our estimates for known foodborne pathogens (*1*).

Acute Gastroenteritis

We estimated the number of episodes of acute gastroenteritis by using combined data from the Foodborne Diseases Active Surveillance Network (FoodNet) Population Surveys conducted in 2000–2001, 2002–2003, and 2006–2007 (Centers for Disease Control and Prevention [CDC], unpub. data). These methods are described in detail elsewhere (*10*). In brief, FoodNet Population Surveys are random-digit-dial telephone surveys of the general population in FoodNet sites. At the time of these surveys, the population in FoodNet sites included 11% (in 2000) to 15% (in 2007) of the US population. In 2005, the demographic features of this population were similar to those of the US population, but the proportion of Hispanics was lower (*11*).

Surveys were conducted over 12-month periods and collected information about episodes of diarrhea and vomiting in the past month. Our estimate of the annual number of episodes of acute gastroenteritis was derived by multiplying the average monthly prevalence by 12. An episode of acute gastroenteritis was defined as diarrhea (≥3 loose stools in 24 hours) or vomiting in the past month, each lasting >1 day or resulting in restricted daily activities. We excluded persons with a chronic condition in which diarrhea or vomiting was a major clinical sign and persons with concurrent cough or sore throat. Data were weighted to compensate for unequal probabilities of selection and to reflect the surveillance population by age and sex.

The estimated rates of acute gastroenteritis according to individual surveys were 0.49 (2000–2001), 0.54 (2002–2003), and 0.73 (2006–2007) episodes per person per year. The number of episodes of acute gastroenteritis was estimated by applying the average rate (0.6 episodes/person/year) from the combined surveys to the 2006 US population estimate. Uncertainty was added by assuming that individual FoodNet site estimates were normally distributed with standard deviations equal to survey standard errors (online Technical Appendix).

Hospitalizations

We estimated the number of hospitalizations for acute gastroenteritis by using 2000–2006 national esti-

mates from 3 sources: CDC National Center for Health Statistics (NCHS) National Hospital Discharge System (NHDS) (*12*,*13*); Healthcare Cost and Utilization Project, Nationwide Inpatient Sample (NIS) (*14*); and combined data from NCHS National Ambulatory and National Hospital Ambulatory Medical Care Surveys (NAMCS and NHAMCS) (*15*).

Codes from International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM), were used to extract hospital discharge data from NHDS and NIS when acute gastroenteritis was listed as 1 of the first 3 diagnoses. Acute gastroenteritis was defined as ICD-9-CM diagnostic codes 001–008 (infectious gastroenteritis of known cause), 009 (infectious gastroenteritis), 558.9 (other and unspecified noninfectious gastroenteritis and colitis), or 787.9 (other symptoms involving digestive system: diarrhea), excluding 008.45 (*C. difficile* colitis) and 005.1 (botulism). Many infectious illnesses from which a pathogen was not isolated may be coded as other and unspecified noninfectious gastroenteritis and colitis. Annual national estimates from 2000–2006 NHDS and 2000–2006 NIS data were obtained by weighting the sample data according to NCHS and the Healthcare Cost and Utilization Project criteria (*12*,*14*).

To estimate hospitalizations for acute gastroenteritis from NAMCS and NHAMCS data, we combined data from both surveys and extracted patient visits to clinical settings (including physician offices, hospital emergency and outpatient departments) with a diagnosis of acute gastroenteritis resulting in hospitalization, when acute gastroenteritis was listed as 1 of the 3 codes. Acute gastroenteritis was defined by using the ICD-9-CM codes described above. Annual national estimates were obtained by weighting the sample data according to NCHS criteria (*15*).

During 2000–2006, mean annual rates of hospitalization for acute gastroenteritis were 203 hospitalizations per 100,000 persons according to NHDS data, 187 per 100,000 according to NIS data, and 109 per 100,000 according to NAMCS and NHAMCS data. To estimate the number of hospitalizations for acute gastroenteritis, we chose the PERT distribution with a low, modal, and high value determined by the lowest (90), mean (166), and highest (211) annual rate per 100,000 persons across all 3 surveys and applied this distribution to the 2006 US population (online Technical Appendix).

Deaths

We estimated the number of deaths caused by acute gastroenteritis by using multiple cause-of-death data from the National Vital Statistics System (2000–2006) (*16*,*17*) when acute gastroenteritis was listed as the underlying or a contributing cause. Acute gastroenteritis was defined as ICD, 10th Revision, codes A00.9–A08.5 (infectious gastroenteritis of known cause), A09 (diarrhea and gastroenteritis of presumed infectious origin), and K52.9 (noninfectious gastroenteritis and colitis, unspecified), excluding A04.7 (enterocolitis caused by *C. difficile*) and A05.1 (botulism). To estimate the number of deaths, we chose the mean death rate (1.5 deaths/100,000 population) as the modal value of a PERT distribution, used the lowest and highest annual death rates (1.2 and 2.4 deaths/100,000 population) as the lower and upper bounds, and applied this distribution to the 2006 US population.

Domestically Acquired and Foodborne Acute Gastroenteritis

To estimate acute gastroenteritis caused by unspecified agents, we subtracted the estimated number of illnesses, hospitalizations, and deaths caused by the 24 known gastroenteritis pathogens from our estimate of the overall number of illnesses, hospitalizations, and deaths from acute gastroenteritis (Figure 2). To estimate the number that were domestically acquired and transmitted by food, we used the overall weighted distribution of the proportions of illnesses, hospitalizations, and deaths that were domestically acquired and foodborne from the 24 known gastroenteritis pathogens to describe the lower, modal, and upper values of the PERT distribution and applied these separately to the estimates of unspecified illnesses, hospitalizations, and deaths (online Technical Appendix).

Uncertainty Analysis

The parametric distribution used for almost all multipliers was a 4-parameter beta (modified PERT) distribution (*18*). The first 3 parameters are low, modal, and high. The fourth parameter is related to the variability of the distribution. We typically fixed this last parameter at 4, which yields the simple PERT distribution (*18*). However, when describing the proportions domestically acquired and foodborne from the 24 known gastroenteritis pathogens, we used a value of 2 to reflect greater uncertainty (online Technical Appendix). On the basis of 100,000 iterations, we obtained empirical distributions of counts corresponding to Bayesian posterior distributions and used these posterior distributions to generate a point estimate (posterior mean) and upper and lower 5% limits for 90% CrIs. We used SAS version 9.2 (SAS Institute, Cary, NC, USA) for these analyses.

Results

Foodborne Illnesses

We estimate that 38.4 million (90% CrI 19.8–61.3 million) episodes of domestically acquired foodborne gastroenteritis were caused by unspecified agents (Figure 2) as follows. We estimated that 178.8 million acute gastro-

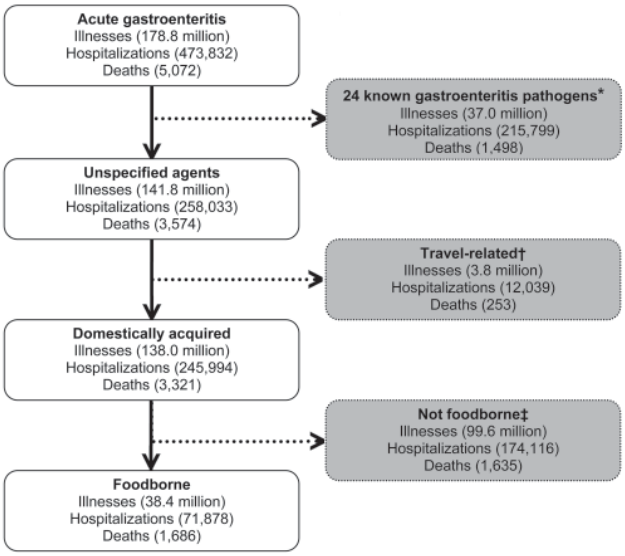

Figure 2. Schematic of estimates of illnesses, hospitalizations, and deaths caused by unspecified acute gastroenteritis agents. *The estimated numbers of illnesses, hospitalizations, and deaths (hereafter, illnesses refers to illnesses, hospitalizations, or deaths as appropriate) caused by the 24 known gastroenteritis pathogens (*1*) were subtracted to estimate the number of illnesses caused by unspecified agents. †The estimated numbers of illnesses related to travel were subtracted to estimate the number of domestically acquired illnesses. The estimates of the proportion related to travel were based on the overall weighted distribution of the proportions of illnesses that were related to travel from the 24 known gastroenteritis pathogens. ‡The estimated numbers of nonfoodborne illnesses were subtracted to estimate foodborne illnesses. The estimates of the proportion foodborne were based on the overall weighted distribution of the proportions of illnesses that were foodborne from the 24 known gastroenteritis pathogens. All estimates were based on US population in 2006.

enteritis illnesses occurred each year in the United States. Subtracting 37.0 million estimated illnesses caused by the 24 known gastroenteritis pathogens leaves 141.8 million acute gastroenteritis illnesses caused by unspecified agents. The proportion of these unspecified agents acquired through domestic foodborne transmission is unknown; however, applying the distribution of the proportion of illnesses from the 24 known gastroenteritis pathogens that were domestically acquired (98%) and foodborne (25%) yields an estimate of 38.4 million domestically acquired foodborne illnesses caused by unspecified agents.

Hospitalizations

We estimate that 473,832 hospitalizations resulted from acute gastroenteritis each year in the United States (Figure 2). Subtracting the 215,799 estimated hospitalizations caused by the 24 known gastroenteritis pathogens leaves 258,033 hospitalizations for acute gastroenteritis caused by unspecified agents. The proportion of these unspecified agents that were acquired as a result of domestic foodborne transmission is unknown; however, applying the distributions of the proportion of hospitalizations among the 24 known gastroenteritis pathogens that were domestically acquired (97%) and foodborne (23%) yields an estimate of 71,878 hospitalizations (90% CrI 9,924–157,340) caused by domestically acquired unspecified agents that were transmitted by food.

Deaths

We estimate that an estimated 5,072 persons died of acute gastroenteritis each year in the United States (Figure 2). Subtracting the 1,498 deaths caused by the 24 known gastroenteritis pathogens leaves 3,574 acute gastroenteritis deaths caused by unspecified agents. The proportion of these unspecified agents acquired as a result of domestic foodborne transmission is unknown; however, applying the distributions of the proportion of deaths among the 24 known gastroenteritis pathogens that were domestically acquired (95%) and foodborne (50%) yields an estimate of 1,686 (90% CrI 369–3,338) deaths caused by domestically acquired unspecified agents that were transmitted by food.

Discussion

Unspecified agents are major contributors to the total number of episodes of acute gastroenteritis and foodborne diseases. If distribution of domestically acquired and foodborne agents is similar to that of the 24 known gastroenteritis pathogens (*1*), then these agents cause 38.4 million episodes of foodborne gastroenteritis each year in the United States, resulting in 78,878 hospitalizations, and 1,686 deaths. Combining the estimates for unspecified agents and major known pathogens provides an estimate of the total effect of contaminated food consumed in the United States: 47.8 million episodes of illness, 127,839 hospitalizations, and 3,037 deaths (Table).

Our estimate of foodborne illness caused by unspecified agents is lower than that estimated by CDC in 1999 (38.4 million vs. 62 million, respectively) (*19*). A major reason for this decrease is our lower estimate of episodes of acute gastroenteritis, which probably resulted from changes in data sources and methods rather than a real decline in the rate of illness. Our estimate is derived from the 3 most recent FoodNet Population Surveys, which had a sample size 5× greater than that in the 1996–1997 FoodNet survey used for the 1999 estimates. Additionally, the 1999 estimates relied on respiratory symptom and vomiting data from US studies conducted before 1980 (*20*,*21*). The current and the 1999 estimates excluded persons reporting concurrent cough or sore throat, but the proportion of respondents reporting these signs was higher in the current than in the earlier surveys (38% vs. 25%), contributing to a lower estimated prevalence of acute gastroen-

Table. Estimated annual number of episodes of domestically acquired, foodborne illness, hospitalizations, and deaths caused by 31 pathogens and unspecified agents transmitted through food, United States*

	Illnesses		Hospitalizations		Deaths	
Cause	Mean (90% CrI)	%	Mean (90% CrI)	%	Mean (90% CrI)	%
Major known pathogens†	9,388,075 (6,641,440–12,745,709)	20	55,961 (39,534–75,741)	44	1,351 (712–2,268)	44
Unspecified agents‡	38,392,704 (19,829,069–61,196,274)	80	71,878 (9,924–157,340)	56	1,686 (369–3,338)	56
Total	47,780,779 (28,658,973–71,133,833)	100	127,839 (62,529–215,562)	100	3,037 (1,492–4,983)	100

*All estimates were based on US population in 2006. CrI, credible interval.
†The 31 known pathogens are astrovirus, *Bacillus cereus*, *Brucella* spp., *Campylobacter* spp., *Clostridium botulinum*, *Clostridium perfringens*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, enterotoxigenic *Escherichia coli* (ETEC), Shiga toxin–producing *E. coli* (STEC) O157, STEC non-O157, diarrheagenic *E. coli* other than STEC and ETEC, *Giardia intestinalis*, hepatitis A virus, *Listeria monocytogenes, Mycobacterium bovis*, norovirus, rotavirus, sapovirus, nontyphoidal *Salmonella* spp., *S. enterica* serotype Typhi, *Shigella* spp., *Staphylococcus aureus, Streptococcus* spp. group A, *Toxoplasma gondii*, *Trichinella spp.*, *Vibrio cholerae*, *V. vulnificus*, *V. parahemolyticus*, other *Vibrio* spp., and *Yersinia* spp. (*1*).
‡Unspecified agents are defined as agents that cause acute gastroenteritis other than the 31 major known pathogens listed above. They include known agents with insufficient data to estimate agent-specific episodes of illness; known agents not yet recognized as causing foodborne illness; microbes, chemicals, and other substances known to be in food but whose pathogenicity is unproven; and agents not yet described.

teritis (0.60 vs. 0.79 episodes/person/year). In addition, the current study excluded persons with vomiting who had been ill for <1 day or whose illness did not result in restricted daily activities, whereas the 1999 estimate included all persons with vomiting. All these factors contributed to the current estimate of acute gastroenteritis being 24% lower than the 1999 estimate.

The proportion of illnesses estimated to be foodborne was also a major driver of the current lower estimate of illness caused by unspecified foodborne agents. Because no data existed with which to directly estimate the proportions of unspecified agents that were domestically acquired and foodborne, distributions of these proportions were estimated to be similar to those of the 24 known gastroenteritis pathogens (*1*). Because norovirus accounts for 59% of illnesses caused by the 24 known gastroenteritis pathogens, the foodborne proportion was driven largely by norovirus. The proportion of foodborne norovirus used for the current estimate is 26%, a marked decrease from 40% used for the 1999 estimates. Additionally, unlike the 1999 estimates, the current estimates exclude international travel-related illnesses. As a result of these newer data and revised methods, the mean proportion of unspecified agents that were estimated to be transmitted by food was 25%, which is lower than 36% used for the 1999 estimate.

Estimating the number of hospitalizations and deaths caused by unspecified foodborne agents is challenging. For an illness caused by a pathogen to be recorded, a physician must order the appropriate diagnostic tests and the pathogen must be detected. Without identification of a pathogen, infections producing signs and symptoms of gastroenteritis may be coded as nonspecific signs or symptoms or as noninfectious illnesses (*22*). Our overall estimates of hospitalizations and deaths from acute gastroenteritis, derived from national data sources, include codes for infectious and noninfectious gastroenteritis. To avoid overestimating the number of hospitalizations, we selected outpatient visits resulting in hospitalization and we selected hospital discharge records on the basis of only the first 3 listed diagnoses. This approach was a compromise between limiting the analysis to hospitalizations for which acute gastroenteritis was listed as the primary cause and including hospitalizations for which signs or symptoms of gastroenteritis may have been a manifestation of another illness. This approach has been taken in other studies (*23*,*24*). For deaths, we included all records in which acute gastroenteritis was listed as an underlying or a contributing cause.

Our approach to estimating illness caused by unspecified agents has many limitations. First, the accuracy of our estimate of the number of acute episodes of gastroenteritis from the FoodNet Population Surveys has not been validated. This estimate was based on responses to questions about diarrhea and vomiting in the past month. These data provide a measure of prevalent cases; however, we lack sufficient data on duration of illness, onset date, and multiple episodes in the past month necessary to estimate incidence. An analysis of the FoodNet Population Surveys reported a 2-day median duration of acute gastroenteritis (*10*), suggesting that the increase in the estimate of illnesses based on incidence versus prevalence would probably be small and would be included within the range of sampling variability and uncertainty associated with our estimate of acute gastroenteritis. The accuracy of the 1-month recall period for acute gastroenteritis is also unknown. Some evidence suggests that shorter recall periods (e.g., past week) may result in higher reported prevalence (*25*). Which recall period is more accurate is not known. Our estimate of the number of episodes of acute gastroenteritis may be too high. Although our survey attempted to eliminate other causes of illness by asking about chronic diseases, some of the vomiting illnesses classified as acute gastroenteritis, for example, could have been caused by medications, alcohol withdrawal, or other causes, and some of the diarrheal illnesses could be caused by medications or other causes.

However, our criteria for acute gastroenteritis were fairly strict, and foodborne illnesses probably occurred in some persons who were excluded because they had concurrent cough or sore throat or because their illness lasted for only 1 day. Second, we may have underestimated the episodes of illness caused by the 24 known gastroenteritis pathogens. When our method is used, any increase in the estimate for the major known pathogens will result in a decrease in the estimate for unspecified agents. Recent serologic data from European countries suggest that infection with *Salmonella* spp. is more common than estimated by other methods, including ours; however, many of these infections may be asymptomatic (*26*). Finally, the proportion of illnesses transmitted by food for unspecified agents is unknown and may differ from that for the 24 known gastroenteritis pathogens. Studies estimating foodborne disease in England and Wales and in Australia (which also attributed a large proportion of foodborne illness to unspecified agents: 73% in Australia and 48% in England and Wales vs. 80% in the United States) have estimated a similar proportion of acute gastroenteritis episodes to be transmitted by food (32%, and 26%, respectively vs. 25% in the United States) (*27*,*28*). A study of illness caused by known chemical agents, with estimates of the proportion that include acute gastroenteritis and that are foodborne, could help improve these estimates.

Combining the estimate for unspecified agents with that for the 31 major known pathogens to arrive at an estimate of overall foodborne illness has limitations. The method used for unspecified agents began with an estimate of acute gastroenteritis episodes, hospitalizations, and deaths and scaled down to a number for domestically acquired foodborne illnesses, hospitalizations, and deaths. Conversely, for most known pathogens, our estimate scaled counts of laboratory-confirmed illnesses up to an estimated number of ill persons, accounting for underreporting and underdiagnosis factors that contribute to an illness not being reported to public health agencies. Combining different approaches is not optimal because the methods themselves may affect the estimates derived. Also, our estimates do not include unspecified foodborne illnesses that do not typically cause signs of acute gastroenteritis. Most foodborne outbreak–associated illnesses caused by chemical agents reported to CDC during 2001–2006 (*29*) were not due to agents characterized by acute gastroenteritis and so would not be included in our estimates.

Although the number of episodes of foodborne disease caused by unspecified agents is substantial, the claim that 80% of foodborne illnesses are unspecified must be treated with caution. Illnesses caused by the 24 known gastroenteritis pathogens were, in most instances, estimated by using models that scaled counts of laboratory-confirmed illnesses up to an estimated number of illnesses with aggregate multipliers to adjust for underreporting and underdiagnosis factors that contribute to an illness not being reported to public health agencies (*1*). These multipliers are sensitive to the methods and modeling approaches used, and different choices could have increased estimates for the 24 known gastroenteritis pathogens, thus decreasing the estimate of foodborne illness caused by unspecified agents. For example, we took a conservative approach to estimating the underreporting multiplier for pathogens for which illness counts were derived from outbreak data (*1*); a less conservative approach would have increased estimated illnesses for these pathogens.

Future estimates might be improved by validating them by using other data on acute gastroenteritis episodes, hospitalizations, and deaths, such as by reviewing acute gastroenteritis coded as a secondary discharge diagnosis or assessing the accuracy of acute gastroenteritis coding on death certificates. The diagnostic gap might be narrowed by identifying additional agents linked to foodborne transmission. Systematic laboratory investigation of specimens from well-investigated outbreaks of foodborne disease of undetermined cause, and detailed investigations of specific syndromes, may identify new agents (*4*,*5*,*30*).

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References

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011;17:7–15.
2. Songer JG, Trinh HT, Killgore GE, Thompson AD, McDonald LC, Limbago BM. *Clostridium difficile* in retail meat products, USA, 2007. Emerg Infect Dis. 2009;15:819–21. DOI: 10.3201/eid1505.081071
3. Pereira KS, Schmidt FL, Guaraldo AM, Franco RM, Dias VL, Passos LA. Chagas' disease as a foodborne illness. J Food Prot. 2009;72:441–6.
4. Dekeyser P, Gossuin-Detrain M, Butzler JP, Sternon J. Acute enteritis due to related *Vibrio*: first positive stool cultures. J Infect Dis. 1972;125:390–2.
5. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N Engl J Med. 1983;308:681–5. DOI: 10.1056/NEJM198303243081203
6. Mintz E. A riddle wrapped in a mystery inside an enigma: Brainerd diarrhoea turns 20. Lancet. 2003;362:2037–8. DOI: 10.1016/S0140-6736(03)15148-3
7. Hall JA, Goulding JS, Bean NH, Tauxe RV, Hedberg CW. Epidemiologic profiling: evaluating foodborne outbreaks for which no pathogen was isolated by routine laboratory testing: United States, 1982–9. Epidemiol Infect. 2001;127:381–7. DOI: 10.1017/S0950268801006161

8. Tauxe RV. Emerging foodborne pathogens. Int J Food Microbiol. 2002;78:31–41. DOI: 10.1016/S0168-1605(02)00232-5
9. US Census Bureau. Population estimates [cited 2010 Mar 9]. http://www.census.gov/popest/states/NST-ann-est.html
10. Jones TF, McMillian MB, Scallan E, Frenzen PD, Cronquist AB, Thomas S, et al. A population-based estimate of the substantial burden of diarrhoeal disease in the United States; FoodNet, 1996–2003. Epidemiol Infect. 2007;135:293–301. DOI: 10.1017/S0950268806006765
11. Scallan E. Activities, achievements, and lessons learned during the first 10 years of the Foodborne Diseases Active Surveillance Network: 1996–2005. Clin Infect Dis. 2007;44:718–25. DOI: 10.1086/511648
12. Centers for Disease Control and Prevention. National Hospital Discharge Survey [cited 2010 Feb 2]. http://www.cdc.gov/nchs/nhds.htm
13. Dennison C, Pokras R. Design and operation of the National Hospital Discharge Survey: 1988 redesign. Vital Health Stat 1. 2000;39:1–42.
14. Healthcare Cost and Utilization Project. Overview of the Nationwide Inpatient Sample (NIS) [cited 2010 Feb 8]. http://www.hcup-us.ahrq.gov/nisoverview.jsp
15. Centers for Disease Control and Prevention. Ambulatory health care data [cited 2010 Feb 8]. http://www.cdc.gov/nchs/ahcd/about_ahcd.htm
16. Centers for Disease Control and Prevention. Mortality data, multiple cause-of-death public-use data files [cited 2010 Feb 8]. http://www.cdc.gov/nchs/products/elec_prods/subject/mortmcd.htm
17. Heron M, Hoyert DL, Murphy SL, Xu J, Kochanek KD, Tejada-Vera B. Deaths: final data for 2006. Natl Vital Stat Rep. 2009;57:1–134.
18. Vose D. Risk analysis. A quantitative guide. 2nd ed. Chichester (UK): John Wiley & Sons, Ltd; 2000.
19. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5:607–25. DOI: 10.3201/eid0505.990502
20. Badger GF, Curtiss C, Dingle JH, Hodges RG, Jordan WS Jr, McCorkle LP. A study of illness in a group of Cleveland families. XI. The occurrence of gastrointestinal symptoms. Am J Hyg. 1956;64:349–56.
21. Monto AS, Koopman JS. The Tecumseh study. XI. Occurrence of acute enteric illness in the community. Am J Epidemiol. 1980;112:323–33.
22. Pinner RW, Teutsch SM, Simonsen L, Klug LA, Graber JM, Clarke MJ, et al. Trends in infectious diseases mortality in the United States. JAMA. 1996;275:189–93. DOI: 10.1001/jama.275.3.189
23. Mounts AW, Holman RC, Clarke MJ, Bresee JS, Glass RI. Trends in hospitalizations associated with gastroenteritis among adults in the United States, 1979–1995. Epidemiol Infect. 1999;123:1–8. DOI: 10.1017/S0950268899002587
24. Gangarosa RE, Glass RI, Lew JF, Boring JR. Hospitalizations involving gastroenteritis in the United States, 1985: the special burden of the disease among the elderly. Am J Epidemiol. 1992;135:281–90.
25. Cantwell LB, Henao OL, Hoekstra RM, Scallan E. The effect of different recall periods on estimates of acute gastroenteritis in the United States, FoodNet Population Survey 2006–2007. Foodborne Pathog Dis. 2010;10:1225–8.
26. Simonsen J, Teunis P, VAN Pelt W, VAN Duynhoven Y, Krogfelt KA, Sadkowska-Todys M, et al. Usefulness of seroconversion rates for comparing infection pressures between countries. Epidemiol Infect. 2010;12:1–8. DOI: 10.1017/S0950268810000750
27. Adak GK, Long SM, O'Brien SJ. Trends in indigenous foodborne disease and deaths, England and Wales: 1992 to 2000. Gut. 2002;51:832–41. DOI: 10.1136/gut.51.6.832
28. Hall G, Kirk MD, Becker N, Gregory JE, Unicomb L, Millard G, et al. Estimating foodborne gastroenteritis, Australia. Emerg Infect Dis. 2005;11:1257–64.
29. Centers for Disease Control and Prevention. Surveillance for foodborne disease outbreaks—United States, 2006. MMWR Morb Mortal Wkly Rep. 2009;58:609–15.
30. 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.

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Completeness of Communicable Disease Reporting, North Carolina, USA, 1995–1997 and 2000–2006

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Despite widespread use of communicable disease surveillance data to inform public health intervention and control measures, the reporting completeness of the notifiable disease surveillance system remains incompletely assessed. Therefore, we conducted a comprehensive study of reporting completeness with an analysis of 53 diseases reported by 8 health care systems across North Carolina, USA, during 1995–1997 and 2000–2006. All patients who were assigned an International Classification of Diseases, 9th Revision, Clinical Modification, diagnosis code for a state-required reportable communicable disease were matched to surveillance records. We used logistic regression techniques to estimate reporting completeness by disease, year, and health care system. The completeness of reporting varied among the health care systems from 2% to 30% and improved over time. Disease-specific reporting completeness proportions ranged from 0% to 82%, but were generally low even for diseases with great public health importance and opportunity for interventions.

Surveillance has been the cornerstone of public health since the US Congress authorized the Public Health Service to collect morbidity data for cholera, smallpox, plague, and yellow fever in 1878. Currently, all states conduct notifiable disease surveillance following guidelines from the Centers for Disease Control and Prevention (CDC) and the Council for State and Territorial Epidemiologists. The current list of nationally notifiable communicable diseases has expanded to >60 diseases and includes vaccine-preventable diseases (e.g., pertussis, measles), emerging infectious diseases (e.g., severe acute respiratory syndrome, West Nile virus encephalitis), foodborne diseases (e.g., Shiga toxin–producing *Escherichia coli* and *Salmonella* spp. infections), sexually transmitted diseases (e.g., syphilis, HIV), and aerosol and droplet transmitted diseases (e.g., tuberculosis, meningococcal meningitis). Active surveillance programs conducted by CDC in conjunction with certain states include Active Bacterial Core surveillance, FoodNet, and influenza-related hospitalization surveillance. Surveillance of epidemiologically important diseases provides critical information to clinicians and public health officials for use in measuring disease incidence in communities, recognizing disease outbreaks, assessing prevention and control measure effectiveness, allocating public health resources, and further clarifying the epidemiology of new and emerging pathogens (*1*).

Like all US states, North Carolina has state laws and regulations mandating communicable disease reporting (*2–4*). The state relies on physicians and laboratories to comply with the directive to report diseases and laboratory results indicative of diseases considered a threat to public health. During the periods of this study (1995–1997, 2000–2006), mandatory reporting was required for >60 diseases. Conditions and disease reports consisted of paper communicable-disease report forms that contained demographic, clinical, and risk factor data for the case-patient. These reports were required to be submitted to the health department within a specified period (i.e., immediately, within 24 hours, or within 7 days), depending on the disease. An important change to the communicable disease surveillance system of the North Carolina Department of Health and Human Services (NC DHHS) occurred when the state administrative code was amended in September

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1998 to require that persons in charge of diagnostic laboratories report positive laboratory results for most diseases already reportable by physicians (*2*). This dual reporting mechanism was intended to improve completeness, timeliness, and accuracy of surveillance. More recently, in 2002, surveillance efforts have also expanded with the introduction of 7 regional public health teams and 11 hospital-based public health epidemiologists.

Despite the widespread use of these surveillance data, systematic data collection based on mandatory physician and laboratory reporting has never been extensively evaluated. To date, only 2 evaluations have examined reporting proportions for >5 diseases (*5*,*6*). Previous studies examining the completeness of disease reporting have differed considerably in terms of the following factors: size of geographic region (e.g., from clinics at a single university to multiple states), range of study period (e.g., several months to several years), heterogeneity of reporting systems (e.g., health care provider–based passive reporting vs. health care provider– and laboratory-based passive reporting), and various patient ascertainment methods (e.g., laboratory records, billing records, active surveillance, death certificates). This variability renders study results difficult to compare or aggregate. Therefore, we undertook a comprehensive study of reporting completeness with an analysis of 53 reportable diseases and conditions in selected health care systems across North Carolina over a 10-year period to estimate disease-specific reporting proportions, describe changes to reporting over time, and examine the variability of reporting completeness between health care facilities.

Methods

A retrospective cohort study was conducted at 8 large nonfederal acute care health care systems that experience 32% of all inpatient visits and 23% of all outpatient visits in North Carolina (*7*). These health care systems ranged in size from 581 to 1,324 site-licensed beds, spanned the Eastern Coastal, Central Piedmont, and Western Mountain regions of the state, and were selected from a network of 11 health care systems staffed with hospital-based public health epidemiologists. The study cohort was defined as all inpatients and outpatients at the 8 health care systems who were assigned a discharge diagnostic code from the International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM), that corresponded with a reportable communicable diseases during a 10-year time period (1995–1997, 2000–2006). The years 1998–1999 were excluded from the study because this period marked the transition when the state law was changed to include a reporting requirement for laboratories.

Diseases were excluded if they were chronic infectious diseases that resulted in a recurring assignment of ICD-9-CM code (e.g., HIV, hepatitis B carrier), if no specific ICD-9-CM code was available (e.g., for viral hemorrhagic fever), or if the NC DHHS did not record patient identifiers in their surveillance database during the entire study period (e.g., for syphilis, gonorrhea, chlamydia). Approval for the study was granted by the institutional review boards of all health care systems as well as by the North Carolina Division of Public Health because identifiable patient data were required to match the hospital and health department databases.

The cohort of patients assigned ICD-9-CM diagnostic codes by the health care systems for a reportable communicable disease were matched to the NC DHHS reported case-patients by using a unique identifier created by either Social Security number, or a combination of the first 2 letters of the last name, first letter of the first name, date of birth, and a 2-digit disease code. Repeat patient visits within a 31-day window for the same disease were enumerated and only the first visit was retained, with the exception of tuberculosis, which had a 365-day window, and hepatitis A and paralytic polio, which were restricted to only the first visit. Patients who had dates of reporting to the NC DHHS before the date of diagnosis at the health care system were excluded because they represented cases that had already been reported.

Unadjusted disease-specific reporting completeness proportions were calculated by dividing the number of case-patients that were reported to NC DHHS by the total number of patients identified in the health care systems who were assigned an ICD-9-CM diagnostic code for a reportable disease. In addition, completeness proportions were estimated by year (1995–1997, 2000–2006) for the 3 health care systems that had complete data available for all 10 years, and generalized linear regression models were used to examine the time trends. For the years 2000–2006, reporting completeness proportions and 95% confidence intervals (CIs) were estimated for each health care system by using a binomial logistic regression model that included as covariates whether or not specific health care system personnel were designated for disease reporting.

For disease-specific completeness proportions, empirical continuity corrections were used when no patients were reported for a disease (*8*). In addition, adjusted completeness proportions and 95% uncertainty intervals (UIs) were calculated by using semi-Bayesian analysis (*9*) as recommended to reduce the mean squared error when an ensemble of measures are estimated (*10*). This semi-Bayesian hierarchical regression analysis uses prior covariates that help explain the mean of the ensemble of estimates and a specified prior variance (τ^2) of the distribution. Traditional maximum-likelihood estimates (i.e., unadjusted estimates as presented here) can be viewed as a special case of semi-Bayesian analysis in which the prior variance is infinite. By specifying even a moderately informative prior variance such as a τ^2

indicating that 95% of all completeness proportions lie between 7.3% and 85%, an appreciable reduction in the overall mean squared error can be expected with a shift in the point estimate and a narrowing of the 95% UI for each completeness proportion, with the relative degree of narrowing being greater for diseases with less information.

A sensitivity analysis was conducted on the specified prior variance, τ^2, by using high, medium, and low τ^2 values that assumed 95% of the completeness proportions were within the following ranges: 2.2%–95%, 7.3%–85%, and 12.9%–75%, respectively. Sensitivity analyses were also conducted on the inclusion or exclusion of prior covariates, which were the time frame for reporting the disease (i.e., 24 hours vs. 7 days), whether or not the disease had a reportable laboratory result, whether or not the disease had reportable serologic test results, whether or not the disease is classified as a CDC category A bioterrorism agent, and the mode of transmission of the disease (person-to-person, arthropod-borne, food/water-borne, droplet/aerosol).

Results

Unadjusted and adjusted disease-specific completeness proportions for 2000–2006 with 95% CIs and UIs, respectively, are summarized in the Table. The adjusted disease-specific, completeness proportions ranged from 0% to 82.0%, and almost all diseases (49/53) had completeness proportions <50%. Eleven diseases accounted for 90% of disease reporting: salmonellosis, tuberculosis, meningococcal disease, Rocky Mountain spotted fever, campylobacteriosis, shigellosis, acute hepatitis A, pneumococcal meningitis, legionellosis, malaria, and *Haemophilus influenzae* invasive disease. Some unexpected diseases had cases identified with an ICD-9-CM code; for example, anthrax had 14 cases identified, paralytic polio had 32 cases identified, human rabies had 12 cases identified, and smallpox had 9 cases identified. The most dramatic adjustments in the unadjusted to adjusted point estimates were noted for staphylococcal foodborne disease, and for foodborne diseases caused by *Vibrio vulnificus* and other *Vibrio* spp., with an ≈80% change in point estimate for the latter. However, wide UIs reflect the imprecision in these estimates.

Figure 1 displays the overall reporting proportions by year for the 2 periods, 1995–1997, when only physicians were required to report most diseases, and 2000–2006, when laboratories and physicians were required to report. Reporting increased significantly in the second period, but was still low overall; the linear trend line slope was ≈0 and the intercept was 10.2%. Figure 2 displays the reporting proportions by health care system for the years 2000–2006. The completeness proportions ranged from 1.8% to 29.7% with an overall median proportion of 8.0%. The covariates that described whether or not each health care system designated persons to report had no effect on a health care system's reporting proportion.

The sensitivity analysis of the τ^2 values showed that the point estimates and UIs were relatively insensitive to dramatic changes in τ^2; for example, for meningococcal meningitis with a low τ^2, the reporting proportion was estimated as 21% (95% UI 16%–28%), with a medium τ^2, 22% (95% UI 16%–28%); and with a high τ^2, 22% (95% UI 16%–29%), and the sensitivity analyses examining the use of prior covariates were shown only to have effects on the reporting proportion and 95% UI for diseases with sparse data; for example, cholera with all prior covariates 22% (95% UI 3%–74%), no prior covariates 10% (95% UI 1%–51%), time covariate alone 50% (95% UI 10%–89%).

Discussion

The public health surveillance system in North Carolina is similar to surveillance systems used nationwide, and, although federal funding in addition to state and local budgets support the infrastructure and maintenance of these systems, they are rarely evaluated with respect to the completeness of the communicable disease data reported. North Carolina's size (ranked 11th in the 2000 US Census) and population diversity enabled a thorough evaluation of the completeness of reporting many reportable communicable diseases that have rarely been evaluated in previous studies.

Disease-specific reporting completeness proportions were estimated to be low and varied greatly according to disease. Notably, even for diseases that require immediate public health intervention, we found that a low proportion of cases were reported to the health department (e.g., meningococcal meningitis 21.2%, pertussis 20.3%). Further research studies should be undertaken to focus on methods to improve completeness and timeliness of case reporting, especially for these diseases that are severe and require immediate public health intervention.

Variations in disease reporting can occur for several reasons. First, clinicians may have the perception that some diseases are a greater public health threat based on communicability or severity of the illness and the likelihood of death (e.g., tuberculosis vs. salmonellosis). Second, some diseases have relatively straightforward and primarily laboratory-based case definitions (e.g., stool culture positive for *Salmonella* spp. infections with a clinically compatible illness), whereas others are more complex, either requiring multiple laboratory results (e.g., 4-fold increase between acute-phase and convalescent-phase serologic results for Rocky Mountain spotted fever) or a combination of multiple clinical signs and symptoms without any specific laboratory result (e.g., toxic shock syndrome which, requires the presence of at least 4 symptoms). One clear pattern that emerged in our findings was that diseases with fewer clini-

Table . Disease-specific reporting completeness proportions in North Carolina, USA, 2000–2006*

Communicable disease	No. reported to NC DHHS	No. identified by ICD-9-CM codes	Unadjusted RCP, % (95% CI)	Semi-Bayesian adjusted RCP, % (95% UI)
Anthrax	0	14	0.01 (0.00–100.00)	0.00 (0.00–100.00)
Arboviral encephalitis	0	18	0.00 (0.00–100.00)	8.67 (0.80–52.77)
Botulism	0	4	0.02 (0.00–100.00)	0.08 (0.00–100.00)
Brucellosis	0	33	0.00 (0.00–100.00)	23.02 (1.36–86.62)
Campylobacteriosis	39	97	40.21 (30.94–50.22)	39.96 (30.82–49.85)
Cholera	0	6	0.01 (0.00–100.00)	18.58 (2.24–69.41)
CJD/vCJD	0	32	0.00 (0.00–100.00)	0.87 (0.03–22.97)
Cryptosporidiosis	10	84	11.90 (6.53–20.73)	12.59 (7.07–21.42)
Cyclosporiasis	0	3	0.03 (0.00–100.00)	18.59 (2.25–69.42)
Dengue	4	25	16.00 (6.14–35.69)	14.48 (5.92–31.31)
Diphtheria	0	5	0.02 (0.00–100.00)	8.28 (0.82–49.70)
Escherichia coli infection	1	3	33.33 (4.34–84.65)	24.67 (5.82–63.45)
Foodborne staphylococcal infection	0	14	0.01 (0.00–100.00)	74.74 (16.74–97.76)
Granulocytic ehrlichiosis	0	67	0.00 (0.00–100.00)	8.66 (0.80–52.74)
Hantavirus infection	0	3	0.03 (0.00–100.00)	10.10 (0.62–67.06)
Hemolytic uremic syndrome	5	429	1.17 (0.49–2.77)	2.20 (0.99–4.84)
Hemophilus Influenzae	14	1,086	1.29 (0.76–2.16)	1.45 (0.87–2.42)
Hepatitis A	27	866	3.12 (2.15–4.51)	3.34 (2.31–4.81)
Legionellosis	24	98	24.49 (16.99–33.95)	24.04 (16.72–33.27)
Leptospirosis	0	33	0.00 (0.00–100.00)	23.02 (1.36–86.62)
Listeriosis	10	64	15.63 (8.62–26.67)	16.14 (9.12–26.95)
Lyme disease	8	790	1.01 (0.51–2.01)	1.18 (0.60–2.30)
Malaria	17	155	10.97 (6.93–16.94)	10.71 (6.80–16.47)
Measles	0	14	0.01 (0.00–100.00)	15.98 (1.41–71.63)
Meningococcal disease	38	179	21.23 (15.85–27.83)	21.19 (15.85–27.73)
Monocytic ehrlichiosis	1	4	25.00 (3.35–76.22)	14.84 (3.12–48.52)
Mumps	1	96	1.04 (0.15–7.02)	1.07 (0.20–5.49)
Plague	0	28	0.00 (0.00–100.00)	0.00 (0.00–100.00)
Pneumococcal meningitis	20	191	10.47 (6.86–15.67)	10.61 (6.99–15.80)
Polio, paralytic	0	32	0.00 (0.00–100.00)	18.56 (2.24–69.38)
Psittacosis	0	21	0.00 (0.00–100.00)	17.45 (1.57–73.69)
Q fever	3	14	21.43 (7.07–49.43)	25.68 (9.14–54.28)
Rabies, human	0	12	0.01 (0.00–100.00)	59.69 (8.00–96.19)
Rocky Mountain spotted fever	40	986	4.06 (2.99–5.48)	4.19 (3.10–5.66)
Rubella	0	39	0.00 (0.00–100.00)	15.97 (1.41–71.61)
Rubella congenital syndrome	0	10	0.01 (0.00–100.00)	1.08 (0.07–15.32)
Salmonellosis	263	594	44.28 (40.33–48.30)	44.82 (40.87–48.83)
SARS (coronavirus infection)	0	1	0.08 (0.00–100.00)	5.71 (0.28–56.27)
Shigellosis	38	213	17.84 (13.26–23.57)	18.17 (13.56–23.93)
Smallpox	0	9	0.01 (0.00–100.00)	0.00 (0.00–100.00)
Streptococcal infection, group A	8	111	7.21 (3.65–13.75)	7.40 (3.80–13.92)
Tetanus	1	20	5.00 (0.70–28.22)	5.25 (1.09–21.78)
Toxic shock syndrome	4	142	2.82 (1.06–7.26)	3.22 (1.28–7.83)
Trichinosis	0	23	0.00 (0.00–100.00)	20.21 (1.82–77.58)
Tuberculosis	100	1,439	6.95 (5.74–8.38)	7.10 (5.87–8.55)
Tularemia	0	6	0.01 (0.00–100.00)	0.04 (0.00–100.00)
Typhoid, acute	3	12	25.00 (8.28–55.18)	21.57 (7.49–48.30)
Typhus, epidemic (louse-borne)	0	2	0.04 (0.00–100.00)	2.93 (0.12–42.63)
Vaccinia	0	13	0.01 (0.00–100.00)	8.27 (0.82–49.68)
Vibrio spp. infection, other	0	1	0.08 (0.00–100.00)	81.58 (20.46–98.71)
Vibrio vulnificus infection	0	2	0.04 (0.00–100.00)	81.57 (20.45–98.71)
Whooping cough (pertussis)	11	54	20.37 (11.65–33.16)	20.31 (11.78–32.72)
Yellow fever	0	3	0.03 (0.00–100.00)	8.69 (0.80–52.81)

*NC DHHS, North Carolina Department of Health and Human Services; ICD-9-CM, International Classification of Diseases, 9th Revision, Clinical Modification; RCP, reporting completeness proportions; CI, confidence interval; UI, uncertainty interval; CJD, Creutzfeldt-Jakob disease; vCJD, variant CJD; SARS, severe acute respiratory syndrome.

cal criteria and laboratory-based case definitions tended to have higher reporting rates (e.g., salmonellosis 44.8% vs. toxic shock syndrome 3.2%). Laboratory-based case definitions ensure that a dual reporting system exists, and the process is more straightforward because less time is required for reviewing medical records for clinical signs and symptoms. This finding underscores the need for simplicity of case definitions, an essential attribute in surveillance system development and maintenance. Future research on predictors for reporting completeness would be useful for designing interventions to improve reporting and for guiding the future direction of surveillance.

Notably, we identified some patients by ICD-9-CM diagnostic codes for some diseases known to be eliminated in the United States (e.g., smallpox and polio) and others that were highly unlikely to have occurred (e.g., anthrax and human rabies). Numerous previous studies that have evaluated reporting completeness have also used ICD-9-CM codes (*5*,*6*,*11*) because they are standard codes that can be queried relatively easily and should capture clinical cases of disease regardless of laboratory confirmation. The accuracy of the ICD-9-CM codes was a potential limitation in our study. Therefore, we also conducted a separate validation study of the positive predictive values of ICD-9-CM codes for communicable disease surveillance by using as the standard a complete medical record review and concordance with published CDC case classification criteria (*12*). These results showed that for most diseases with higher incidence and relatively straightforward diagnoses, the positive predictive values (PPVs) were high (>80%) with the exception of tuberculosis, which had a PPV of 29% (*13*). For diseases with low PPVs, the estimates we present here are likely to be underestimates of the true reporting completeness because the completeness proportion denominator, or the number of patients identified by ICD-9-CM codes for reportable diseases, is likely to be an overestimate (i.e., contain false-positive cases). However, an additional limitation of this study was that we were unable to assess the sensitivity of ICD-9-CM codes (i.e., false-negative cases) for communicable disease reporting. Quantification of the sensitivity and PPVs of ICD-9-CM codes for communicable disease surveillance is essential in the interpretation of all ICD-9-CM data because these codes are used frequently for research studies and have been proposed as adjuncts to electronic, automated surveillance systems.

Bayesian analyses have been shown in theory, simulation, and prediction problems to offer better estimates for measures as varied as baseball batting averages (*14*) and toxoplasmosis prevalence (*15*). We believe that the semi-Bayesian adjusted estimates offer improved overall accuracy for our ensemble of reporting completeness estimates. For example, for completeness proportions where the maximum-likelihood estimation methods result in 0% proportions, it is unlikely that the true proportion is actually 0%. The use of semi-Bayesian methods enables us to incorporate additional prior covariate data to produce results that are likely better and more plausible than maximum-likelihood estimation results. However, for estimates that were based on less information, we still observed wide UIs around the adjusted estimates. Specifically, we did note a dramatic shift in the reporting completeness proportions after semi-Bayesian adjustments for several diseases, including staphylococcal foodborne disease and *V. vulnificus* and other *Vibrio* spp. infections. This shift reflects the imprecision in each disease's measured estimates of reporting completeness and the adjustment or shrinkage of their proportions to the mean of the prior covariate probability groups (i.e., food/water-borne transmission, and reporting time of 24 hours). These estimates are shrunk toward the mean of the food/water-borne transmission group of diseases which includes many of those with the highest reporting proportion (e.g., campylobacteriosis, salmonellosis). This finding reinforces the importance of careful specification of prior covariates as well as judicious examination and interpretation of the unadjusted and semi-Bayesian adjusted estimates along with their precision.

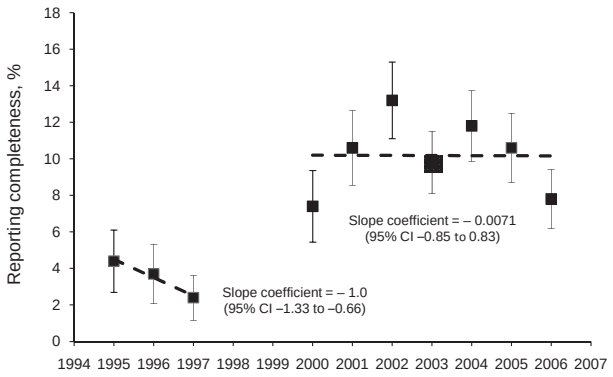

Figure 1. Reporting completeness of communicable diseases in North Carolina, USA, by year, with 95% confidence intervals, 2000–2006.

The reporting variation seen among health care systems (Figure 2) may be explained in part by health care systems' internal policies that assign the responsibility for communicable disease reporting to the infection prevention department. For example, the health care system with the highest reporting proportion (health care system A) has hospital-based public health epidemiologists or infection preventionists responsible for disease reporting, and the health care system with the lowest reporting proportion (health care system G) does not assign any additional reporting responsibility beyond the state-mandated reporting by physicians and laboratories. However, adjusting for these

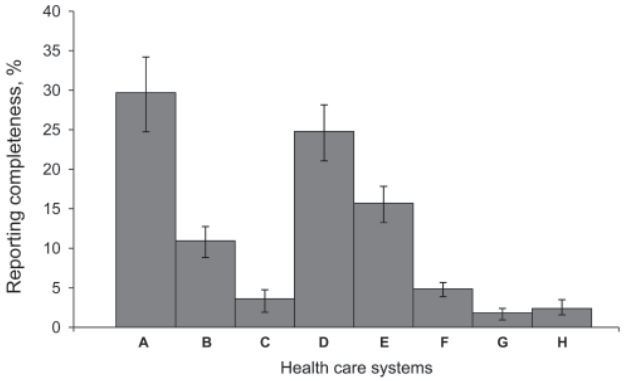

Figure 2. Reporting completeness of communicable diseases in North Carolina, USA, by health care system, 2000–2006. Error bars indicate 95% confidence intervals.

health care system policies did not modify the reporting completeness proportions. Currently, the North Carolina general statute states that medical facilities may report (*16*) as opposed to physicians and persons in charge of laboratories who shall report (*17*,*18*). Because infection preventionists typically receive laboratory data daily, are well-trained on case definition application, and share disease prevention goals with the health department, they can serve as partners to the local health department in ensuring that diseases are reported and investigated appropriately. However, redundancy in disease reporting responsibilities could also cause reporting fatigue and the mistaken assumption that someone else has reported the case-patient (*19*,*20*). In addition, external generalizations of these findings to other health care systems should be approached with caution because the participating sites were part of an existing network that includes the largest health care systems in North Carolina and therefore may have been more likely to treat patients who had more severe illnesses or who did not receive a diagnosis at a local clinic or smaller hospital.

Conclusions

The general trend of the yearly reporting completeness proportions suggests that disease reporting has improved over time yet remains low. Several notable changes occurred in North Carolina's surveillance system during this period. First, in 1998, the inclusion of laboratory-mandated reporting served as a secondary reporting mechanism in addition to the already mandated physician-based reporting. Regional public health teams were established in 2002 to assist health departments with outbreak investigations. In 2003, a network of public health epidemiologists (funded through the state's Public Health Emergency Preparedness cooperative agreement with CDC) were placed in hospitals to facilitate disease reporting and case investigation, and, also in 2003, a statewide emergency department–based syndromic surveillance system (North Carolina Disease Event Tracking and Epidemiologic Collection Tool) was created for early case identification. Despite the likely positive effects of these regulatory and programmatic changes on disease reporting, the proportion of diseases reported remains low, as is consistent with data from other passive reporting surveillance systems (*21*).

More recently, automated alerting and data collection for case-patients with reportable diseases (e.g., a positive blood culture result with gram-negative diplococci triggers an alert with case-patient contact information to infection preventionists, local health department staff, or both) has been shown to increase reporting rates when applied to traditional passive surveillance systems (*22*,*23*). Although North Carolina, like many states, has developed and implemented an electronic disease surveillance system, the reporting of communicable diseases by local health departments still remains largely passive in that reporting is accomplished by accessing a secure Internet site and entering patient information. Physicians who practice outside local health departments currently use paper-based reporting.

When health information exchange becomes a reality, public health surveillance can benefit significantly by automating processes that currently rely on manual data entry. Disease reporting could be automated by standardized queries directly from the electronic health records for key laboratory results (e.g., positive acid-fast bacillus sputum smear) and for simplified or proxy clinical case definitions by using ICD-9-CM diagnosis codes or free-text admission diagnoses. Upon recognition of these potential case-patients, automating surveillance data collection directly from electronic health records to populate data fields for basic patient demographics and laboratory results could also reduce administrative time for physicians and health department officials and expedite communicable disease investigations.

This type of automated technology for electronic health records is consistent with The American Recovery and Reinvestment Act of 2009, which authorizes the Centers for Medicare and Medicaid Services to provide reimbursement incentives for health care entities who are "meaningful users" of certified electronic health record technology. In fact, the recent draft recommendations for defining "meaningful use" from the Health Information Technology Policy Council to the National Coordinator propose that hospitals be capable of providing electronic submission of reportable laboratory results to public health agencies by 2011 (*24*). Such an undertaking will require implementation of national laboratory reporting standards for hospitals and can only be accomplished with resource allocation and partnerships between health departments and health care systems. Furthermore, additional surveillance research should investigate the sensitivity, specific-

ity, and feasibility of using different key laboratory results and proxy clinical case definitions (e.g., ICD-9-CM codes) for automating the identification of potential case-patients. The "meaningful use" of the electronic health record for automated case-finding and data collection will transition our current public health surveillance system from passive to active and thereby overcome the major barriers to complete, accurate and timely communicable disease reporting and surveillance.

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References

1. Thacker SB, Berkelman RL. Public health surveillance in the United States. Epidemiol Rev. 1988;10:164–90.
2. Reportable Diseases. North Carolina Administrative Code. Vol 10A NCAC 41A.0101.
3. Method of Reporting. North Carolina Administrative Code. Vol 10A NCAC 41A.102.
4. 130A–134 North Carolina General Statutes. Vol 130A–134.
5. Marier R. The reporting of communicable diseases. Am J Epidemiol. 1977;105:587–90.
6. Campos-Outcalt D, England R, Porter B. Reporting of communicable diseases by university physicians. Public Health Rep. 1991;106:579–83.
7. American Hospital Association. American Hospital Association guide: America's directory of hospitals and health care systems. Chicago: Health Forum; 2007.
8. Sweeting MJ, Sutton AJ, Lambert PC. What to add to nothing? Use and avoidance of continuity corrections in meta-analysis of sparse data. Stat Med. 2004;23:1351–75. DOI: 10.1002/sim.1761
9. Witte JS, Greenland S, Kim LL. Software for hierarchical modeling of epidemiologic data. Epidemiology. 1998;9:563–6. DOI: 10.1097/00001648-199809000-00016
10. Greenland S, Robins JM. Empirical-Bayes adjustments for multiple comparisons are sometimes useful. Epidemiology. 1991;2:244–51. DOI: 10.1097/00001648-199107000-00002
11. Finger R, Auslander MB. Results of a search for missed cases of reportable communicable diseases using hospital discharge data. J Ky Med Assoc. 1997;95:237–9.
12. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. MMWR Recomm Rep. 1997;46(RR-10):1–55.
13. Sickbert-Bennett EE, Weber DJ, Poole C, MacDonald PDM, Maillard J-M. Utility of ICD-9-CM codes for communicable disease surveillance. Am J Epidemiol. 2010 Sep 28; [Epub ahead of print].
14. Efron B, Morris C. Stein's Paradox in statistics. Sci Am. 1977;236:119–27. DOI: 10.1038/scientificamerican0577-119
15. Greenland S, Robins JM. Empirical-Bayes adjustments for multiple comparisons are sometimes useful. Epidemiology. 1991;2:244–51. DOI: 10.1097/00001648-199107000-00002
16. 130A–137 North Carolina General Statutes. Vol 130A–137.
17. 130A–135 North Carolina General Statutes. Vol 130A–135.
18. 130A–139 North Carolina General Statutes. Vol 130A–139.
19. Ktsanes VK, Lawrence DW, Kelso K, McFarland L. Survey of Louisiana physicians on communicable disease reporting. J La State Med Soc. 1991;143:27–8, 30–1.
20. Schramm MM, Vogt RL, Mamolen M. The surveillance of communicable disease in Vermont: who reports? Public Health Rep. 1991;106:95–7.
21. Henderson DA. Surveillance of smallpox. Int J Epidemiol. 1976;5:19–28.
22. Hoffman MA, Wilkinson TH, Bush A, Myers W, Griffin RG, Hoff GL, et al. Multijurisdictional approach to biosurveillance, Kansas City. Emerg Infect Dis. 2003;9:1281–6.
23. Effler P, Ching-Lee M, Bogard A, Ieong MC, Nekomoto T, Jernigan D. Statewide system of electronic notifiable disease reporting from clinical laboratories: comparing automated reporting with conventional methods. JAMA. 1999;282:1845–50. DOI: 10.1001/jama.282.19.1845
24. US Department of Health and Human Services. Health Information Technology. Meaningful use. 2009 [cited 2009 Nov 19]. http://healthit.hhs.gov/portal/server.pt?open=512&objID=1325&parentname=Community Page&parentid=1&mode=2

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Hepatitis E Virus Infection without Reactivation in Solid-Organ Transplant Recipients, France

Florence Legrand-Abravanel, Nassim Kamar, Karine Sandres-Saune, Sebastien Lhomme, Jean-Michel Mansuy, Fabrice Muscari, Federico Sallusto, Lionel Rostaing, and Jacques Izopet

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Learning Objectives

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

- Describe factors associated with higher risk for HEV seroconversion and development of chronic disease among liver and kidney transplant patients.

Editor

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Infections with hepatitis E virus (HEV) in solid-organ transplant recipients can lead to chronic hepatitis. However, the incidence of de novo HEV infections after transplantation and risk for reactivation in patients with antibodies against HEV before transplantation are unknown. Pretransplant prevalence of these antibodies in 700 solid-organ transplant recipients at Toulouse University Hospital in France was 14.1%. We found no HEV reactivation among patients with antibodies against HEV at the first annual checkup or by measuring liver enzyme activities and HEV RNA. In contrast, we found 34 locally acquired HEV infections among patients with no antibodies against HEV, 47% of whom had a chronic infection, resulting in an incidence of 3.2/100 person-years. Independent risk factors for HEV infection were an age <52 years at transplantation and receiving a liver transplant. Effective prophylactic measures that include those for potential zoonotic infections should reduce the risk for HEV transmission in this population.

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Large epidemics of hepatitis E have occurred in Asia, Africa, and Latin America where sanitation is suboptimal (*1*). The numbers of non–travel-associated hepatitis E

virus (HEV) infections have also increased in industrialized countries in recent years, and these infections are now considered an emerging infectious disease in Western countries (*2*). HEV belongs to the family *Hepeviridae*. At least 4 major genotypes of HEV have been recognized. Genotypes 1 and 2 are restricted to humans and associated with epidemics in developing countries, and genotypes 3 and 4 are zoonotic and infect humans and several other animals in developing and industrialized countries (*3*). The discovery of animal strains of HEV in pigs, wild boars, deer, and rodents and the finding of other animal species with antibodies against HEV has broadened the host range and diversity of HEV (*3*).

Although HEV is transmitted mainly by the fecal–oral route in developing countries, there are other routes in industrialized countries. These routes include contact with animal reservoirs (*4*,*5*) and consumption of undercooked pig, deer, or wild boar meat (*6*–*8*). Nosocomial infection and transfusion-transmitted infection have also been reported (*9*–*11*). HEV infection generally has a self-limiting symptomatic course or an asymptomatic course that includes acute hepatitis. Fulminant hepatitis may occur in pregnant women and persons with underlying liver disease (*1*,*12*,*13*). HEV can lead to chronic infection in solid-organ transplant (SOT) patients (*14*–*16*), in patients who have had chemotherapy (*11*,*17*,*18*), and in patients infected with HIV (*19*,*20*). HEV infection can evolve to chronic hepatitis in up to 60% of infected SOT patients (*16*).

Reactivation of an HEV infection was reported recently in a patient in Germany who had acute lymphoblastic leukemia after allogeneic stem cell transplantation (*21*). This reactivation was diagnosed as acute limited hepatitis E before stem cell transplantation. HEV viremia reappeared 14 weeks after stem cell transplantation with increased aminotransferase activity. Analysis of virus RNA sequences showed reactivation of endogenous HEV genotype 3, which indicated that the virus had persisted despite the recovery of the patient from acute hepatitis E (*21*).

Southwestern France is characterized by a high prevalence of antibodies against HEV in blood donors (*22*), and several cases of HEV infections have been reported in immunocompetent and immunocompromised patients (*23*,*24*). However, the incidence and risk for reactivation of HEV infection in SOT patients in this area are unknown. To identify these factors, we examined a large cohort of immunocompromised patients at Toulouse University Hospital, France, during January 2004–December 2009 to determine the prevalence of antibodies against HEV in SOT recipients before transplantation, the frequency of HEV reactivation, the incidence of HEV infections relative to the organ transplanted, and the risk for HEV transmission from the organ donor or from a blood transfusion.

Patients and Methods

Patients

A total of 808 adult patients underwent transplantation of a kidney or liver during January 2004–December 2008 at Toulouse University Hospital. All adult patients for whom a blood sample obtained on the day of transplantation was available were included in the study (n = 700, 529 kidney transplant recipients and 171 liver transplant recipients). Serum samples obtained on the day of transplantation and during annual checkups until December 2009 were tested for immunoglobulin (Ig) M and IgG against HEV. Positive samples were tested for HEV RNA.

Patients with pretransplant antibodies against HEV were investigated for HEV reactivation by testing for HEV RNA at the first annual checkup and for increased liver enzyme activities during follow-up. Patients without pretransplant antibodies who underwent IgG seroconversion and patients who had unexplained acutely increased liver enzyme activities during follow-up were also tested for HEV RNA. Patients whose test results were antibody negative before transplantation were monitored from the date of transplantation until the date of IgG seroconversion or HEV infection, death, last health care encounter, or study end (December 31, 2009).

Frozen serum samples from donors (obtained at Toulouse University Hospital) who had donated an organ to an HEV-infected recipient were also tested for IgG and IgM against HEV and HEV RNA to determine whether transmission had occurred by organ transplantation. Similarly, frozen serum samples from donors who gave blood to an HEV-infected recipient were also tested for IgG and IgM against HEV and HEV RNA to determine whether HEV infection had occurred by blood transfusion.

Case Definitions

Patients who had IgG, IgM, or both against HEV but did not have HEV RNA were considered uninfected. Patients who were negative for antibodies against HEV at transplantation and who showed HEV IgG seroconversion during follow-up were considered to have a de novo HEV infection. Patients who were positive for HEV RNA were also considered to have an HEV infection that was independent of IgG or IgM against HEV.

Laboratory Investigation for HEV Infection

We detected IgM and IgG against HEV by using EIAgen HEV IgG and EIAgen HEV IgM Kits (Adaltis Ingen, Paris, France). Ratios of antibodies against HEV were sample optical densities/cutoff optical densities. A result was considered positive if the sample ratio was ≥1. We used real-time PCRs and a hydrolysis probe to detect HEV RNA (*24*,*25*). Virus genotype was determined by sequencing

a 189-nt fragment within the open reading frame 2 gene (*26*). Phylogenetic analyses were performed by using genotype information of reference sequences based on the HEV classification proposed by Lu et al. (*27*).

Statistical Analysis

Analyses were performed by using Stata version 9.2 (StataCorp LP, College Station, TX, USA). The χ^2 test or Fisher exact test was used to compare proportions. Student *t* test was used to compare continuous variables. A p value <0.05 was considered significant.

Demographic and clinical factors associated with pretransplant antibodies against HEV were evaluated by using univariate analyses. We analyzed sex, age at transplantation (<52 years or ≥52 years), place of residence on the basis of community size (<20,000 or ≥20,000 inhabitants), type and cause of transplantation, and immunosuppressive therapy at discharge (each therapy or triple and double immunosuppressive regimens). Variables with a p value ≤0.10 by univariate analysis were entered into a multivariate, backward, stepwise logistic regression analysis to identify variables independently associated with pretransplant HEV seroprevalence.

Incidence of HEV infection and 95% confidence intervals (CIs) among SOT patients negative for antibodies against HEV was calculated. For the purpose of estimating the date of HEV infection for patients with HEV IgG seroconversion, we used the date midway between the last available seronegative test result and the first available seropostive test result. Demographic and clinical risk factors were entered into a Poisson regression to assess factors associated with de novo HEV infection. Risks are expressed as incidence rate ratios and their 95% CIs.

Results

Pretransplant HEV Seroprevalence

Blood samples from 700 SOT recipients were screened on the day of transplantation. Median age of patients at transplantation was 52 years (range 18–79 years); 459 (65.6%) of the patients were men (Table 1). No demographic or clinical factors were associated with pretransplant antibod-

Table 1. Characteristics of 700 solid-organ transplant recipients and seroprevalence of antibodies against HEV before transplant, France, January 2004–December 2008*

Characteristic	No. patients	HEV IgG positive, no. (%)	HEV IgM positive, no. (%)
Sex			
M	459	57 (12.4)	8 (1.7)
F	241	32 (13.2)	9 (3.7)
Age at transplantation, y			
<52	347	43 (12.4)	8 (2.3)
≥52	363	46 (13.0)	9 (2.5)
Transplant type			
Liver	171	21 (12.3)	4 (2.3)
Kidney	529	68 (12.8)	13 (2.4)
Cause of kidney transplantation			
Glomerulonephritis	188	27 (14.3)	4 (2.1)
Genetic nephritis	102	15 (14.7)	1 (0.9)
Pyelonephritis or interstitial nephritis	92	7 (7.6)	2 (2.1)
Nephroangiosclerosis	82	9 (10.9)	2 (2.4)
Other	57	10 (17.5)	4 (7)
Cause of liver transplantation			
Alcoholic cirrhosis	67	6 (8.9)	0
Hepatitis B or C	60	7 (11.6)	2 (3.3)
Autoimmune cirrhosis	9	2 (22)	1 (11)
Other	35	6 (17.1)	1 (2.8)
Hepatocarcinoma (yes/no)	64/107	9 (14)	2 (3.1)
Induction treatment (yes/no)	501/199	73 (14.5)	14 (2.8)
Interleukin-2 receptor blockers	292	40 (7.3)	4 (1.3)
Rabbit antithymocyte globulins	160	25 (15.6)	8 (5)
Immunosuppressive therapy at discharge			
Belatacept	49	8 (16.3)	2 (16.3)
Cyclosporine A	182	27 (15.3)	1 (0.5)
Tacrolimus	442	52 (11.7)	14 (3.1)
Steroids	657	85 (12.9)	17 (2.5)
Mycophenolate	593	74 (12.5)	15 (2.6)
Azathioprine	2	0	0

*HEV, hepatitis E virus; Ig, immunoglobulin.

ies against HEV. IgG, IgM, or both antibodies against HEV were found in 99 patients (14.1%): 77 of the 529 kidney-transplant patients (14.5%) and 22 of the 171 liver-transplant patients (12.9%) (Figure 1). Serum from 17 patients (2.4%) contained HEV IgM (7 of these patients were also IgG positive). None of the 99 patients who had antibodies against HEV had HEV RNA. Pretransplant seroprevalence varied according to year of transplantation (range 8.7%–16.3%), although these variations were not significant.

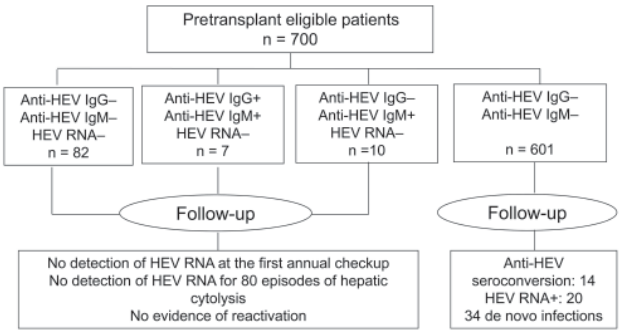

Figure 1. Hepatitis E virus (HEV) markers in 700 solid-organ transplant recipients, France, January 2004–December 2008. Ig, immunoglobulin.

HEV Reactivation in Pretransplant Seropositive Patients

We determined HEV reactivation in 99 pretransplant HEV seropositive patients whose blood samples obtained at the first annual checkup were negative for HEV RNA (Figure 1). All blood samples were negative for HEV RNA. We also checked clinical records to identify any episodes of hepatic cytolysis. Liver enzyme activities of 38 patients were not increased during follow-up, but there were 80 episodes of hepatic cytolysis in the other 61 patients. None of the blood samples obtained during episodes of hepatic cytolysis was positive for HEV RNA.

The 89 pretransplant patients who were positive for HEV IgG included 84 patients who underwent a serologic test after a median of 24 months (interquartile range [IQR] 14–36 months). Thirty-two (35.9%) patients who were positive for HEV IgG became negative. Disappearance of antibodies against HEV was not associated with type of immunosuppressive therapy at discharge. The 17 pretransplant HEV IgM–positive patients included 16 patients who underwent a serologic test after a median of 16 months (IQR 12–23 months). Five of the 16 patients were still positive for HEV IgM but negative for HEV RNA at this time.

Incidence of HEV Infection

The 601 pretransplant HEV IgG– and HEV IgM–negative patients included 452 kidney transplant recipients and 149 liver transplant recipients. Of these patients, 76 kidney transplant recipients and 26 liver transplant recipients were lost to follow-up. Median follow-up time was 22 months (IQR 12–36 months); total follow-up time was 1,064 person-years after transplantation. A total of 34 HEV infections were identified: 22 were in kidney transplant recipients and 12 were in liver transplant recipients (Figure 1). Median interval between transplantation and de novo HEV infections was 15 months (IQR 6–30 months).

We diagnosed HEV infections in 20 patients with increased liver enzyme activities by detecting HEV RNA; HEV infections in 14 other patients were diagnosed by detection of HEV IgG during posttransplant follow-up. Nine of these 14 patients also had HEV IgM (Figure 2). Investigation of clinical records led to detection of HEV RNA–positive samples in 4 of these patients by retrospective analysis of frozen stored blood samples. HEV RNA was not detected in the other 10 patients because no samples were available when alanine aminotransferase levels peaked.

Incidence of HEV infection was 3.2 cases/100 person-years (95% CI 2.06–4.13 cases). The incidence in liver transplant recipients (4.8 cases/100 person-years; 95% CI 2.2–7.4 cases) tended to be higher than in kidney transplant recipients (2.7 cases/100 person-years; 95% CI 1.52–3.68 cases; p = 0.09). Incidence rates for each year after transplantation (Table 2) ranged from 0.9 to 4.3 cases/100 person-years in kidney transplant recipients and from 0 to 7.1 cases/100 person-years in liver transplant recipients, but the differences were not significant. The incidence according to calendar year of transplantation varied from 1.2 to 4.3 cases/100 person-years in kidney transplant recipients and from 2.5 to 9.1 cases/100 person-years in liver transplant recipients. Incidence of HEV infection according to calendar year of transplantation did not differ significantly over the 5-year study period.

HEV Transmission by Organ Transplant or Blood Transfusion

We studied the risk for HEV transmission by organ transplant by identifying virologic markers in 15 persons who provided organs for 15 HEV-infected SOT recipients (44%). Eight recipients were infected with HEV in the first year posttransplantation, 3 were HEV positive 1–2 years posttransplantation, 3 were HEV positive 2–3 years posttransplantation, and 1 was HEV positive >4 years after transplantation. Of the 15 organ donors, 14 were negative for HEV IgG and HEV IgM, and 1 was negative for HEV IgG and positive for HEV IgM. All 15 organ donors were negative for HEV RNA.

We determined how many of the 34 HEV-infected patients had required a blood transfusion during the 3-month period before onset of hepatitis or during the year before

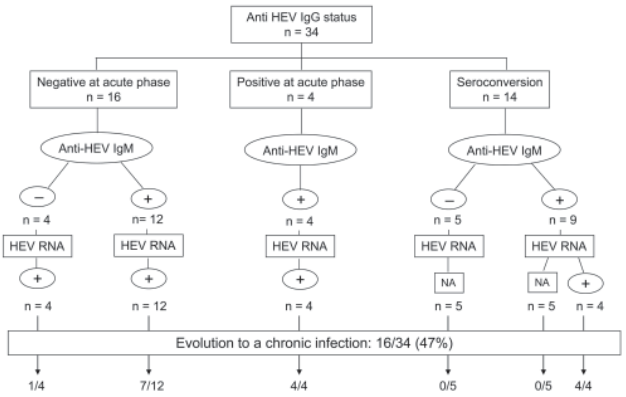

Figure 2. Diagnosis and characteristics of 34 patients with hepatitis E virus (HEV) infection, France, January 2004–December 2008. Ig, immunoglobulin; NA, not available.

HEV seroconversion. Only 2 liver transplant recipients (17%) were given transfusions of concentrated erythrocytes (one 2 months before and one 3 weeks before infection with HEV was diagnosed). All 12 donors of these blood transfusions were negative for HEV RNA. Eleven of them were negative for HEV IgG and HEV IgM, and 1 was positive for HEV IgG and negative for HEV IgM.

Description of HEV Case-Patients

We identified 34 patients with HEV infection (9 women and 25 men, mean ± SD age at diagnosis 46 ± 13 years). HEV genotypes for 21 patients were identified: 15 genotype 3f strains, 5 genotype 3c strains, and 1 genotype 3 strain with an undetermined subtype. Infections became chronic in 16 patients (47%), which was defined as HEV RNA in plasma for >6 months. None of the 18 patients who cleared virus after the acute phase showed any reactivation or reinfection during follow-up. Multivariate analysis showed that the independent risk factors associated with HEV infection (Table 3) were a younger age at transplantation (<52 years) and having a liver transplant.

Discussion

We followed up 700 patients who had received a kidney or liver transplant during 2004–2008. The incidence of de novo HEV infection was high (3.2 cases/100 person-years), but no infections were reactivated in this population.

The pretransplant HEV seroprevalence (14.7%) was similar to that of blood donors in the same area (16.6%) (*22*), but higher than in liver transplant recipients in the Netherlands (2.1%) (*28*), Germany (4%) (*29*), and Spain (2.7%) (*30*). Seven patients had HEV IgG and IgM at transplantation, but none had HEV RNA. These results suggest that HEV infection occurred shortly before transplantation. Ten patients were positive for HEV IgM but negative for HEV IgG and HEV RNA. None of the 10 patients showed delayed seroconversion for IgG during follow-up. Although the Adaltis EIAgen anti-HEV IgM Kit is highly specific (*31*), a false-positive result cannot be excluded. However, prolonged persistence of HEV IgM for >1 year after resolution of HEV infection has been reported (*32*). Because the anti-HEV IgG test we used could be relatively insensitive (*33*,*34*), absence of IgG seroconversion is compatible with a recent infection.

A recent study reported HEV reactivation in a patient with acute lymphoblastic leukemia after allogeneic stem cell transplantation (*21*). HEV may persist in hepatocytes or macrophages, as do other RNA viruses (*35*,*36*). We therefore investigated the possibility of reactivation in our patients who had antibodies against HEV but no HEV RNA at transplantation. We tested for HEV RNA at the first annual checkup and whether liver enzyme activities were increased during follow-up. We found no HEV infection in these patients. In addition, 32 (35.9%) of these patients no longer had HEV IgG after transplantation. The delay in disappearance of HEV IgG is poorly documented in immunocompetent or immunocompromised patients. The sensitivity of the IgG test may influence this delay, as recently documented by Bendall et al. in immunocompetent patients (*33*). This disappearance of antibodies against HEV may be caused by immunosuppressive treatment. Mycophenolic acid and rapamycin can decrease antibody synthesis (*37*,*38*), but we found no association between use of mycophenolic acid and loss of antibodies against HEV.

The incidence of HEV infection after transplantation was high; we identified 34 patients with HEV infection. Most patients were infected with HEV genotype 3f, which is the most prevalent genotype in the study area (*26*). Incidence rates for each year after transplantation were similar. Likewise, incidence of HEV infection in SOT patients did not vary according to year of transplantation. This finding is consistent with that of a study in immunocompetent

Table 2. Incidence of HEV per year after transplantation in 601 solid-organ transplant recipients, France, January 2004–December 2008*

Patient group	Incidence, cases/100 person-years (95% confidence interval)					p value
	Global	First year	Second year	Third year	Fourth year	
All	3.2 (2.06–4.13)	3.5 (1.9–5.1)	1.1 (0.03–2.19)	3.7 (1.09–6.1)	3.0 (0.1–7.52)	0.08
Kidney transplant	2.7 (1.52–3.68)	2.4 (0.8–3.9)	0.9 (0.01–4.4)	3.3 (0.47–6.120)	4.3 (1.28–11.48)	0.18
Liver transplant	4.8 (2.2–7.4)	7.12 (2.63–11.62)	1.6 (0.04–7.60)	5.1 (0.52–14.54)	0 (0–10)	0.24

*HEV, hepatitis E virus.

Table 3. Analysis of risk factors for acquiring HEV infection in 601 solid-organ transplant recipients, France, January 2004–December 2008*

Characteristic	Univariate analysis		Multivariate analysis	
	Relative risk (95% CI)	p value	Relative risk (95% CI)	p value
Male sex	1.56 (0.73–3.35)	0.24	–	–
Age at transplantation <52 y	2.58 (1.20–5.53)	0.01	2.8 (1.3–6.0)	0.008
Living in rural area (<20,000 inhabitants)	0.6 (0.28–1.32)	0.21		
Having a liver transplant	1.78 (0.88–3.60)	0.10	2.03 (1–4.13)	0.05
Indication of liver transplantation				
Alcohol	0.95 (0.29–3.10)	0.94	–	–
Hepatitis B or C	1.34 (0.47–3.80)	0.58	–	–
Autoimmune	3.1 (0.75–13.15)	0.11	–	–
Other causes	2.52 (0.77–8.24)	0.12	–	–
Indication of kidney transplantation				
Glomerulonephritis	0.49 (0.19–1.28)	0.15	–	–
Genetic nephritis	1.06 (0.37–3.02)	0.90	–	–
Pyelonephritis or interstitial nephritis	0.20 (0.02–1.52)	0.13	–	–
Nephroangiosclerosis	1.45 (0.55–3.68)	0.46	–	–
Other causes	2.52 (0.77–8.24)	0.12	–	–
Induction therapy				
Yes	0.83 (0.39–1.74)	0.62	–	–
ATG	1.19 (0.53–2.65)	0.66	–	–
Anti-IL2R	0.95 (0.49–1.94)	0.95	–	–
Immunosuppressive therapy at discharge				
Belatacept	0.49 (0.06–3.60)	0.48	–	–
Cyclosporine A	0.72 (033–1.55)	0.41	–	–
Tacrolimus	1.49 (0.73–3.07)	0.27	–	–
Steroids	0.74 (0.17–3.11)	0.68	–	–
Mycophenolate	0.84 (0.34–2.02)	0.69	–	–
Azathioprine	0	1		
Double regimen	1.07 (0.50–2.30)	0.85	–	–
Triple regimen	0.99 (0.46–2.13)	0.99		

*HEV, hepatitis E virus: CI, confidence interval; –, not significant; ATG, antithymoglobulin; anti-IL2R, interleukin-2 receptor blockers.

patients in the same area, in which the frequency of HEV infections did not vary over time (*24*). No studies have investigated the incidence of HEV in immunocompromised patients. The prevalence of acquired HEV after liver transplantation in the Netherlands was only 1% (*28*). This discrepancy with our data reinforces the need for studies on factors associated with HEV infection in different regions of Europe.

We investigated HEV transmission by organ transplant by detection of antibodies against HEV and HEV RNA in the blood of donors who provided organs to 15 patients infected with HEV. No HEV RNA was detected in blood samples of organ donors; thus, they were considered to be noninfectious. Because transfusion-associated hepatitis E has been described in Europe (*9*,*10*), we assayed virologic markers in frozen blood samples of 12 blood donors involved in transfusions for 2 HEV-infected recipients, but none of the samples were positive.

Multivariate analysis identified an age <52 years as a factor associated with HEV infection in SOT patients. A previous report found that a younger age was associated with primary cytomegalovirus infection in liver transplant recipients (*39*). However, the mechanisms underlying these observations for cytomegalovirus and HEV infections are not clear. HEV infections are more frequent in older persons in industrialized countries (*2*,*24*). Being a liver transplant recipient is another risk factor for acquiring HEV infection. Local inflammation may cause liver transplant patients to have a greater risk for HEV infection.

Immunocompromised patients have a higher risk for chronic infection with HEV, which may lead to cirrhosis (*15*,*23*). The high incidence of HEV infections in the study area and the fact that no vaccine against HEV is available make it essential to identify routes of transmission so that preventive measures can be taken. A case–control study showed that factors associated with HEV infection in immunocompromised patients in the study area were the consumption of game meat, food products made with pork, and mussels (*32*). Thorough cooking of game meat and pork products and better information on HEV transmission would help minimize the risk for HEV infection.

Our study had several limitations. We investigated HEV reactivation at the first annual checkup and in patients with hepatic cytolysis. Thus, we may have missed asymptomatic reactivation that occurred during the rest of the follow-up period. In addition, 102 patients who were

HEV negative at transplantation were lost to follow-up, which may have affected the incidence of HEV cases. The de novo cases were identified by detection of HEV RNA or HEV IgG. Low sensitivity of the HEV IgG assay may have also influenced incidence of HEV infection. Lastly, we screened only 44% of those who donated an organ to the HEV-infected patients and may have missed a transplant-associated infection.

In conclusion, we detected a high incidence of HEV infection in SOT patients in southern France. Liver transplant recipients may be at greater risk for HEV infection than kidney transplant recipients. Patients on a transplant waiting list could be vaccinated when a suitable vaccine becomes available. Until then, the high risk for HEV infection becoming chronic indicates that the diets of SOT patients should be closely monitored.

Acknowledgments

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Dr Legrand-Abravanel is a research scientist in the Virology Department of Toulouse University Hospital. Her primary research interests are genetic variability of hepatitis viruses and chronic forms of HEV infection in organ-transplant recipients.

References

1. Emerson SU, Purcell RH. Hepatitis E virus. Rev Med Virol. 2003;13:145–54. DOI: 10.1002/rmv.384
2. Dalton HR, Bendall R, Ijaz S, Banks M. Hepatitis E: an emerging infection in developed countries. Lancet Infect Dis. 2008;8:698–709. DOI: 10.1016/S1473-3099(08)70255-X
3. Meng XJ. Recent advances in hepatitis E virus. J Viral Hepat. 2010;17:153–61. DOI: 10.1111/j.1365-2893.2009.01257.x
4. Christensen PB, Engle RE, Hjort C, Homburg KM, Vach W, Georgsen J, et al. Time trend of the prevalence of hepatitis E antibodies among farmers and blood donors: a potential zoonosis in Denmark. Clin Infect Dis. 2008;47:1026–31. DOI: 10.1086/591970
5. Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol. 2002;40:117–22. DOI: 10.1128/JCM.40.1.117-122.2002
6. Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, et al. Hepatitis E virus transmission from wild boar meat. Emerg Infect Dis. 2005;11:1958–60.
7. Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet. 2003;362:371–3. DOI: 10.1016/S0140-6736(03)14025-1
8. Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, et al. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol. 2003;84:2351–7. DOI: 10.1099/vir.0.19242-0
9. Boxall E, Herborn A, Kochethu G, Pratt G, Adams D, Ijaz S, et al. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. Transfus Med. 2006;16:79–83. DOI: 10.1111/j.1365-3148.2006.00652.x
10. Colson P, Coze C, Gallian P, Henry M, De Micco P, Tamalet C. Transfusion-associated hepatitis E, France. Emerg Infect Dis. 2007;13:648–9. DOI: 10.3201/eid1304.061387
11. Mansuy JM, Huynh A, Abravanel F, Recher C, Peron JM, Izopet J. Molecular evidence of patient-to-patient transmission of hepatitis E virus in a hematology ward. Clin Infect Dis. 2009;48:373–4. DOI: 10.1086/595893
12. Dalton HR, Thurairajah PH, Fellows HJ, Hussaini HS, Mitchell J, Bendall R, et al. Autochthonous hepatitis E in southwest England. J Viral Hepat. 2007;14:304–9. DOI: 10.1111/j.1365-2893.2006.00800.x
13. Péron JM, Bureau C, Poirson H, Mansuy JM, Alric L, Selves J, et al. Fulminant liver failure from acute autochthonous hepatitis E in France: description of seven patients with acute hepatitis E and encephalopathy. J Viral Hepat. 2007;14:298–303. DOI: 10.1111/j.1365-2893.2007.00858.x
14. Gérolami R, Moal V, Picard C, Colson P. Hepatitis E virus as an emerging cause of chronic liver disease in organ transplant recipients. J Hepatol. 2009;50:622–4. DOI: 10.1016/j.jhep.2008.12.008
15. Haagsma EB, van den Berg AP, Porte RJ, Benne CA, Vennema H, Reimerink JH, et al. Chronic hepatitis E virus infection in liver transplant recipients. Liver Transpl. 2008;14:547–53. DOI: 10.1002/lt.21480
16. Kamar N, Selves J, Mansuy JM, Ouezzani L, Peron JM, Guitard J, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. N Engl J Med. 2008;358:811–7. DOI: 10.1056/NEJMoa0706992
17. Ollier L, Tieulie N, Sanderson F, Heudier P, Giordanengo V, Fuzibet JG, et al. Chronic hepatitis after hepatitis E virus infection in a patient with non-Hodgkin lymphoma taking rituximab. Ann Intern Med. 2009;150:430–1.
18. Péron JM, Mansuy JM, Recher C, Bureau C, Poirson H, Alric L, et al. Prolonged hepatitis E in an immunocompromised patient. J Gastroenterol Hepatol. 2006;21:1223–4. DOI: 10.1111/j.1440-1746.2006.04209.x
19. Colson P, Kaba M, Moreau J, Brouqui P. Hepatitis E in an HIV-infected patient. J Clin Virol. 2009;45:269–71. DOI: 10.1016/j.jcv.2009.06.002
20. Dalton HR, Bendall RP, Keane FE, Tedder RS, Ijaz S. Persistent carriage of hepatitis E virus in patients with HIV infection. N Engl J Med. 2009;361:1025–7. DOI: 10.1056/NEJMc0903778
21. le Coutre P, Meisel H, Hofmann J, Rocken C, Vuong GL, Neuburger S, et al. Reactivation of hepatitis E infection in a patient with acute lymphoblastic leukaemia after allogeneic stem cell transplantation. Gut. 2009;58:699–702. DOI: 10.1136/gut.2008.165571
22. Mansuy JM, Legrand-Abravanel F, Calot JP, Peron JM, Alric L, Agudo S, et al. High prevalence of anti-hepatitis E virus antibodies in blood donors from south west France. J Med Virol. 2008;80:289–93. DOI: 10.1002/jmv.21056
23. Kamar N, Abravanel F, Selves J, Garrouste C, Esposito L, Lavayssiere L, et al. Influence of immunosuppressive therapy on the natural history of genotype 3 hepatitis-E virus infection after organ transplantation. Transplantation. 2010;89:353–60. DOI: 10.1097/TP.0b013e3181c4096c
24. Mansuy JM, Abravanel F, Miedouge M, Mengelle C, Merviel C, Dubois M, et al. Acute hepatitis E in south-west France over a 5-year period. J Clin Virol. 2009;44:74–7. DOI: 10.1016/j.jcv.2008.09.010
25. Mansuy JM, Peron JM, Abravanel F, Poirson H, Dubois M, Miedouge M, et al. Hepatitis E in the south west of France in individuals who have never visited an endemic area. J Med Virol. 2004;74:419–24. DOI: 10.1002/jmv.20206

26. Legrand-Abravanel F, Mansuy JM, Dubois M, Kamar N, Peron JM, Rostaing L, et al. Hepatitis E virus genotype 3 diversity, France. Emerg Infect Dis. 2009;15:110–4. DOI: 10.3201/eid1501.080296
27. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Rev Med Virol. 2006;16:5–36. DOI: 10.1002/rmv.482
28. Haagsma EB, Niesters HG, van den Berg AP, Riezebos-Brilman A, Porte RJ, Vennema H, et al. Prevalence of hepatitis E virus infection in liver transplant recipients. Liver Transpl. 2009;15:1225–8. DOI: 10.1002/lt.21819
29. Pischke S, Suneetha PV, Baechlein C, Barg-Hock H, Heim A, Kamar N, et al. Hepatitis E virus infection as a cause of graft hepatitis in liver transplant recipients. Liver Transpl. 2010;16:74–82. DOI: 10.1002/lt.21958
30. Buti M, Cabrera C, Jardi R, Castells L, Esteban R. Are recipients of solid organ transplantation a high-risk population for hepatitis E virus infection? Liver Transpl. 2010;16:106–7; author reply 108. DOI: 10.1002/lt.21925
31. Legrand-Abravanel F, Thevenet I, Mansuy JM, Saune K, Vischi F, Peron JM, et al. Good performance of immunoglobulin M assays in diagnosing genotype 3 hepatitis E virus infections. Clin Vaccine Immunol. 2009;16:772–4. DOI: 10.1128/CVI.00438-08
32. Legrand-Abravanel F, Kamar N, Sandres-Saune K, Garrouste C, Dubois M, Mansuy JM, et al. Characteristics of autochthonous hepatitis E virus infection in solid-organ transplant recipients in France. J Infect Dis. 2010;202:835–44. DOI: 10.1086/655899
33. Bendall R, Ellis V, Ijaz S, Ali R, Dalton H. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. J Med Virol. 2010;82:799–805. DOI: 10.1002/jmv.21656
34. Mast EE, Alter MJ, Holland PV, Purcell RH. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. Hepatology. 1998;27:857–61. DOI: 10.1002/hep.510270331
35. Labadie K, Larcher T, Joubert C, Mannioui A, Delache B, Brochard P, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. J Clin Invest. 2010;120:894–906. DOI: 10.1172/JCI40104
36. Way SJ, Lidbury BA, Banyer JL. Persistent Ross River virus infection of murine macrophages: an in vitro model for the study of viral relapse and immune modulation during long-term infection. Virology. 2002;301:281–92. DOI: 10.1006/viro.2002.1587
37. Luo H, Chen H, Daloze P, Chang JY, St-Louis G, Wu J. Inhibition of in vitro immunoglobulin production by rapamycin. Transplantation. 1992;53:1071–6. DOI: 10.1097/00007890-199205000-00019
38. Rentenaar RJ, van Diepen FN, Meijer RT, Surachno S, Wilmink JM, Schellekens PT, et al. Immune responsiveness in renal transplant recipients: mycophenolic acid severely depresses humoral immunity in vivo. Kidney Int. 2002;62:319–28. DOI: 10.1046/j.1523-1755.2002.00425.x
39. Arthurs SK, Eid AJ, Pedersen RA, Dierkhising RA, Kremers WK, Patel R, et al. Delayed-onset primary cytomegalovirus disease after liver transplantation. Liver Transpl. 2007;13:1703–9. DOI: 10.1002/lt.21280

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Concurrent Conditions and Human Listeriosis, England, 1999–2009

Piers Mook, Sarah J. O'Brien, and Iain A. Gillespie

The epidemiology of listeriosis in England and Wales changed during 2001–2008; more patients ≥60 years of age had bacteremia than in previous years. To investigate these changes, we calculated risk for listeriosis by concurrent condition for non–pregnancy-associated listeriosis cases reported to the national surveillance system in England during 1999–2009. Conditions occurring with *L. monocytogenes* infection were coded according to the International Classification of Diseases, 10th Revision, and compared with appropriate hospital episode statistics inpatient denominator data to calculate incidence rates/million consultations. Malignancies (especially of the blood), kidney disease, liver disease, diabetes, alcoholism, and age ≥60 years were associated with an increased risk for listeriosis. Physicians should consider a diagnosis of listeriosis when treating patients who have concurrent conditions. Providing cancer patients, who accounted for one third of cases, with food safety information might help limit additional cases.

Listeriosis is a rare but serious foodborne disease caused by the bacterium *Listeria monocytogenes*. Three groups of persons are disproportionately affected: the elderly, the immunocompromised, and pregnant women and their unborn or newborn infants. The clinical signs of disease in these persons include septicemia, meningitis, and miscarriage. Pregnant women can transmit the infection to the fetus, for whom the result can be deadly. However, these women may not have clearly overt signs or symptoms of infection. Case-fatality rates range from 20% to 50% (*1*). The susceptibility of healthy persons to symptomatic listeriosis is substantially less than that of persons with underlying conditions.

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Persons with cancer, diabetes, AIDS, and liver or kidney disease are often predisposed to severe infection and death after infection with *L. monocytogenes*. This predisposition is a consequence of suppressed T-cell–mediated immunity (*2*) caused by the condition or its treatment. Similarly, pregnant women, the elderly, and those receiving immunosuppressive therapy are also at risk because of impaired or modulated immune function.

The epidemiology of listeriosis in England and Wales has changed since 2001 (*3*). Incidence has increased (2.1 cases/million population during 1990–2000 vs. 3.6 cases/million population during 2001–2009), and more cases have been found in persons ≥60 years of age who had bacteremia (but not meningitis). Similar patterns have been reported in other countries in Europe (*4*–*6*). The reasons for these changes are not fully understood, but they do not seem to be caused by surveillance artifacts and are not associated with sex, season, geography, ethnic or socioeconomic differences, underlying conditions, or *L. monocytogenes* subtype (*3*). We have showed that the increase occurred in persons with cancer or other conditions whose treatment included acid-suppressing medication (*7*). In view of recent trends, we examined national surveillance data for England to quantify the role of concurrent conditions in persons with listeriosis and stratified these conditions to examine risks for persons ≥60 years of age.

Methods

The Health Protection Agency Centre for Infections has coordinated national surveillance of listeriosis in England and Wales since 1990. Cases are included in the system by voluntary referral of cultures to the national reference laboratory or by electronic reporting of confirmed cases from local laboratories. Clinical data, including details of patients' concurrent conditions, are subsequently sought from the consultant clinical microbiologist involved in the

care of the case-patient. Microbiologic data from local and reference laboratories and clinical and risk factor data are linked for each case, deduplicated as necessary, and stored in a bespoke Microsoft Access database (Microsoft, Redmond, WA, USA) Access database.

A case of listeriosis is defined as a person with clinically compatible illness and from whom *L. monocytogenes* was isolated from a normally sterile site. Cases are subsequently classified as either non–pregnancy-associated (persons >1 month of age) or pregnancy-associated (a maternal–fetal or maternal–neonatal pair; such pairs were considered a single case). In this study, we included non–pregnancy-associated cases reported from laboratories in England for which a clinical questionnaire was available and showed that at least 1 reported concurrent condition was present. We included cases reported during April 1, 1999–March 31, 2009 because denominator data were arranged by fiscal years. These cases included sporadic cases and cases that were identified as being part of common source foodborne outbreaks.

Authors (P.M. and I.A.G.) reviewed each reported concurrent condition and assigned an International Classification of Diseases, 10th Revision (ICD-10) (*8*) code when appropriate. Rules for assigning codes were developed at the outset to ensure standardized coding throughout the study (online Technical Appendix, www.cdc.gov/EID/content/17/1/38-Techapp.pdf). These rules were validated by a third author (S.J.O.), a clinically qualified investigator, who also reviewed any coding disparities. Counts were calculated of all persons and those ≥60 years of age for each ICD-10 chapter (ICD-10 codes are aggregated into 22 chapters) and subgroup (within each chapter).

Hospital episode statistics finished consultant episodes (FCE) data, which were aggregated by ICD-10 code, age group (0–14 years, 15–59 years, 60–74 years, and ≥75 years), and fiscal year, were obtained from the Health and Social Care Information Centre (*9*) and used as denominator data. These data describe episodes of continuous admitted patient care under a specific consultant for National Health Service hospital inpatients in England, and a primary diagnosis is assigned to each episode by using ICD-10 coding. To ensure reliable confidence intervals (CIs), we calculated incidence rates/million FCEs and 95% CIs for each ICD-10 chapter and subgroup in which there were ≥10 cases. Two ICD-10 chapters not used by hospital episodes statistics to code primary diagnoses, external causes of morbidity and mortality (V01–Y98) and codes for special purposes (U00–U99), were not considered. Relative risks (RRs) and corresponding 95% CIs were calculated as appropriate when ≥10 cases were reported for a concurrent condition subgroup or chapter. Analysis was then repeated for case-patients ≥60 years of age.

Data were stored, manipulated, and summarized by using Microsoft Access, and incidence rates and RRs were calculated by using Microsoft Excel. Differences in proportions and changes in proportions over strata were assessed by using the χ^2 test and the χ^2 test for trend, respectively.

Results

A total of 1,239 ICD-10–coded concurrent conditions were reported by 1,413 case-patients with non–pregnancy-associated listeriosis in England during April 1, 1999–March 31, 2009 (Figure). Of those patients who reported ≥1 underlying condition, 21 (2.2%) were identified as being part of a common source outbreak. Characteristics of case-patients with and without a completed clinical questionnaire are shown in Table 1. Overall, 9.1 cases of listeriosis/million FCEs were reported over the study period (95% CI 8.6–9.6) (online Appendix Table, www.cdc.gov/EID/content/17/2/38-appT.htm). Compared with all other reported conditions, higher rates of disease were reported for the following chapters (in order of highest to lowest RR): endocrine, nutritional, and metabolic diseases (RR 5.3, 95% CI 4.2–6.6); neoplasms (RR 4.9, 95% CI 4.4–5.5); mental and behavior disorders (RR 3.1, 95% CI 2.4–4.1); diseases of the circulatory system (RR 1.4, 95% CI 1.2–1.6); diseases of the digestive system (RR 1.3, 95% CI 1.1–1.5); and diseases of the musculoskeletal system and connective tissue (RR 1.3, 95% CI 1.1–1.6) (Table 2).

Within these chapters, only certain subgroups showed increased rates: diabetes mellitus; malignant neoplasms of the lymphoid, hematopoietic, and related tissues; eye, brain, and other parts of the central nervous system (CNS); respiratory and intrathoracic organs; digestive organs; breast; male and female genital organs; thyroid and other endo-

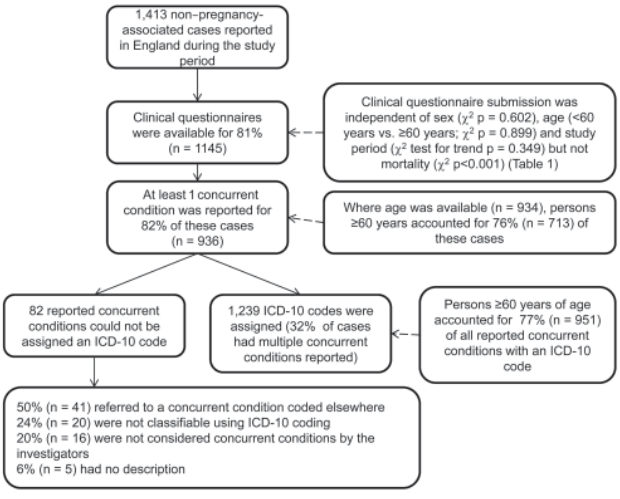

Figure. Study population and reported International Classification of Diseases, 10th Revision (ICD-10)–coded concurrent conditions for 1,413 case-patients with non–pregnancy-associated listeriosis, England, April 1, 1999–March 31, 2009.

Table 1. Characteristics of case-patients with non–pregnancy-associated listeriosis, England, 1999–2009*

Characteristic	No. (%) case-patients	
	CQR, n = 1,145	No CQR, n = 268
Fiscal years		
1999–2000 and 2000–2001	133 (85.3)	23 (14.7)
2001–2002 and 2002–2003	229 (89.8)	26 (10.2)
2003–2004 and 2004–2005	228 (63.9)	129 (36.1)
2005–2006 and 2006–2007	253 (81.1)	59 (18.9)
2007–2008 and 2008–2009	302 (90.7)	31 (9.3)
Sex		
M	642 (56.1)	145 (54.1)
F	503 (43.9)	122 (45.5)
Unknown	0	1 (0.4)
Age group, y		
<60	277 (24.2)	63 (23.5)
≥60	866 (75.6)	193 (72)
Unknown	2 (0.2)	12 (4.5)
Status		
Died	445 (38.9)	25 (9.3)
Did not die	664 (58)	159 (59.3)
Unknown	36 (3.1)	84 (31.3)

*CQR, clinical questionnaire received.

crine glands; mental and behavior disorders caused by psychoactive substances (alcohol-related in 96% of reports); hypertensive diseases, other forms of heart disease, and diseases of arteries, arterioles, and capillaries; diseases of the liver and noninfective enteritis and colitis; and systemic connective tissue disorders (Table 2). In addition, several subgroups were associated with increased risk even when the corresponding chapter was not: renal failure, diseases of blood and blood-forming organs, and chronic lower respiratory diseases (Table 2).

Concurrent conditions were disproportionately reported for persons ≥60 years of age (χ^2 $p<0.001$), and the rate of listeriosis for this age group (16.8/million; 95% CI 15.8–17.9) was significantly higher than that for younger persons (RR 4.6, 95% CI 4.1–5.3) (Table 2). When the RR for each chapter for persons ≥60 years of age (using persons <60 years of age as the reference population) was calculated, the following were associated with increased risk: endocrine, nutritional and metabolic diseases; genitourinary system diseases; diseases of the musculoskeletal system and connective tissue; neoplasms; certain infectious and parasitic diseases; diseases of the digestive system; and mental and behavior disorders (Table 2). In instances where the risk for each subgroup in persons ≥60 years of age could be calculated and compared with that for persons <60 years of age, all subgroups of previously identified chapters were associated with increased risk.

Discussion

We analyzed surveillance data that included detailed denominator data by using an internationally recognized diagnostic classification system and found that a wide variety of conditions seem to increase the risk for serious infection with *L. monocytogenes*. Malignancies accounted for more than one third of conditions, and cancer patients had a 5-fold increased risk for development of listeriosis. Cancers of the blood seemed to have the greatest effect. Other high-risk conditions included diabetes mellitus; alcoholism; certain diseases of the circulatory system and the musculoskeletal system and connective tissue; noninfective enteritis and colitis; and diseases of the liver and kidney. For most high-risk conditions, the risk for infection was higher among older patients.

Case identified by the national surveillance program in England are laboratory confirmed, and most cases result in serious illness requiring hospitalization or death. Given this finding, a hospitalized population better represents the population at risk than a community population, which was used in previous studies (*10,11*).

The response rate to the clinical questionnaire that captured information on concurrent conditions was high and not influenced by age or sex of the case-patient, which minimized differential ascertainment of clinical data. However, we could not assess concurrent conditions for which completed clinical questionnaires were not returned. This issue indicates that the role of some conditions might be underestimated if clinicians were unwilling to return questionnaires and disclose information for certain case-patients (e.g., those with AIDS). Similarly, but less likely, reporting bias might exist if the propensity to report certain concurrent conditions were affected by the presence or absence of others conditions, or if only concurrent conditions considered relevant to *L. monocytogenes* infection were reported. Concurrent conditions were reported by the clinical microbiologist rather than by the consultants responsible for the care of the patients with concurrent conditions. These consultants might be better informed of existing concurrent conditions. However, hospital microbiologists need to be aware of such conditions to provide treatment accordingly, and questioning several consultants for each case-patient may have a negative effect on questionnaire response because questionnaires might be lost if passed between multiple consultants.

Misclassification was minimized by grouping conditions only to 3-character ICD-10 code levels. Although we acknowledge that such grouping might mask high-risk conditions apparent at the 4-character ICD-10 code level, routine surveillance data were not specific enough to further discriminate among conditions. In some instances, in which treatments were reported in the absence of relevant conditions (e.g., chemotherapy, dialysis, splenectomy), we made assumptions about the conditions requiring such treatment and coded accordingly (online Technical Appendix). Although these assumptions could inflate the inci-

dence rates for certain conditions, they occurred relatively infrequently and were not used for treatments that could be prescribed for a range of conditions (e.g., broad-spectrum antimicrobial drugs).

Table 2. Relative risks for ICD-10 conditions for case-patients with non–pregnancy-associated listeriosis, England, 1999–2009*

Chapter and subgroup (code)	Relative risk (95% CI)	
	Versus other conditions	Age ≥60 y vs. <60 y
Certain infectious and parasitic diseases (A00–B99)	1.3 (0.9–2.0)	2.5 (1.1–5.9)
Neoplasms (C00-D48)	4.9 (4.4–5.5)	2.9 (2.3–3.6)
Digestive organs (C15–C26)	3.1 (2.4–3.9)	NC
Respiratory and intrathoracic organs (C30–C39)	4.8 (3.5–6.5)	NC
Breast (C50)	2.9 [2.1–4.1)	2.6 (1.4–5.2)
Female genital organs (C51–C58)	1.9 (1.07–3.5)	NC
Male genital organs (C60–C63)	2.9 (1.7–5.1)	NC
Eye, brain, and other parts of central nervous system (C69–C72)	7.3 (4.2–12.7)	NC
Thyroid and other endocrine glands (C73–C80, C97)	2.7 (2.0–3.6)	3.2 (1.6–6.4)
Lymphoid, hematopoietic, and related tissues (C81–C96)	17.6 (15.1–20.6)	2.8 (2.0–3.9)
In situ and benign neoplasms and others of uncertainty D00–D48)	0.7 (0.4–1.1)	NC
Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism (D50–D89)	1.3 (0.9–2.0)	0.8 (0.4–1.8)
Anemias (D50–D64)	1.0 (0.6–1.7)	NC
Diseases of blood and blood-forming organs (D65–D89)	2.3 (1.3–4.0)	NC
Endocrine, nutritional and metabolic diseases (E00–E90)	5.3 (4.2–6.6)	6.3 (3.5–11.2)
Diabetes mellitus (E10–E14)	11.4 (9.0–14.5)	4.9 (2.7–8.8)
Mental and behavior disorders (F00–F99)	3.1 (2.4–4.1)	1.7 (1.01–2.8)
Due to psychoactive substance (F10–F19)	12.3 (9.4–16.1)	4.7 (2.7–8.1)
Diseases of the nervous system (G00–G99)	0.6 (0.4–1.0)	NC
Diseases of the eye and adnexa (H00–H59)	NC	NC
Diseases of the ear and mastoid process (H60–H95)	NC	NC
Diseases of the circulatory system (I00–I99)	1.4 (1.2–1.6)	NC
Hypertensive diseases (I10–I15)	8.0 (5.2–12.2)	NC
Ischemic heart diseases (I20–I25)	0.8 (0.5–1.1)	NC
Other forms of heart disease (I30–I52)	2.4 (1.9–3.1)	NC
Cerebrovascular diseases (I60–I69)	0.7 (0.4–1.2)	NC
Diseases of arteries, arterioles, and capillaries (I70–I79)	2.1 (1.2–3.5)	NC
Diseases of the respiratory system (J00–J99)	0.9 (0.7–1.1)	NC
Chronic lower respiratory diseases (J40–J47)	1.8 (1.3–2.5)	NC
Other diseases of respiratory system (J80–J99)	1.7 (0.95–3.1)	NC
Diseases of the digestive system (K00–K93)	1.3 (1.1–1.5)	1.9 (1.4–2.6)
Noninfective enteritis and colitis (K50–K52)	4.3 (3.3–5.6)	2.3 (1.4–3.8)
Other diseases of intestines (K55–K63)	0.5 (0.3–0.9)	NC
Diseases of liver (K70–K77)	22.4 (17.7–28.4)	2.2 (1.4–3.6)
Diseases of the skin and subcutaneous tissue (L00–L99)	NC	NC
Diseases of the musculoskeletal system and connective tissue (M00–M99)	1.3 (1.1–1.6)	4.5 (2.7–7.3)
Arthropathies (M00–M25)	1.7 (1.3–2.2)	NC
Systemic connective tissue disorders (M30–M36)	18.3 (12.6–26.6)	NC
Diseases of the genitourinary system (N00–N99)	1.2 (0.99–1.5)	5.3 (3.2–8.6)
Renal failure (N17–N19)	12.2 (9.8–15.1)	1.7 (1.02–2.7)
Pregnancy, childbirth, and puerperium (O00–O99)	NC	NC
Certain conditions originating in the perinatal period (P00–P96)	NC	NC
Congenital malformations, deformations, and chromosomal abnormalities (Q00–Q99)	NC	NC
Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified (R00–R99)	NC	NC
Injury, poisoning, and certain other consequences of external causes (S00–T98)	NC	NC
External causes of morbidity and mortality (V01–Y98)	–	–
Factors influencing health status and contact with health services (Z00–Z99)	NC	NC
Codes for special purposes (U00–U99)	–	–
Total	NC	4.6 (4.1–5.3)

*ICD-10, International Classification of Diseases, 10th Revision; CI, confidence interval; NC, not calculated (for conditions with <10 cases); –, data not available.

Because only single-variable analysis could be performed, we could not assess the extent to which concurrent conditions were correlated, which led to the potential for uncontrolled confounding. Such method limitations might explain the high incidence associated with both diabetes and kidney disease and reinforce the need to consider these findings as highly refined hypotheses to be tested by other methods (*12*).

To our knowledge, few studies have attempted to quantify the risk for listeriosis by patient concurrent conditions. As part of a risk assessment of *L. monocytogenes* in ready-to-eat foods, researchers from the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) calculated the relative susceptibility to listeriosis for certain conditions (*10*). Furthermore, risk levels for listeriosis by predisposing condition in Denmark have also been estimated (*11*). Despite differences in methods between those studies and our study, several high-risk conditions were also identified in those studies: malignancies (most notably those of the blood), kidney disease (recorded as dialysis [*10*] and renal transplant [*11*]), diabetes, alcoholism, and increased age in all 3 studies; liver disease and pulmonary cancer in the WHO/FAO study and our study; and systemic lupus erythematosus in the study in Denmark and our study (as systemic connective tissue disorders). Such commonality would seemingly validate our estimates.

The absence of AIDS as a high-risk condition in our study and its presence in both previous studies (*10*,*11*), might reflect improved treatment for HIV infection that prevents AIDS and, consequently, *L. monocytogenes* infection (*13*) or highlight a reporting bias by the consultant microbiologist. A general transplantation status, identified as a condition leading to the highest relative susceptibility in the WHO/FAO study, was not coded in our study because it is a treatment. Noninfective enteritis and colitis and certain diseases of the circulatory system were identified as additional high-risk conditions in our study but not in the previous studies. These additional conditions might be the result of improved accuracy, use of ICD-10 coding and a hospitalized reference population instead of the general population, different susceptibility calculations, or changes in the prevalence of certain conditions in the interim period (the previous studies used data from 1992 [*10*] and 1989–1990 [*11*]). However, we acknowledge that links between these conditions and listeriosis have been reported (*14*–*18*).

With these caveats in mind, our findings have implications for clinical practice and food safety policy makers. The number and diversity of conditions that appear to increase the risk for listeriosis imply that physicians working in all specialties should consider listeriosis when treating patients with concurrent conditions and provide appropriate food safety advice. Similarly, current UK government food safety advice on avoidance of listeriosis, which is delivered passively and is specific mainly for pregnant women (*19*,*20*), should be communicated actively to all high-risk groups. In prioritizing advice, policy makers should consider not only the associated risk but also the prevalence of the concurrent condition. Cancer patients accounted for more than one third of listeriosis cases, and high risks were observed for most cancer subgroups. Because we are not aware of any appropriate food safety advice that is tailored specifically for cancer patients in the UK, emphasis on this group might help to prevent further cases.

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References

1. Rocourt J, Bille J. Foodborne listeriosis. World Health Stat Q. 1997;50:67–73.
2. Farber JM, Peterkin PI. *Listeria monocytogenes*, a food-borne pathogen. Microbiol Rev. 1991;55:476–511.
3. Gillespie IA, McLauchlin J, Grant KA, Little CL, Mithani V, Penman C, et al. Changing pattern of human listeriosis, England and Wales, 2001–2004. Emerg Infect Dis. 2006;12:1361–6.
4. Goulet V, Hedberg C, Le Monnier A, de Valk H. Increasing incidence of listeriosis in France and other European countries. Emerg Infect Dis. 2008;14:734–40. DOI: 10.3201/eid1405.071395
5. Koch J, Stark K. Significant increase of listeriosis in Germany—epidemiological patterns 2001–2005. Euro Surveill. 2006;11:85–8.
6. Kvistholm JA, Ethelberg S, Smith B, Moller NE, Larsson J, Molbak K, et al. Substantial increase in listeriosis, Denmark 2009. Euro Surveill. 2010;15:pii: 19522.
7. Gillespie IA, McLauchlin J, Little CL, Penman C, Mook P, Grant K, et al. Disease presentation in relation to infection foci for non-pregnancy-associated human listeriosis in England and Wales, 2001 to 2007. J Clin Microbiol. 2009;47:3301–7. DOI: 10.1128/JCM.00969-09
8. World Health Organization. International statistical classification of diseases and related health problems, 10th revision; 2007 [cited 2009 Mar 6]. http://apps.who.int/classifications/apps/icd/icd10online
9. Hospital Episode Statistics (HES) Online [cited 2009 Feb 23]. http://www.hesonline.nhs.uk/Ease/servlet/ContentServer?siteID=1937&categoryID=202
10. World Health Organization. Risk assessment of *Listeria monocytogenes* in ready-to-eat foods. Microbiological risk assessment series 5; 2004 [cited 2010 Oct 18]. http://www.who.int/foodsafety/publications/micro/en/mra5_contents.pdf
11. Jensen A, Frederiksen W, Gerner-Smidt P. Risk factors for listeriosis in Denmark, 1989–1990. Scand J Infect Dis. 1994;26:171–8. DOI: 10.3109/00365549409011781

12. Gradel KO, Schonheyder HC, Kristensen B, Dethlefsen C, Ejlertsen T, Nielsen H. Are host characteristics or exposure factors mainly involved in the acquisition of zoonotic *Salmonella* and *Campylobacter* coinfection in humans? Foodborne Pathog Dis. 2009;6:251–5. DOI: 10.1089/fpd.2008.0148
13. Bennion JR, Sorvillo F, Wise ME, Krishna S, Mascola L. Decreasing listeriosis mortality in the United States, 1990–2005. Clin Infect Dis. 2008;47:867–74. DOI: 10.1086/591131
14. Dalton CB, Merritt TD, Unicomb LE, Kirk MD, Stafford D, Lalor K; the OzFoodNet Working Group. A national case-control study of risk factors for listeriosis in Australia. Epidemiol Infect. 2010;Apr 30:1–9 [Epub ahead of print].
15. Goulet V, Marchetti P. Listeriosis in 225 non-pregnant patients in 1992: clinical aspects and outcome in relation to predisposing conditions. Scand J Infect Dis. 1996;28:367–74. DOI: 10.3109/00365549609037921
16. McLauchlin J. Human listeriosis in Britain, 1967–85, a summary of 722 cases. 2. Listeriosis in non-pregnant individuals, a changing pattern of infection and seasonal incidence. Epidemiol Infect. 1990;104:191–201. DOI: 10.1017/S0950268800059355
17. Nieman RE, Lorber B. Listeriosis in adults: a changing pattern. Report of eight cases and review of the literature, 1968–1978. Rev Infect Dis. 1980;2:207–27.
18. Siegman-Igra Y, Levin R, Weinberger M, Golan Y, Schwartz D, Samra Z, et al. *Listeria monocytogenes* infection in Israel and review of cases worldwide. Emerg Infect Dis. 2002;8:305–10.
19. Food Standards Agency. Eat well, be well. Pregnancy [cited 2009 Sep 15]. http://www.eatwell.gov.uk/asksam/agesandstages/pregnancy
20. Food Standards Agency. Eat well, be well. Look out for *Listeria* [cited 2010 Oct 21]. http://eatwell.gov.uk/healthissues/foodpoisoning/listeria/whatisit

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Genotyping Rotavirus RNA from Archived Rotavirus-Positive Rapid Test Strips

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Genotyping circulating rotaviruses before and after introduction of rotavirus vaccine is useful for evaluating vaccine-associated changes in genotype distribution. We determined frequency of rotavirus genotypes among 61 rotavirus-positive children hospitalized in Israel during the 2005–06 rotavirus season. Accurate molecular epidemiologic data were recovered from affinity-concentrated rotavirus immobilized in rotavirus-positive bands from air-dried, diagnostic rotavirus rapid test strips (dipstick) stored at room temperature from 1 week to 5 years. G genotypes were identical for 21 paired dipsticks and suspensions, whereas dipsticks or suspensions detected an additional G genotype in 2 samples. RNA sequences from 7 pairs were identical. Phylogenetic analysis suggested previously unreported G2 sublineages and G9 lineages. The ease with which dipsticks can be stored at local facilities and transported to central reference laboratories can reverse increasing difficulties in obtaining geographically representative stool samples and expand surveillance to regions lacking adequate laboratory facilities.

Rotavirus infection is a leading cause of gastroenteritis in children <5 years of age worldwide; children with severe dehydration and electrolyte imbalance require hospitalization (*1*). In 2007 and 2008, the annual economic cost for hospitalization of rotavirus case-patients in Israel was US $4,578,489 in direct costs to the health system plus an additional household cost of $3,101,955 (*2*). Poor hygienic conditions and lack of appropriate medical facilities in developing countries result in high infant mortality rates (*1*,*3*–*5*).

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The genes encoding the outer capsid viral proteins VP7 and VP4 form the basis of classification of group A rotaviruses into G and P genotypes, respectively (*6*,*7*). Two live oral vaccines, monovalent Rotarix vaccine (GlaxoSmithKline, Research Triangle Park, NC, USA) and pentavalent Rotatek vaccine (Merck, Rahway, NJ, USA) effectively reduced hospitalizations for subsequent infections with G1P[8], G2P[4], G3P[8], and G4P[8] rotaviruses by >85% (*8*,*9*). Rotavirus vaccines that are scheduled for inclusion in Israel's national vaccination program after 2010 may selectively change relative distributions of naturally cocirculating rotaviruses of different G and P genotypes. According to analysis of voluntary immunizations before universal vaccination, the effectiveness of rotavirus vaccine in preventing rotavirus gastroenteritis–associated hospitalizations in Israel is already evident (*10*).

Simple to use, inexpensive, diagnostic rotavirus rapid test strips (dipsticks) identify group A rotavirus–positive stools in <30 minutes by lateral flow immunochromatography without indicating the genotype. Dipsticks are dipped into saline stool suspensions. If rotavirus is present, a colored band appears when indicator-linked rotavirus antibodies bind to the virus, and this complex is trapped on a band of membrane-bound rotavirus antibody on the dipstick. With their growing use at point-of-care facilities and in local hospital laboratories, fewer samples are available for genotyping by centralized reference laboratories. Obtaining geographically representative stool samples and expanding surveillance to regions lacking adequate laboratory facilities is becoming increasingly difficult. The ease with which dipsticks can be stored at local facilities and transported to central reference laboratories can help overcome these difficulties. We hypothesized that rotavirus trapped on rotavirus-specific antibody bands, equivalent to affinity concentrated rotavirus, might yield genotyping-quality RNA

from dipsticks that are easily archived locally and transportable to centralized laboratories. In this study, our main objective was to determine the feasibility of recovering molecular epidemiologic data from rotavirus immobilized on air-dried diagnostic rotavirus dipsticks to compensate for the decreasing number of fecal samples reaching central reference laboratories.

Methods

Stool samples from 311 children hospitalized with acute gastroenteritis at Sheba Medical Center, Tel Hashomer, Israel, during May 15, 2005–May 15, 2006, were suspended in saline. Rotavirus dipsticks (Hy Laboratories LTD, Rehovot, Israel) were used to identify rotavirus-positive stool samples. (For comparison with rotavirus ELISA with Dako reagents [Dako Diagnostics LTD, Glostrup, Denmark], diagnostic dipsticks from Hy-Laboratories and Novamed [Novamed, Jerusalem, Israel] had sensitivities of 98.5% ± 3% and 92.3%, and specificities of 92.5% ± 1% and 93.5%, respectively [L.M. Shulman et al., unpub data].) The section of the dipstick containing the rotavirus-specific bands from 14 rotavirus-positive dipsticks that had been stored at room temperature for at least 1 week were excised and placed into 120 μL saline.

Rotaviral RNA was extracted from these rotavirus-specific bands and from the corresponding saline stool suspensions by using QIAmp Viral RNA Mini kits (QIAGEN, Valencia, CA, USA). RNA amplified from rotavirus-specific bands from dipsticks archived at room temperature 5 years earlier was similarly extracted. RNA was amplified by using generic primers for reverse transcription–PCR (RT-PCR) (QIAGEN OneStep RT-PCR kits, QIAGEN) followed by heminested PCR (AmpliTaq Gold, Applied Biosystems, Foster City, CA, USA) with genotype-specific primers to identify G and P genotypes as described by Gouvea et al. (*11*) and Gunasena et al. (*12*), respectively. RT-PCR products were gel purified (QIAQuick Gel Extraction Kit, QIAGEN) or purified directly (HiPure PCR Product Purification Kit; Roche Applied Science, Indianapolis, IN, USA) from the PCR mix and sequenced by using an ABI PRISM Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems) and the genotype-specific primer and common primer from the heminested PCR reaction described. Reaction mixtures were analyzed on Applied Biosystems model 373 DNA automatic sequencing system to confirm the RT-PCR genotyping results and for phylogenetic analysis.

Sequences of the same genotype were truncated to the longest common length by using the Sequencher program (Genecodes, Ann Arbor, Michigan, USA). Nearest-neighbor phylogenetic trees were constructed from these sequences after the data were bootstrapped 1,000 times with ClustalX (*13*). The tree was analyzed by using NJplot (*14*). Sequences have been deposited in European Molecular Biology Laboratory/GenBank/DNA Data Bank of Japan under accession nos. FN298858–FN298875, FN582119–FN582124, and HQ174462–HQ174463. Isolate names are linked to their specific accession numbers in the online Appendix Table (www.cdc.gov/EID/content/17/1/44-appT.htm).

This study was approved by the institutional review board of Chaim Sheba Medical Center (SMC-7606-09). Experiments were not performed on humans. All personal identification was removed from the remnants of fecal samples sent to the National Center for Viral Gastroenteritis for rotavirus analysis. The viral RNA used for this study was obtained from these anonymous samples.

Results

To assess the suitability of RNA extracted from dipsticks for determining the VP7 genotype, we first determined the VP7 G genotype of rotavirus in rotavirus-positive clinical samples from children hospitalized during May 15, 2005–May 15, 2006. Of 311 stool samples, 61 were determined to be rotavirus positive by using diagnostic rotavirus dipsticks. The G genotype and P genotype of RNA in 54 of the 61 samples was determined by the size of the heminested PCR amplification product as described. Mixed infections, e.g., >1 G (6 samples) and P genotypes (2 samples) in the same fecal suspension, were found in 8 (15%) of the 54 positive samples. Taking into account mixed infections, we found G1, G2, G3, and G9 G genotypes in 79%, 5%, 2%, and 15%, respectively, of the 62 rotaviruses identified in the 54 clinical samples. The frequencies of associated G and P genotypes for isolates from the 2005–2006 rotavirus season were 3% G1P[4], 76% G1P[8], 5% G2P[4], 2% G3P[8], 3% G9P[4], and 11% G9P[8].

We chose 18 rotavirus-positive clinical samples with which to study the feasibility of recovering molecular information from dipsticks. Thirteen were from children infected with 1 G genotype of rotavirus and 5 from children simultaneously infected with rotaviruses belonging to 2 different G genotypes. Of the 13 infections by a single G genotype, 8 were G1, 3 were G2, and 1 each were G3 and G9. All 5 mixed infections, RoV-24_ISR05, RoV_41_ISR05, RoV_56_ISR06, RoV_57_ISR06, and RoV_60_ISR06, were simultaneous infections with G1 and G9 genotypes. Mixed infections were identified by specific product size on gels and confirmed by partial sequencing of the G1 product for all 4 patients whose G9 sequences appear in the Figure.

RNA was extracted from rotavirus-positive bands of dipsticks from the 18 clinical samples. The dipsticks had been stored for ≥1 week at room temperature. The quality of the RNA from all 18 extractions was sufficient to enable determination of VP7 G genotypes by heminested RT-PCR. The genotypes obtained from the RNA extracted from dipsticks were identical to those from RNA extracted

Figure. Neighbor-joining phylogenetic trees for viral protein (VP) 7 G1, G2, G3, and G9 genotypes of hospitalized children in Israel, including sequences recovered from archived rotavirus dipsticks (indicated by **boldface**). Representative isolates for lineages and sublineages of VP7 genotypes G1 (A), G2 (B), G3 (C), and G9 (D) were chosen from Phan et al. (*15*) Page and Steele (*16*), Wang et al. (*17*), and Martinez-Laso et al. (*18*), respectively, and the sequences were downloaded from the European Molecular Biology Laboratory/GenBank/DNA Data Bank of Japan. These sequences were aligned with Israeli sequences by using the Sequencher program (Genecodes, Ann Arbor, MI, USA) and truncated to the longest segment common to all sequences in the alignment; 515 nt for G1, 547 nt for G2, 274 nt for G3, and 207 nt for G9. Each of the 4 phylogenetic trees was prepared by using ClustalX (*13*) for data bootstrapped 1,000× and was analyzed with NJplot (*14*). Whole numbers indicate bootstrap values for branches; fractional numbers indicate genetic distances. The genotype, lineage, and, where relevant, sublineage of each isolate appears in brackets after the name of the isolate: for example, KY3303[G2.2a] is VP7 genotype G 2, lineage 2, sublineage a for isolate KY3303. A letter at the end of the name of the Israeli sequences indicates the source of the RNA (D for dipstick or S for fecal suspension). D&S appears when the sequences were identical. The GenBank accession numbers for all sequences in this figure appear in the online Appendix Table (www.cdc.gov/EID/content/17/1/44-appT.htm). Scale bars indicate percent of nucleotide substitutions per site.

from corresponding saline suspensions for 14 of the 18 rotavirus-positive stool samples, including 3 samples from children with dual infections. In 2 mixed G1–G9 infections, G9, but not G1 RNA, was recovered, and G1 RNA was recovered from dipstick RNA in addition to G9 from a G9-infected child. A fourth dipstick from a G3 sample yielded a G3 and an equivocal G1 that did not appear in subsequent replicate heminested amplifications. In other words, 21 (92%) of 23 genotypes identified from RNA extracted from 18 fecal suspensions were also identified from RNA extracted from dipsticks, including 8 of 10 genotypes from 5 samples simultaneously infected with rotaviruses of 2 different genotypes.

Rotaviral VP7 RNA suitable for genotyping was also recovered from all 5 air-dried rotavirus-positive dipsticks that had been stored at room temperature for 5 years. The G genotypes of the rotavirus in the fecal suspensions from the 5 archived dipsticks from 2002 were unknown, and the suspensions were no longer available for comparison. One of the dipsticks was manufactured by Hy Laboratories; the remaining 4 were manufactured by Novamed. Initial amplification with generic G-type primers yielded generic G genotype amplification products of expected size (≈1,062 nt) upon gel electrophoresis. Three of the dipsticks yielded G3 RNA; the other 2 yielded G1 RNA. As proof of concept that P genotypes could be determined in addition to G genotypes, the P genotypes of RNA recovered from 2 of the 5-year-old rotavirus dipsticks with G3 RNA were also determined by direct sequencing of the RT-PCR products by using the generic P-type

primers. Both were P[8] by sequence analysis (online Appendix Table).

The G genotypes of 3 G1s, 2 G2s, 1 G3, and 1 G9 RNA extracted from dipsticks were confirmed by sequencing their heminested amplification products. Moreover, the sequences of the RNA extracted from each of the 7 dipsticks were identical to the sequence of the RNA extracted directly from the corresponding stool suspension. The lineages and sublineages of these and other isolates from the 2005–06 season were inferred from phylogenetic comparisons to equivalent segments of the 11 lineages of G1, the 2 lineages of G2, the 4 lineages of G3, and the 6 lineages of G9 described by Phan et al. (*15*), Page and Steele (*16*), Wang et al. (*17*), and Martinez-Laso et al. (*18*), respectively. The G3 phylogenetic tree also includes 3 sequences obtained from the 5-year-old dipsticks and 4 G3 isolates from among those with highest homology to G3 isolates from Israel identified in BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (*19*). Specifically, all VP7 sequences for a given G genotype were aligned and truncated to the longest common segment. Four separate phylogenetic trees (Figure) were constructed because of the size difference between G genotype–specific heminested amplification products. The lineage or sublineage of each isolate from Israel (online Appendix Table) was inferred from the relative difference of each sequence compared with equivalent segments of previously determined lineages. For each genotype, a new lineage or sublineage was suggested when the sequence from Israel failed to closely group with the equivalent segment of ≥1 reference strains and the nucleotide differences between the strain from Israel and the reference strains were similar or greater than the differences among reference lineages and sublineages. The existence of such new lineages and sublineages would need to be confirmed with longer full-length open reading frame sequences. Finally, sequences from RNA recovered from dipsticks and/or from saline suspensions were used in BLAST searches to identify the most similar contemporary sequences. G1 isolates were most similar to isolates from Europe, the Far East, and South America; G2 isolates to isolates from the Far East; and G3 isolates to isolates from Europe and the Far East (Figure). G9 isolates were most similar to isolates from Africa, Europe, and the Far East.

Discussion

The G1P[8] genotype predominated in central Israel during the 2005–06 rotavirus season as it did throughout Israel during 1991–1994 (*20*) and in northern Israel during 2007–2009 (*2*,*10*). However, the relative distributions of other genotypes differed. For example, G4P[8], absent during 2005–2008, was present in 32.3% of samples a decade earlier and reappeared in 2008–09 (*2*). Conversely, G9 genotypes, absent a decade earlier, were present in 15.7% of the 2005–06 isolates and 9.3% of the 2006–07 isolates. This substantial prevalence of G9 mirrors the global emergence of G9 among hospitalized children in the mid-1990s (*21*,*22*). In addition, double infections indicated by >1 G or P genotype rose from <2% during 1991–1994 (*20*) to 16.4% for the isolates in this study and 27% during 2007–08 (*2*). As indicated by Muhsen et al (*2*), evidence is good for rotavirus reassortants emerging in vivo from appropriate double infections. Phylogenetic analysis in the present study suggested that some G2 and G9 rotaviruses from Israel belonged to new sublineages or lineages, respectively.

In conclusion, air-dried affinity-concentrated virus from rotavirus-positive bands of dipsticks yielded RNA suitable for G genotyping and sequencing, even for dipsticks stored at room temperature for 5 years. Even though P genotyping and sequencing was performed only on RNA isolated from 2 of the 5-year old dipsticks, it is reasonable to assume that P genotyping and sequencing would have been possible for the rest based on the equivalent quality of the RNA extracted from the dipsticks and RNA extracted from stool suspensions. Thus, dipsticks can be used for recovery of molecular epidemiologic data by reference laboratories from diagnostic tests routinely performed in many clinical laboratories and point-of-care facilities. RNA extracted from these archived dipsticks will enable centralized laboratories to easily recover epidemiologically useful data from the samples of other laboratories and from regions lacking adequate laboratory facilities. In addition, centralized laboratories will be able to assess whether the introduction of universal rotavirus vaccination changed the distribution of rotavirus genotypes associated with severe rotavirus-associated acute gastroenteritis.

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References

1. Parashar UD, Hummelman EG, Bresee JS, Miller MA, Glass RI. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis. 2003;9:565–72.
2. Muhsen K, Shulman L, Rubinstein U, Kasem E, Kremer A, Goren S, et al. Incidence, characteristics, and economic burden of rotavirus gastroenteritis associated with hospitalization of Israeli children <5 years of age, 2007–2008. J Infect Dis. 2009;200(Suppl 1):S254–63. DOI: 10.1086/605425
3. Widdowson MA, Bresee JS, Gentsch JR, Glass RI. Rotavirus disease and its prevention. Curr Opin Gastroenterol. 2005;21:26–31.
4. Parashar UD, Gibson CJ, Bresse JS, Glass RI. Rotavirus and severe childhood diarrhea. Emerg Infect Dis. 2006;12:304–6.
5. 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. DOI: 10.1086/605025
6. Estes MK, Cohen J. Rotavirus gene structure and function. Microbiol Rev. 1989;53:410–49.
7. Estes MK, Kapikian AZ. Rotaviruses. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, et al., editors. Fields virology. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1917–74.
8. Vesikari T, Matson DO, Dennehy P, Van Damme P, Santosham M, Rodriguez Z, et al. Safety and efficacy of a pentavalent human–bovine (WC3) reassortant rotavirus vaccine. N Engl J Med. 2006;354:23–33. DOI: 10.1056/NEJMoa052664
9. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, Abate H, Breuer T, Clemens SC, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. N Engl J Med. 2006;354:11–22. DOI: 10.1056/NEJMoa052434
10. Muhsen K, Shulman L, Kasem E, Rubinstein U, Shachter J, Kremer A, et al. Effectiveness of rotavirus vaccines for prevention of rotavirus gastroenteritis–associated hospitalizations in Israel: a case–control study. Hum Vaccin. 2010;6. Epub ahead of print.
11. 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.
12. Gunasena S, Nakagomi O, Isegawa Y, Kaga E, Nakagomi T, Steele AD, et al. Relative frequency of VP4 gene alleles among human rotaviruses recovered over a 10-year period (1982–1991) from Japanese children with diarrhea. J Clin Microbiol. 1993;31:2195–7.
13. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. 1997;25:4876–82. DOI: 10.1093/nar/25.24.4876
14. Perrière G, Gouy M. WWW-query: an on-line retrieval system for biological sequence banks. Biochimie. 1996;78:364–9. DOI: 10.1016/0300-9084(96)84768-7
15. Phan TG, Khamrin P, Quang TD, Dey SK, Takanashi S, Okitsu S, et al. Detection and genetic characterization of group A rotavirus strains circulating among children with acute gastroenteritis in Japan. J Virol. 2007;81:4645–53. DOI: 10.1128/JVI.02342-06
16. Page NA, Steele AD. Antigenic and genetic characterization of serotype G2 human rotavirus strains from the African continent. J Clin Microbiol. 2004;42:595–600. DOI: 10.1128/JCM.42.2.595-600.2004
17. Wang YH, Kobayashi N, Zhou X, Nagashima S, Zhu ZR, Peng JS, et al. Phylogenetic analysis of rotaviruses with predominant G3 and emerging G9 genotypes from adults and children in Wuhan, China. J Med Virol. 2009;81:382–9. DOI: 10.1002/jmv.21387
18. Martinez-Laso J, Roman A, Head J, Cervera I, Rodriguez M, Rodriguez-Avial I, et al. Phylogeny of G9 rotavirus genotype: a possible explanation of its origin and evolution. J Clin Virol. 2009;44:52–7. DOI: 10.1016/j.jcv.2008.08.022
19. Zhang Z, Schwartz S, Wagner L, Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol. 2000;7:203–14. DOI: 10.1089/10665270050081478
20. Silberstein I, Shulman LM, Mendelson E, Shif I. Distribution of both rotavirus VP4 genotypes and VP7 serotypes among hospitalized and nonhospitalized Israeli children. J Clin Microbiol. 1995;33:1421–2.
21. 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. DOI: 10.1002/rmv.448
22. Santos N, Volotao EM, Soares CC, Campos GS, Sardi SI, Hoshino Y. Predominance of rotavirus genotype G9 during the 1999, 2000, and 2002 seasons among hospitalized children in the city of Salvador, Bahia, Brazil: implications for future vaccine strategies. J Clin Microbiol. 2005;43:4064–9. DOI: 10.1128/JCM.43.8.4064-4069.2005

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Seroprevalence of African Swine Fever in Senegal, 2006

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In Senegal, during 2002–2007, 11 outbreaks of African swine fever (ASF) were reported to the World Organisation for Animal Health. Despite this, little was known of the epidemiology of ASF in the country. To determine the prevalence of ASF in Senegal in 2006, we tested serum specimens collected from a sample of pigs in the 3 main pig-farming regions for antibodies to ASF virus using an ELISA. Of 747 serum samples examined, 126 were positive for ASF, suggesting a prevalence of 16.9%. The estimated prevalences within each of the regions (Fatick, Kolda, and Ziguinchor) were 13.3%, 7.8%, and 22.1%, respectively, with statistical evidence to suggest that the prevalence in Ziguinchor was higher than in Fatick or Kolda. This regional difference is considered in relation to different farming systems and illegal trade with neighboring countries where the infection is endemic.

African swine fever (ASF) is a disease caused by a DNA virus in the family *Asfarviridae*. The disease is highly contagious and often lethal for pigs and is of considerable economic importance, due to the direct loss of animals as well as resulting trade restrictions. No vaccine is available against the virus. The epidemiology of ASF is complex, transmission is direct and vector-borne, and the disease has well recognized sylvatic and domestic cycles.

ASF is currently considered enzootic in eastern and southern Africa, and the epidemiologic cycles of importance in many of the countries in these regions are well understood (*1*). In contrast, little is known about the epidemiology of the infection in West Africa, despite evidence of considerable spread of disease in this region in the late 1990s. Since it was first identified in Senegal in 1959, frequent reports of outbreaks of ASF in the country have been made to the World Organisation for Animal Health (OIE). Since 1986, a total of 54 outbreaks have been reported, with periods of frequent reports (19 outbreaks during 1986–1989; 15 outbreaks during 1999–2003) and periods with a lower frequency of reports (15 outbreaks during 1989–1998; 5 outbreaks during 2004–2006). The sylvatic cycle likely plays little role in the epidemiology of ASF in West Africa.

The suggestion has been made that in Senegal a domestic cycle of infection involving ticks may be possible because of the enzootic nature of disease in the country and the identification of infected soft ticks in some pig pens (*2*). Nevertheless, a pig-to-pig domestic cycle appears to be the main cycle of infection in the country, due to the large free-ranging pig population, along with regular reintroduction from disease-endemic countries. The pig sector plays a large part in the economy in several regions of Senegal, and has dramatically increased in size in recent years (from 191,000 pigs in 1997 [*3*] to 320,000 in 2005 [*4*]). The consequences of ASF outbreaks in many countries are catastrophic, with major economic losses, and in developing countries, considerable social effects may result: the loss of employment for farm workers, the loss of an major source of income for farmers, the loss of a major source of high quality and cheap protein for poor communities, and the consequences for traditional ceremonies (for which pigs are often required, as is seen in Cameroon and Côte d'Ivoire) (*5*,*6*).

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Because of religious dietary restrictions, pigs within Senegal are principally clustered within regions containing the majority of the non-Muslim population, such as the population of the Casamance (the Ziguinchor and Kolda regions), and in areas where tourism has increased the demand for pork, such as Sine Saloum (the Fatick region). Although ASF has been identified as one of the 6 major diseases in need of epidemiologic surveillance in Senegal, few structured surveys have been conducted (*7*). A problem of underreporting of disease in the country was identified by Lefèvre in 1998; he explained that a large gap exsisted between declarations and reality (*3*). To date, no statistical data are available on the epidemiology of ASF in Senegal. Therefore, this study was designed to fill that information gap and document the seroprevalence of African swine fever in Senegal.

Materials and Methods

Sampling Protocol

The sampling protocol adopted in this study was based on the information obtained during a survey of pig production systems in Senegal (*7*). As mentioned earlier, the regions of Fatick, Kolda, and Ziguinchor were selected for the study because they corresponded to the area with the largest pig populations in Senegal. Kolda and Ziguinchor are located in the area of Casamance, which borders both Guinea-Bissau and the Gambia (within which, ASF is enzootic); and Fatick (an area where pig production is more dedicated to pig fattening) is located between Casamance and Dakar, the main area of pork meat consumption. Casamance and the Fatick region contain 75% of all pigs in Senegal (*4*) (Figure).

Free-range farming has been identified previously as the most widespread pig farming system in Senegal, with a recent study estimating that 76% (95% confidence interval 72%–80%) of all farms in the country were free-range systems (*7*). Therefore, villages as well as individual farms were considered as potential clusters.

A multistage sampling approach was adopted: the random selection of villages was followed by the random selection of farms within these villages. To estimate the required sample size, a prevalence of 50% was assumed (to maximize the required sample size), with a required precision of 6% and an α-error of 5%. Villages were considered as clusters of animals, and a decision was made to sample 10 pigs per village, from as many different farms as possible to maximize the representativeness of the sample. The formulas used in determining sample size, while accounting for clustering at the village level, are shown below (*8*,*9*):

$$n = \mathrm{P}(1-\mathrm{P})\frac{Z_\alpha^{\ 2}}{(\mathrm{AP})^2}$$

$$n' = n(1 + \rho(m-1))$$

Figure. Sampled villages (black dots) in the 3 main regions of Senegal for pig production, Fatick, Ziguinchor, and Kolda (gray shading). Dashed lines indicate the 700 mm (gray) and 800 mm (black) rainfall isohyets for 2006. The southern limit range of *Ornithodoros sonrai* tick distribution (750 mm) can be estimated.

where n is the sample size without correction, Zα is the percentile point relating to the required α error under the z distribution, AP is the absolute precision, P is the estimated prevalence, n′ is the final sample size, ρ is the intracluster correlation, and m is the number of units sampled in each village. An intracluster correlation of 0.2 was used, because it rarely exceeds this value in the case of infectious disease (*10*).

The required sample size was 748, from a total of 75 villages; 756 pigs were actually sampled, from 82 villages and 205 farms (Table 1; Figure). Due to logistical and cost issues, the number of villages sampled in each region was not equal. Following a single exposure to ASF virus, antibodies will persist for at least 2 years (*11*). Therefore, to reduce the effect of past exposure to virus, only pigs from 6 months to 2 years of age were sampled. All pigs appeared healthy at the time of sampling.

Dates and Laboratory Analysis

Sampling was undertaken in May and July 2006, during the dry season. Blood samples were collected from the jugular vein in plain tubes and were centrifuged to obtain serum. Serologic analysis was performed by using an Ingezim PPA Compac 1.1.PPA K3 ELISA kit (Ingenasa, Madrid, Spain), which is a blocking ELISA that uses a purified protein extract from the virus (VP73) as the antigen. According to C. Gallardo (researcher at Centro de Inestigacion en Sanidad Animal, Madrid, Spain; pers. comm.) the sensitivity and specificity of this test were both in the

Table 1. Numbers of villages and farms sampled and number of realized samples for African swine fever virus in each study region, Senegal, 2006

Characteristic	Fatick	Kolda	Ziquinchor
No. villages	15	24	43
No. farms	72	64	69
Realized samples	152	286	318

region of 95% to 98%. The apparent prevalence estimates were therefore corrected to give the true prevalence by using the following formula (*9*):

$$TP = \frac{AP + Sp - 1}{Se + Sp - 1}$$

where TP is the true prevalence; Se and Sp are the sensitivity and specificity of the test, respectively; and AP is the apparent prevalence according to the test results.

Statistical Analysis

To account for clustering within villages and farms when estimating the regional prevalence, a general linear mixed model method was adopted; the 'lme4' package within R software was used (www.r-project.org). Farms were nested within villages, and both were modeled as random effects. A z test was conducted to compare these random effects, to evaluate whether evidence of clustering of seropositivity could be found within villages and farms. The prevalence estimates in different regions were then compared by using the method described by Altman and Bland (*12*).

Results

The z test found evidence of clustering of seropositivity within villages and farms ($p<0.05$). The general linear mixed model method gave seroprevalence estimates of 13.3%, 7.8%, and 22.1% for the regions of Fatick, Kolda, and Ziguinchor, respectively (Table 2). The prevalence estimate for Ziguinchor was significantly higher than that for Kolda ($p<0.05$) and that for Fatick ($p<0.1$).

Test sensitivity and specificity were accounted for to estimate the true seroprevalence for each region. We gave the smallest and higher value taking into account the uncertainty around the true value of sensitivity and specificity. Following this process, the seroprevalence estimates for Fatick, Kolda, and Ziguinchor were expanded to range from 8.9% to 12.1%; from 3.0% to 6.2%; and from 18.3% to 21.6%, respectively. 95% confidence intervals for these estimates are shown in Table 2.

Discussion

The results of this study are corroborated by unpublished data from the Senegalese Institute of Agricultural Research regarding the prevalence of ASF in the Ziguinchor region (*13*). Previous prevalence data from the Kolda region were not available. In 1988, no ASF antibodies were detected in pigs in the Fatick region; whereas 18 years later, the disease seemed to circulate periodically (*13*). This was probably due to virus spread from Guinea-Bissau (where the disease is enzootic) to Casamance, and then to the Gambia (where persons originally from Guinea Bissau, Casamance, and the Gambia all produce pigs).

Haresnape et al. conducted a seroprevalence study for ASF virus in Malawi and also collected information from pig owners about clinical signs and illness duration (*14*,*15*). The virus was considered enzootic in the western part of the central region between 1981 and 1986, where prolonged outbreaks of ASF were common. However, in the southern regions, ASF occurred in intermittent epizootics, and no evidence of ASF virus circulation was found in the northern region. On the basis of these findings, ASF virus strains of low virulence were believed to be present in the country (*14*), although no experimental proof was given. Infected meat introduced from affected areas was proposed to be the main source of ASF outbreaks, although warthogs tested positive for antibodies against ASF virus in the southern region. This finding suggested that a wildlife reservoir could play a role in the epidemiology of ASF in the country. In 9 of the 24 districts of Malawi, ASF virus was also detected in *Ornithodoros moubata* ticks, which are likely acting as a virus reservoir and vector of ASF virus (*16*).

The ASF situation in Mozambique was different from that in Malawi. In a study conducted in 1998, antibodies to ASF virus were detected in healthy pigs in the Angonia district, close to the Malawi border, indicating that these pigs survived an outbreak. However, experiments showed that this resistance was not highly heritable (*11*). ASF virus was considered enzootic in this district and was maintained in the population through a cycle involving domestic pigs only. No evidence of soft ticks, warthogs, or bush pigs was found in the area.

The current study has presented the estimated seroprevalence among pigs sampled within 3 regions and has accounted for clustering of seropositive individual pigs within farms and within villages. A more detailed characterization of the seroprevalence pattern could be conducted by estimating the presence of virus at different hierarchical

Table 2. Individual prevalence of African swine fever in main pig breeding regions, Senegal, 2006

Region	No. pigs sampled	Estimated individual prevalence, %	95% confidence interval for the apparent prevalence	Uncertainty interval for the true prevalence, %
Fatick	149	13.3	8.0–21.2	3.2–20.6
Kolda	281	7.8	4.9–12.2	0–11.0
Ziguinchor	317	22.1	16.9–28.3	12.8–28.3

aggregations; that is, the proportion of infected villages, the proportion of infected farms within infected villages, and the proportion of infected animals within infected farms. However, such an analysis, using hierarchical Bayesian modeling, for example, is beyond the scope of the present study (*17*).

True seroprevalence estimates were calculated by taking into account the sensitivity and specificity of the ELISA, which were estimated by using serum specimens from European domestic pigs (C. Gallardo, pers. comm.). Considering that ASF viruses currently circulating in West Africa are closely related to those circulating in Europe in the second half of the last century (*18*) (which are different from those currently circulating in Russia [*19*]), we can assume that the ELISA is appropriate for analyzing serum samples from Senegal.

Ornithodoros sonrai ticks containing remnants of ASF virus DNA have been identified in the Fatick region (*2*), which suggests the existence of an epidemiologic cycle in which ticks act as a reservoir, as occurs in eastern and southern Africa (*16*). However, the absence of a statistical association between the presence of ticks on pig farms and reported cases (or farmers' suspicions of cases) of ASF, 3 years before the current survey, suggests that even if ticks were responsible for resurgence (*20*), they may not play a major role in the spread or emergence of ASF in this region, in contrast to the situation in Malawi (*16*). The Ziguinchor and Kolda regions are located below the 750 mm isohyet (≈13°30′N in Senegal; Figure), the southern limit of the reported geographic distribution of *O. sonrai* ticks (*21*,*22*). Therefore, the high prevalence of ASF in Ziguinchor is likely to be predominantly due to direct transmission between pigs, with little or no vector contribution. No bush pigs are present in Senegal, and according to local hunters and hunting settlements, warthogs in the Fatick region are scarce (with no available data on warthog numbers in the other regions). As such, the epidemiologic cycling of the virus in the country likely only involves domestic pigs, and the virus persists due to the large free-ranging pig population, as is the case in Mozambique (*11*). Further serologic studies involving warthogs are necessary to confirm the limited role of warthogs in the cycle of ASF in Senegal.

Although ASF virus can persist for long periods after infection and even recovery in pigs, seroprevalence estimates for the antibodies against the virus do not estimate the percentage of pigs with current infection, or even the percentage of carrier pigs. Rather, they indicate the percentage of pigs that have been exposed to the virus at some point in their lifetime. Bech-Nielsen et al. reported the detection of ASF virus in only 4.4% of carrier animals (*23*). Penrith et al. confirm also that fully recovered pigs apparently do not become long-term carriers (*11*). ASF antibody testing is recommended for the study of subacute and chronic forms of the disease (*24*). Also, the presence of antibodies against ASF virus does not imply that pigs are protected against new infections (*25*), since cellular immunity is essential for protection against ASF virus (*26*).

Considering that only pigs from 6 months to 2 years of age were tested, the pigs that tested positive must have become infected between 2004 and 2006. During this period, 5 outbreaks were declared in Senegal, with 646 cases and 561 deaths (*27*,*28*). When our data were compared with these official reports, we concluded that many cases were not declared by the farmers, possibly to avoid the costs of veterinary intervention and prohibition of animal movement.

Furthermore, our results suggest that these pigs survived virus infection, which contrasts with the widespread perception that mortality rates for ASF virus infection are high, approaching 80% (*25*,*29*). This high mortality rate mainly applies to the acute forms of the disease, which are more likely to be reported because of their dramatic effects on farms with large numbers of pigs or because they might have been responsible for the disappearance of farms with small number of pigs. Studies conducted in Spain and Portugal identified animals that have survived infections with ASF virus (*23*). Two possible explanations for the findings of the current study are that strains of ASF virus in Senegal have low virulence or that local breeds of pigs have some form of resistance to circulating ASF virus strains. In either case, the presence of healthy animals with antibodies suggests that ongoing circulation of ASF virus in the pig population in Senegal is a serious issue. This could explain the enzootic state of the disease in Senegal, even if stress factors are often needed to reactivate the transmission (*29*).

ASF virus strains of low virulence have been identified in various countries since 1984, and despite low virulence, could still maintain a high infectivity (*30*). In Senegal, however, in vivo tests on Large White pigs using ASF viruses isolated from pig leukocytes during 1987–1989 showed high virulence (*31*). These strains predominantly originated from Casamance (6 strains from 10 isolations), but more research, with experimental infection, is necessary to confirm whether new strains with low virulence are currently in Senegal. Indeed, outbreaks of ASF with high pig mortality rates have been reported in West Africa in the late 1990s: for example, in Côte d'Ivoire in 1996(*5*), in Benin and Togo in 1997 (*3*), and in Nigeria during 1997–1998 (*32*). However, the 11 reported outbreaks of ASF in Senegal from 2002 through 2007 had mortality rates varying between 100% and 31% (*27*,*28*). Epidemiologic patterns of disease characterized by frequent outbreaks with low mortality have also been described in enzootic areas of southern Africa (Malawi [*14*,*33*] and Mozambique [*11*]).

Antibody-mediated resistance to ASF virus can be acquired through passive transfer of maternal antibodies or

by previous infection with a virus of low pathogenicity or from low doses of highly virulent viruses (*11*). Development of protective immunity to ASF virus infection through either of these mechanisms could explain why healthy pigs with ASF antibodies were identified in the current study and why low mortality rates after exposure have been recorded in Senegal. These findings should be explored in further studies.

Significant differences in seroprevalence were observed between the 3 regions, with a higher seroprevalence identified in Ziguinchor than in Kolda or Fatick. The Ziguinchor region lies between the Gambia and Guinea-Bissau. ASF has been enzootic within Guinea-Bissau for years, and no efforts to control the disease have been reported (*3*). The legal and illegal trade of pigs between these neighboring countries could explain the higher prevalence observed in that region. Although the Kolda region also shares borders with these 2 countries, its eastern geographic location provides a drier and hotter climate, making it less favorable for pig farming. As a result of this and other economic issues, pig farming practices within the Kolda region are not as developed and organized as they are in the Ziguinchor region (*7*).

A study conducted in 1987 and 1988 found no evidence of seropositive animals among the 122 samples in the Fatick region (*13*). The current study, however, identified seropositive pigs in this region. This change may be a result of the development of pig trade between Fatick and the Ziguinchor region and the Gambia. Dakar, which lies north of the Fatick region, has one of the largest markets for pork in Senegal, and Fatick is therefore a crossing point for most of the pigs imported from the Gambia (*34*). Additionally, the recent development of the tourism industry in the Petite Côte and the Sine Saloum areas of the Fatick region over the past 10 years has increased the number of pigs in the region. The recent identification of ASF virus DNA in soft ticks in the Fatick region gives further evidence in support of spread of ASF virus into this region (*2*). The possible regular reintroduction of the virus from the Gambia or the Ziguinchor region could contribute to virus persistence in the region. Because trade is a likely factor affecting virus presence and persistence in all of the regions studied, further investigation of pig trade and the supply chains present in these regions is warranted.

Although ASF has been known to be enzootic in the Ziguinchor region for >10 years (*3*,*27*) (with all ASF reports to the OIE from 2002 through 2006 coming from this region), no cases have been reported in the Kolda region since 1996 (*27*). No cases from Fatick have been officially reported and reports from more northern regions (Thiès, Kaolack) have also been scarce. Lack of reporting of ASF cases could be explained by a limited interest by the authorities in the development of large-scale pig farms (*35*). A more accurate surveillance system, combined with compulsory reporting, could therefore help control the spread of the disease. Developing this system would require development of resources for the local veterinary services. A risk-based surveillance approach, involving the awareness of the pig farming community, would allow more efficient control of the disease, but will require further analysis of risk factors for infection in Senegal. A new public health policy regarding this issue, which includes a strategy of information dissemination about the disease and its risk factors among the pig farming community, is urgently needed.

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References

1. Penrith ML, Vosloo W. Review of African swine fever: transmission, spread and control. J S Afr Vet Assoc. 2009;80:58–62.
2. Vial L, Wieland B, Jori F, Etter E, Dixon L, Roger F. African swine fever virus DNA in soft ticks, Senegal. Emerg Infect Dis. 2007;13:1928–31.
3. Lefèvre P-C. African swine fever in West Africa: Togo, Senegal, Gambia, Guinea-Bissau. Rome: Food and Agricultural Organization of the United Nations, Animal Production and Health Division; 1998. p. 14.
4. Ministère de l'Élevage, Sénégalais. Rapport annuel de la Division de la Protection Zoosanitaire. Dakar (Senegal): Direction de l'Élevage, Ministère de l'Élevage; 2006. p. 7.
5. el Hicheri K, Gomez-Tejedor C, Penrith ML, Davies G, Dovati A, Edoukov GJ, et al. The 1996 epizootic of African swine fever in the Ivory Coast. Rev Sci Tech. 1998;17:660–73.
6. Nana-Nukechap MF, Gibbs EP. Socioeconomic effects of African swine fever in Cameroon. Trop Anim Health Prod. 1985;17:183–4. DOI: 10.1007/BF02356970
7. Ndiaye RK. Epidémiologie de la peste porcine africaine au Sénégal: facteurs de risque en relation avec l'élevage porcin dans les régions de Fatick, Kolda et Ziguinchor [thesis]. Dakar (Senegal): Université Cheikh Anta Diop et Ecole Inter-Etats des Sciences et Médecine Vétérinaire; 2007.
8. Thrusfield M. Veterinary epidemiology. Oxford: Blackwell Scientific Publications;1995.
9. Dohoo I, Martin W, Stryhn H. Veterinary epidemiologic research. Charlottetown (PEI, Canada): AVC Inc.; 2003.

10. Otte MJ, Gumm ID. Intra-cluster correlation coefficients of 20 infections calculated from the results of cluster-sample surveys. Prev Vet Med. 1997;31:147–50. DOI: 10.1016/S0167-5877(96)01108-7
11. Penrith ML, Thomson GR, Bastos AD, Phiri OC, Lubisi BA, Du Plessis EC, et al. An investigation into natural resistance to African swine fever in domestic pigs from an endemic area in southern Africa. Rev Sci Tech. 2004;23:965–77.
12. Altman DG, Bland JM. Interaction revisited: the difference between two estimates. BMJ. 2003;326:219. DOI: 10.1136/bmj.326.7382.219
13. Sarr J, Diop M. Situation épizootiologique de la peste porcine africaine au Sénégal. Dakar (Sénégal); Institut Sénégalais de Recherche Agricole, Laboratoire de l'Élevage et de Recherches Vétérinaires; 1990. p. 13.
14. Haresnape JM, Lungu SA, Mamu FD. A four-year survey of African swine fever in Malawi. J Hyg (Lond). 1985;95:309–23. DOI: 10.1017/S0022172400062732
15. Haresnape JM, Lungu SA, Mamu FD. An updated survey of African swine fever in Malawi. Epidemiol Infect. 1987;99:723–32. DOI: 10.1017/S0950268800066589
16. Haresnape JM, Wilkinson PJ, Mellor PS. Isolation of African swine fever virus from ticks of the *Ornithodoros moubata* complex (Ixodoidea: Argasidae) collected within the African swine fever enzootic area of Malawi. Epidemiol Infect. 1988;101:173–85. DOI: 10.1017/S0950268800029332
17. Suess EA, Gardner IA, Johnson WO. Hierarchical Bayesian model for prevalence inferences and determination of a country's status for an animal pathogen. Prev Vet Med. 2002;55:155–71. DOI: 10.1016/S0167-5877(02)00092-2
18. Bastos AD, Penrith ML, Crucière C, Edrich JL, Hutchings G, Roger F, et al. Genotyping field strains of African swine fever virus by partial p72 gene characterisation. Arch Virol. 2003;148:693–706. DOI: 10.1007/s00705-002-0946-8
19. Rowlands RJ, Michaud V, Heath L, Hutchings G, Oura C, Vosloo W, et al. African swine fever virus isolate, Georgia, 2007. Emerg Infect Dis. 2008;14:1870–4. DOI: 10.3201/eid1412.080591
20. Sanchez-Vizcaino JM, Martinez-Lopez B, Martinez-Avilés M, Martins C, Boinas F, Vial L, et al. Scientific report submitted to European Food Safety Authority: Scientific review on African swine fever [cited 2010 Nov 10]. http://www.efsa.europa.eu/en/scdocs/doc/5e,0.pdf
21. Trape JF, Godeluck B, Diatta G, Rogier C, Legros F, Albergel J, et al. The spread of tick-borne borreliosis in West Africa and its relationship to sub-Saharan drought. Am J Trop Med Hyg. 1996;54:289–93.
22. Lecompte Y, Trape JF. West African tick-borne relapsing fever. Ann Biol Clin (Paris). 2003;61:541–8.
23. Bech-Nielsen S, Fernandez J, Martinez-Pereda F, Espinosa J, Perez Bonilla Q, Sanchez-Vizcaino JM. A case study of an outbreak of African swine fever in Spain. Br Vet J. 1995;151:203–14. DOI: 10.1016/S0007-1935(95)80012-3
24. World Organisation for Animal Health. Terrestrial animal health code. Paris: The Organisation; 2007.
25. Ayoade G, Adeyemi I. African swine fever: an over-view. Rev Elev Med Vet Pays Trop. 2003;56:129–34.
26. Oura CA, Denyer MS, Takamatsu H, Parkhouse RM. In vivo depletion of CD8+ T lymphocytes abrogates protective immunity to African swine fever virus. J Gen Virol. 2005;86:2445–50. DOI: 10.1099/vir.0.81038-0
27. World Organisation for Animal Health. Handistatus II [database online]. Paris: The Organization; 2005 [cited 2010 Nov 10]. http://www.oie.int/fr/info/fr_infold.htm?eld5
28. World Organisation for Animal Health. WAHID [database online]. Paris: The Organization; 2007 [cited 2010 Nov 10]. http://www.oie.int/wahis/public.php?page=home
29. Sánchez Botija C. African swine fever. New developments. Rev Sci Tech. 1982;1:1065–94.
30. Pan IC, Hess WR. Virulence in African swine fever: its measurement and implications. Am J Vet Res. 1984;45:361–6.
31. Sarr J, Diop M. Etude de la peste porcine africaine au Sénégal. Rapport final du projet européen: Contrat n°TSDA 219/CEE. Dakar (Senegal); Institut Sénégalais de Recherche Agricole, Laboratoire de l'Élevage et de Recherches Veterinaries; 1990. p. 32.
32. Odemuyiwa SO, Adebayo IA, Ammerlaan W, Ajuwape AT, Alaka OO, Oyedele OI, et al. An outbreak of African swine fever in Nigeria: virus isolation and molecular characterization of the VP72 gene of a first isolate from West Africa. Virus Genes. 2000;20:139–42. DOI: 10.1023/A:1008118531316
33. Haresnape JM. African swine fever in Malawi. Trop Anim Health Prod. 1984;16:123–5. DOI: 10.1007/BF02239857
34. Laleye FX. La filière porcine au Sénégal: commercialisation et consommation des viandes de porc et de phacochère dans les départements de Dakar, Fatick, Ziguinchor et Kolda [thesis]. Dakar (Senegal): Université Cheikh Anta Diop et École Inter-États des Sciences et Médecine Vétérinaire; 2007.
35. Penrith ML, Lopes Pereira C, Lopes da Silva MM, Quembo C, Nhamusso A, Banze J. African swine fever in Mozambique: review, risk factors and considerations for control. Onderstepoort J Vet Res. 2007;74:149–60.

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Molecular Typing of Protease-Resistant Prion Protein in Transmissible Spongiform Encephalopathies of Small Ruminants, France, 2002–2009

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The agent that causes bovine spongiform encephalopathy (BSE) may be infecting small ruminants, which could have serious implications for human health. To distinguish BSE from scrapie and to examine the molecular characteristics of the protease-resistant prion protein (PrP^{res}), we used a specifically designed Western blot method to test isolates from 648 sheep and 53 goats. During 2002–2009, classical non-Nor98 transmissible spongiform encephalopathy had been confirmed among ≈1.7 million small ruminants in France. Five sheep and 2 goats that showed a PrP^{res} pattern consistent with BSE, or with the CH1641 experimental scrapie source, were identified. Later, bioassays confirmed infection by the BSE agent in 1 of the 2 goats. Western blot testing of the 6 other isolates showed an additional C-terminally cleaved PrP^{res} product, with an unglycosylated band at ≈14 kDa, similar to that found in the CH1641 experimental scrapie isolate and different from the BSE isolate.

Transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases that include scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and Creutzfeldt-Jakob disease (CJD) in humans (*1*,*2*). TSEs are characterized by accumulation in the brain of a disease-associated isoform (PrP^{d}) of a host-encoded cellular prion protein (PrP^{c}) (*3*). PrP^{d}, in comparison with the normal prion protein PrP^{c}, clearly differs in secondary and tertiary structures (*4*,*5*) and in biochemical characteristics (*6*). Proteinase K (PK) digestion destroys PrP^{c}, but in PrP^{d} it generates a protease-resistant fragment known as PrP^{res}. Most TSE diagnostic methods (e.g., ELISA and Western blot tests) are based on detection of PrP^{res} (*7*).

The transmissible agent involved in BSE in cattle is known to cause prion diseases in other species under natural conditions (*8*). BSE can also be experimentally transmitted to sheep and goats, including after oral challenge to test for transmission (*9*). Because BSE-contaminated meat and bone meal may have been fed to small ruminants, BSE may have been transmitted to sheep or goats. Also, the Scientific Steering Committee of the European Commission has hypothesized that the BSE agent might have originated from a scrapie agent in sheep or goats and that these animals may represent a reservoir (*10*). In view of these data, the European Commission defined a strategy to investigate the possible presence of BSE in sheep and goats under natural conditions (*11*).

The standard for strain typing TSE agents is based on analysis of the phenotypic characteristics of the disease after transmission in laboratory rodents. Biological characterization of the BSE agent in inbred wild-type mice appeared to be reliable, because it showed uniform features in mice (*8*). However, this approach is time-consuming and costly. The identification of uniform molecular features of PrP^{res} by Western blot in human variant CJD paved the way to a similar approach for detecting possible BSE in small ruminants (*12*). The molecular criteria defined in these studies included electrophoretic mobilities, glyco-

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sylation characteristics, and immunolabeling with different monoclonal antibodies (*13*). The last criteria enabled mapping of the protease cleavage site of the PrP protein fragment obtained after PK digestion (*14*). More recently, the identification of additional C-terminal PrP^{res} products may contribute to discrimination of the different types of CJD (*15*) or of different scrapie and BSE sources (*16*,*17*). Discriminant molecular features of the prion protein can also be investigated by immunohistochemical analysis (*18*) or ELISA (*19*). In all of these studies, it was assumed that the strain information was closely associated with the structural features of PrP^{d}.

The Western blot method enabled discrimination of experimental BSE in sheep from most scrapie-affected animals (*12*,*13*,*20*–*24*). Nevertheless, discrimination was more difficult with the CH1641 experimental scrapie isolate (*21*,*25*), which otherwise clearly differs from BSE by its absence of transmissibility to wild-type mice (*26*). Similar molecular features to those of CH1641 have been described in a few natural scrapie cases in France (*27*) and in the United Kingdom (*24*). We describe the molecular findings obtained for a large series of TSE infections in France identified in small ruminants by active surveillance during 2002–2009 and for CH1641-like isolates in sheep and in 1 goat.

Materials and Methods

Animals and Tissues

Two samples of BSE in small ruminants were used as controls, 1 (SB1) from a sheep experimentally infected by BSE with a brain homogenate from a BSE-affected cow from France (*21*), and the other (CH41x76) from a goat that had been intracerebrally inoculated with a brain homogenate from cattle BSE from Britain (*28*).

Two experimental scrapie sources in sheep (provided by N. Hunter, Institute for Animal Health, Edinburgh, UK) were also studied. These were the SSBP/1 scrapie isolate, experimentally maintained by serial passages in sheep (*29*), and CH1641 that was derived from a Cheviot sheep and then maintained by serial passages in sheep (*26*).

Natural TSE isolates in small ruminants (Table) were mainly obtained through an active surveillance program in France. In operation since 2002, this program involves the random selection and testing of samples from rendering plants and slaughterhouses (*30*). Some samples originated from the passive surveillance program, which involves collecting samples from animals showing suspected clinical signs of the disease. Brain stems from all selected animals are subjected to a rapid test, and all reactive samples are then retested in the National Reference Laboratory by confirmatory methods based on Western blot (*31*). When a sample is confirmed as TSE positive, it is classified as similar, or not similar, to Nor98 (atypical scrapie), according to the PrP^{res} molecular profile, which in atypical scrapie shows 5 major bands (*31*). Samples that are not similar to Nor98 (classical TSE), i.e, showing a 3-band pattern between 19–30 kDa, are then studied by using a Western blot discriminatory method to identify possible similarities with BSE (*11*).

Table. Results of TSE diagnostic tests and molecular characterization for sheep and goats, France, 2002–2009*

Year	Species	No. animals tested by rapid tests	Confirmed TSE cases	Nor98-like isolates	Molecular characterization			
					Not analyzed	Not defined	Scrapie	BSE-like
2002	Ovine	68,580	153	15	0	16	122	0
	Caprine	27,087	14	1	0	3	9	1
2003	Ovine	63,207	117	28	0	7	81	1
	Caprine	23,161	10	2	0	2	6	0
2004	Ovine	24,639	58	7	3	0	48	0
	Caprine	5,730	3	0	0	0	3	0
2005†	Ovine	34,290	74	9	19	1	44	1
	Caprine	148,338	16	4	2	1	9	0
2006‡	Ovine	492,023	382	182	1	6	190	3
	Caprine	165,606	10	1	0	1	8	0
2007§	Ovine	327,894	264	173	1	4	86	0
	Caprine	183,498	7	4	0	2	1	0
2008¶	Ovine	86,269	71	45	0	0	26	0
	Caprine	79.966	12	8	0	0	3	1
2009	Ovine	55,163	34	22	0	1	11	0
	Caprine	52,248	6	3	0	0	3	0
Total	Ovine	1,152,065	1,153	481	24	35	608	5
	Caprine	685,634	78	23	2	9	42	2

*TSE, transmissible spongiform encephalopathy; BSE, bovine spongiform encephalopathy.
†January 2005, beginning of exhaustive testing of goats in abattoir and rendering plant.
‡First quarter 2006, beginning of exhaustive testing of sheep in abattoir and rendering plant.
§January 2007, end of exhaustive testing of sheep in abattoir.
¶February 2008, end of exhaustive testing of goats in abattoir and sheep in rendering plant.

Western Blot

All confirmed classical TSE (non–Nor98-like) cases were analyzed to determine whether the PrP^{res} looked similar to that found in bovine BSE (i.e., showed a lower molecular mass than that found in bovine BSE) or, on the contrary, was similar as in most scrapie cases. PrP^{res} extracts were obtained from animal brains by using Bio-Rad protocol (the TeSeE Western Blot kit, ref: 355 1169; Bio-Rad, Marnes-la-Coquette, France), which is used to confirm suspected TSE-positive samples and includes PK digestion and rapid protein precipitation steps. Denatured samples were then loaded on two 15% bis-polyacrylamide gels with the same gel plan. After electrophoresis (200 volts for 80 min), the proteins were transferred onto a nitrocellulose membrane (R-Biopharm, St. Didier au Mont d'Or, France). Blocking was performed for 1 h in 5% (wt/vol) milk powder in phosphate-buffered saline (PBS) containing 0.1 % (vol/vol) Tween-20 (PBS-T) for the membrane to be treated with monoclonal antibody Bar233 (1/5000) (144-FGNDYEDRYYRE-155 ovine PrP sequence) (provided by J. Grassi, C.E.A., Saclay, France) or in 3% (wt/vol) bovine serum albumin solution (Sigma, St. Quentin-Fallavier, France) in PBS-T for the membrane to be treated with monoclonal antibody P4 (0.2 mg/mL) (93-WGQGGSH-99 ovine PrPsequence) (R-Biopharm).

Both antibodies were incubated on the membranes for 30 min at room temperature. The membranes were then washed for 20 min in PBS-T and incubated with a solution of streptavidine-peroxidase–conjugated antimouse immunoglobulin (Southern Biotech distributed by Clinisciences, Montrouge, France) in PBS-T for 20 min at room temperature. The membranes were then washed for 30 min in PBS-T and for 5 min in PBS before detection by use of enhanced chemiluminescent substrate (Amersham Biosciences, Orsay, France). The signals were identified on autoradiographic films (Amersham) after a 3-min exposure. Quantitative studies were performed by using Quantity One software (Bio-Rad), and the apparent molecular masses were determined by comparing the positions of the PrP^{res} bands with a biotinylated marker (B2787) (Sigma). All samples were compared, during molecular characterization of field isolates, by expressing the molecular mass of the unglycosylated band in terms of differential molecular mass (dmm). The dmm corresponds to the difference measured between the test sample and the control cattle BSE sample always loaded beside it.

The possible presence of additional C-terminal PrP^{res} products (PrP^{res} #2) (*17*) was detected and quantified by deglycosylation by using peptide N-glycosidase F (PNGase) (kit P07043; BioLabs distributed by Ozyme, Saint-Quentin-en-Yvelines, France) as described (*17*). The deglycosylated PrP^{res} was detected with SAF84 (0.6 mg/mL) (167-RPVDQY-172 ovine PrP sequence) (SPI-Bio, Montigny le Bretonneux, France) mouse monoclonal antibody. The respective proportions of ≈14- and ≈19-kDa bands, observed after PNGase deglycosylation, were quantified by using Quantity One software (Bio-Rad).

Results

Active Surveillance Findings during 2002–2009

Since active surveillance of TSEs in small ruminants began in France in 2002, a total of 1,231 small ruminant (1,153 sheep and 78 goats) samples have been confirmed as TSE-positive by Western blot using Sha 31 antibody; >1.7 million animals have been tested by rapid tests (1,152,065 sheep and 685,634 goats) (Table). Nearly half of the positive isolates (504) have been identified as Nor98 scrapie isolates, on the basis of detection of 5 major bands, including a prominent ≈10–12 kDa band detected by Western blot (*32*). After confirmatory Western blot, insufficient quantities of brain tissue were available for Western blot discriminatory testing in 26 samples, which are shown in the Table as samples not analyzed.

The other classical TSE cases, i.e., non–Nor98 cases (648 sheep and 53 goats), which typically showed a 3-band pattern between 19–30 kDa, have been further characterized by applying a discriminatory Western blot method, described as the Agence française de sécurité sanitaire des aliments (French Food Safety Agency) discriminatory method in the Technical Handbook for National Reference Laboratories (*33*). This method enables rapid identification of PrP^{res} patterns similar to those found in experimental ovine BSE and is essentially based on comparison of the PrP^{res} molecular mass with that of cattle BSE, and comparative labeling with 2 antibodies, P4 and Bar233, against either the N terminal end or core part of the PrP^{res} protein, respectively.

Molecular Characterization of Experimental Isolates

We first analyzed 4 reference experimental isolates in small ruminants: a sheep and a goat with BSE, 2 sheep infected with SSBP/1 or CH1641 scrapie sources, and a bovid with classical BSE. The mean molecular masses of the diglycosylated (H), monoglycosylated (L), and unglycosylated (U) PrP^{res} bands (using Bar233 antibody) and the P4/Bar233 differential labeling, as well as the proportions of glycoforms, are shown in Figure 1 and in Figure 2, panel A. These analyses show the lower molecular mass of the unglycosylated PrP^{res} in BSE in sheep (–0.6 kDa) and 1 goat (–0.5 kDa), and in CH1641 scrapie (–0.8 kDa), compared with the cattle BSE (Figure 1; Figure 2, panel A). In contrast, SSBP/1 showed a higher apparent molecular mass (+0.6 kDa). The molecular masses of the 3 bands obtained for CH1641 (H: –0.8 kDa, L: –0.3 kDa and U: –0.2 kDa) were lower than in BSE in sheep or goats. Differential labeling by Bar233 and P4 antibodies was correlated with the

molecular masses of the protease-resistant cores, with high and low Bar233/P4 ratios in SSBP/1 and BSE in sheep, respectively (Figure 1). Like BSE in sheep, CH1641 showed a decreased signal with P4 compared with Bar 233. bovine BSE showed no labeling with P4 antibody.

Comparison of the proportions of diglycosylated and monoglycosylated bands showed the highest levels of diglycosylated PrP^{res} in experimental BSE in sheep (75 ± 9%/18 ± 4%) and goats (71 ± 7%/20 ± 4%), even compared with cattle BSE (64 ± 10%/27 ± 4%) (Figure 2, panel D). On the contrary, SSBP/1 showed much lower levels of diglycosylated PrP^{res} (46 ± 6%/32 ± 3%), whereas CH1641 was close to cattle BSE (62 ± 9%/29 ± 5%).

Experimental BSE in a goat showed the same molecular features as those observed in experimental BSE in sheep. Both molecular masses, P4 immunolabeling and glycoforms proportions, were involved (Figure 1; Figure 2, panels A, D).

Molecular Characterization of Natural TSE Sheep and Goat Isolates

We then compared the cattle BSE control with the 701 classical samples available for further analyses (648 sheep and 53 goats). Most of the isolates tested (657) were PrP^{res} positive by discriminatory Western blot using Bar233 core antibody and showed a 3-band pattern, whereas the signal was weaker in 44 samples, preventing the identification and characterization of the unglycosylated band.

We chose to express the molecular masses measures as dmm, which corresponds to the difference of molecular masses measured between the tested sample and the control cattle BSE always loaded beside it. Most (650) samples showed a positive dmm (Figure 3) and strong labeling with P4 antibody, as previously described for SSBP/1. However, large variations in molecular mass (1.2 kDa) were observed among these samples, a possible clue pointing to biological diversity among scrapie sources; in contrast, only small variations were observed after repeated measures of a same sample with the Western blot method, as shown by the small standard deviations during repeated analysis of reference samples (Figure 2). A minority of the samples (*12*) showed similar molecular masses to bovine BSE (dmm <0.1 kDa difference). Stronger P4 labeling compared with Bar 233 antibody was observed in all these samples and in the 44 (not defined) samples for which the dmm could not

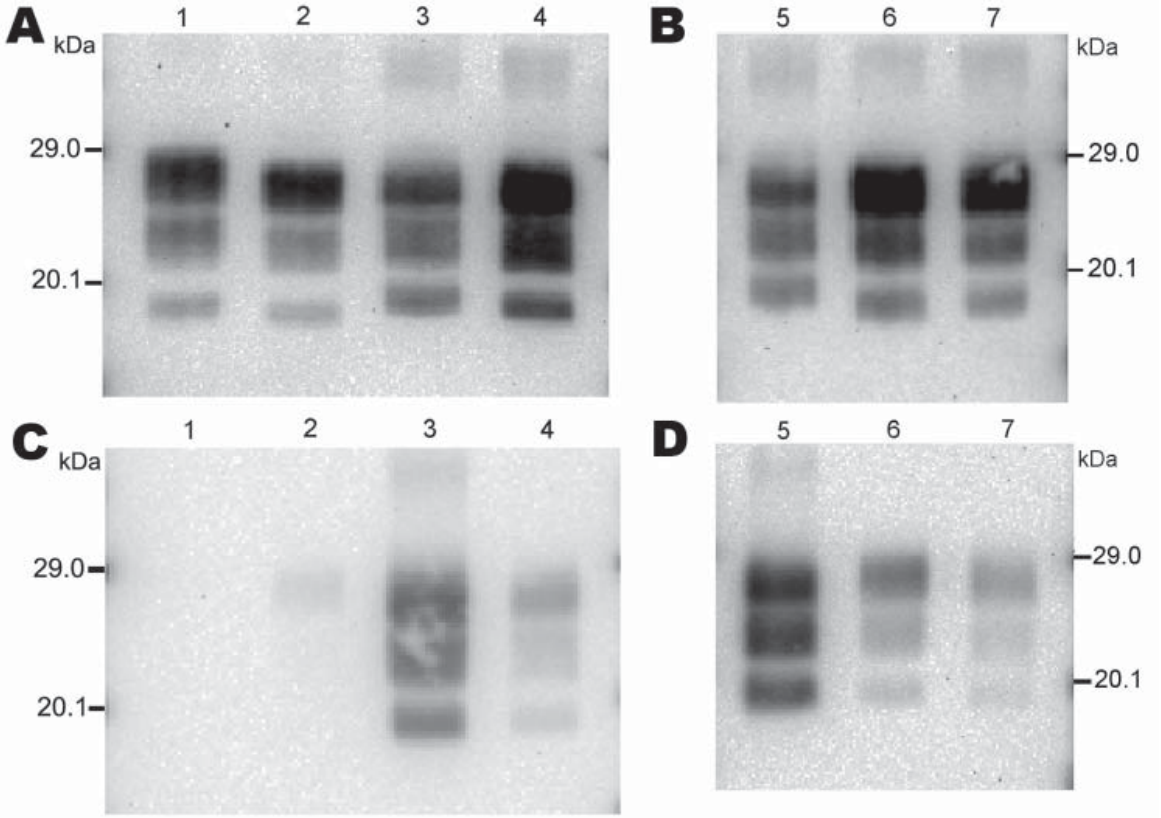

Figure 1. Immunoblots obtained for reference brain samples by discriminatory Western blot method. The first membrane (A, B) was probed with Bar233 antibody. The second membrane (C, D) was probed with monoclonal antibody P4. The 2 immunoblots were loaded with a natural classical bovine spongiform encephalopathy (BSE) isolate (lane 1); an isolate from a sheep experimentally infected with classical BSE 4 (SB1, lanes 2, 6); 2 sheep-passaged scrapie isolates (SSBP/1, lanes 3, 5; CH1641, lane 4); and an isolate from a goat experimentally infected with classical BSE (CH41x76, lane 7).

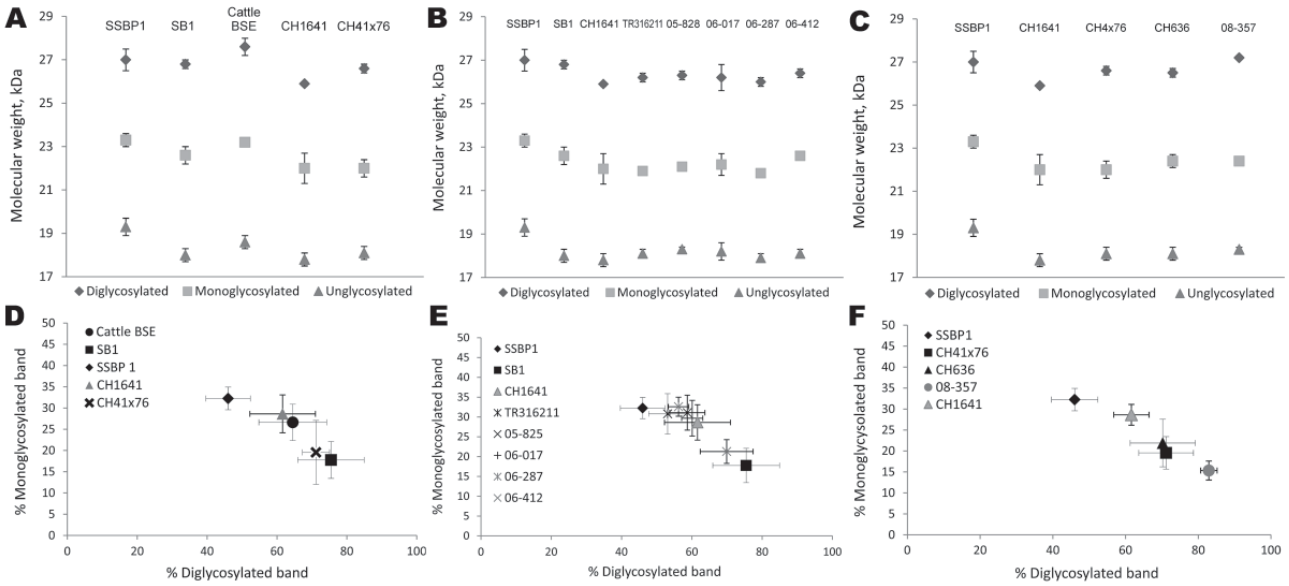

Figure 2. Molecular mass obtained for the di-, mono-, and unglycosylated protein bands (A–C) and the glycoform proportions (D–F) between the diglycosylated band and the monoglycosylated band of the protease-resistant prion protein of the reference transmissible spongiform encephalopathies isolates (A, D), CH1641-like isolates in sheep (B, E), and unusual isolates in goats (C, F). Results were obtained from immunoblots detected by Bar233 antibody. BSE, bovine spongiform encephalopathy.

be measured with Bar233 antibody because quantities of extracted PrPres were too low.

A few samples (5 sheep and 2 goats) showed a lower PrP^{res} molecular mass than the bovine BSE control, i.e., a negative dmm (Figure 3), combined with poor labeling by P4 antibody, as described for BSE in small ruminants and CH1641 scrapie. These samples were suspected to represent BSE in small ruminants and were thus further characterized by repeated Western blot analyses in comparison with experimental controls.

Molecular Studies of TSE Isolates with Low Molecular Mass PrP^{res}

These experiments allowed repeated measures of PrP^{res} molecular masses and P4/Bar233 differential labeling and estimation of the proportions of PrP^{res} glycoforms. Western blot analyses were also performed with the C-terminal SAF84 antibody to identify possible additional C-terminal PrP^{res} cleavage products, as described for the CH1641 experimental isolate (*17*).

The 3 PrP^{res} glycoforms of the 5 sheep isolates showed similar molecular masses close to BSE in sheep or CH1641 scrapie (Figure 2, panel B; Figure 4, panel A). These similarities of PrPres migration were associated with intermediate labeling with P4 but nevertheless varied considerably (up to 3×) according to the isolate (data not shown). Analyses of the glycoform proportions showed lower proportions of the diglycosylated band, compared with BSE in sheep, and also similarity to CH1641 in this respect (Figure 2, panel E). All 5 isolates showed an additional ≈14 kDa PrP^{res} band, after use of the SAF84 antibody (Figure 4, panel C), that in 4 isolates represented 30 ± 7% to 34 ± 7% of the total signal after repeated analyses and PNGase deglycosylation. Among these sheep isolates, the 06-412 sample showed higher molecular masses and proportion of diglycosylated band. Nevertheless, the 5 natural isolates thus appeared similar to the CH1641 experimental scrapie isolate.

For the 2 goat samples with low molecular mass PrP^{res}, the molecular characteristics of the CH636 isolate were indistinguishable from BSE in goat (CH41x76), with regard to PrP^{res} molecular masses (Figure 5, panel A) and the respective proportions of the diglycosylated/monoglycosylated bands (70 ± 9%/22 ± 6% and 71 ± 8%/20 ± 4%, respectively) (Figure 2, panel F) when Bar233 antibody was used, and reduced labeling by P4 antibody compared with Bar233 (Figure 5, panel B). A similar pattern was observed with the other goat sample 08-357, but the levels of the diglycosylated band were even higher (83 ± 2%). However, Western blot analyses with SAF84 antibody showed that these 2 goat samples clearly differed and had an additional ≈14 kDa PrP^{res} band only in the 08-357 isolate, as in the CH1641 experimental scrapie isolate. This ≈14 kDa PrP^{res} band was represented in the same proportions as in the CH1641-like sheep isolates. This sample could not be clearly distinguished from BSE in goat according to other molecular criteria.

Discussion

We investigated the PrP^{res} molecular features of one of the largest series of natural TSE isolates from sheep and

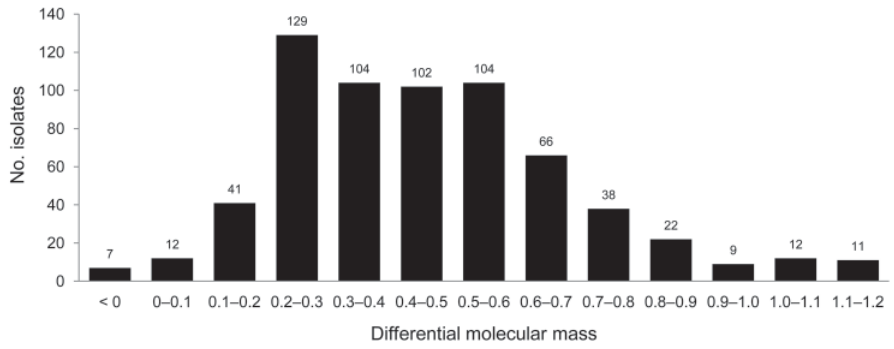

Figure 3. Differences in molecular mass observed between protease-resistant prion protein in cattle bovine spongiform encephalopathy (BSE) and usual transmissible spongiform encephalopathy cases in small ruminants. Differential molecular mass was obtained by subtracting the molecular mass of the unglycosylated band of the cattle BSE control to that of the natural small ruminant isolate from an immunoblot detected by Bar233 antibody.

goats analyzed so far in Europe. Approximately 1.7 million small ruminants were subjected to a rapid test; among these, 1,153 sheep and 78 goats originating from 992 flocks were confirmed as TSE-positive on the basis of identification of PrP^{res} in the brain stem. Another large study using 1,247 sheep originating from 450 flocks in Great Britain has been reported (*24*). Similar studies have been conducted in other European countries such as Germany (*34*), the Netherlands (*14,*) and Italy (*23*), but fewer TSE-positive animals were reported. In our series, animals with classical cases represented 53% of the TSE-affected animals.

The molecular features of most of these isolates (99%) were comparable to those previously described for most scrapie cases, in studies in Europe or France (*21*). In all of these cases, PrP^{res} from sheep and goats showed clearly distinct features from BSE, based on the identification of a higher molecular mass of unglycosylated PrP^{res}, associated with strong labeling by the P4 antibody that recognizes the N terminal end of the protein. However, our observation of large individual variations in this PrP^{res} molecular mass implies that a possible relationship with the biologic diversity of TSE agents, which has been described after transmission of scrapie to inbred wild-type mice (*35*), would be questionable. Only a few animals (5 sheep and 2 goats) in this large series of TSE-affected animals showed molecular characteristics that, in comparison with the usual features of scrapie, could be consistent with the known BSE signature in small ruminants. These samples represented all the suspected isolates that were identified by official surveillance in France during 2002–2009.

An essential molecular criterion defined from the observation of PrP^{res} BSE compared with scrapie was the low molecular mass of the unglycosylated band in PrPres BSE, associated with a decreased PrP^{res} signal lower with an N terminal antibody than with a core antibody (*13*,*22*,*36*). After identification of these molecular features in a few small ruminants, only 1 (CH636) of the 2 cases here described in goats, identified as TSE positive in 2002, has been shown to be infected by the BSE agent after bioassays in mice (*37*). Another goat in the United Kingdom identified by an immunohistochemical discriminatory method as TSE-positive in 1990 showed characteristics that were indistinguishable from BSE (*18*). These results clearly indicate that in a situation characterized by a decrease in the number of cases in cattle in all countries in Europe, the possibility of finding BSE in small ruminants is now remote.

The other unusual isolates showed molecular characteristics that were partly similar, not only to BSE in small ruminants with a low molecular mass of PrP^{res} and faint labeling with P4 antibody, but also to the CH1641 experimental scrapie isolate. However, detailed immunohistochemical investigations of CH1641 showed subtle differences in the cleavage site of the protein compared with BSE in sheep (*38*). As previously described, after transmission in ovine transgenic mice (*17*), the slightly lower PrP^{res} molecular mass in CH1641-like isolates, as recognized in the CH1641 experimental isolate (*13*),was confirmed by Western blot, at least in sheep (Figure 2, panel B). However, these differences in molecular mass are more easily identified on the diglycosylated band. Compared with BSE in small ruminants, lower proportions of this diglycosylated band were found in sheep, whereas the 08-357 goat sample showed very high levels of this diglycosylated band, which would be consistent with BSE. Experimental transmissions of BSE in sheep have shown that, to a certain extent, the PrP^{res} molecular features could be influenced by different factors, such as serial passages in sheep (*39*) or sheep genotypes (*36*), although slight variability did not compromise the discrimination with scrapie. Furthermore, all these CH1641-like natural isolates in sheep and goats clearly differed from BSE by the presence of an additional, C-terminally cleaved, PrP^{res} product specifically recognized by a C-terminal antibody (SAF84), as previously described for the CH1641 experimental scrapie isolate (*17*). Baron et al. described bioassays of 3 CH1641-like sheep isolates (*17*), which are also being conducted for the other isolates.

These 6 CH1641-like isolates were identified among 1,153 sheep and 78 goats with confirmed TSE, and the

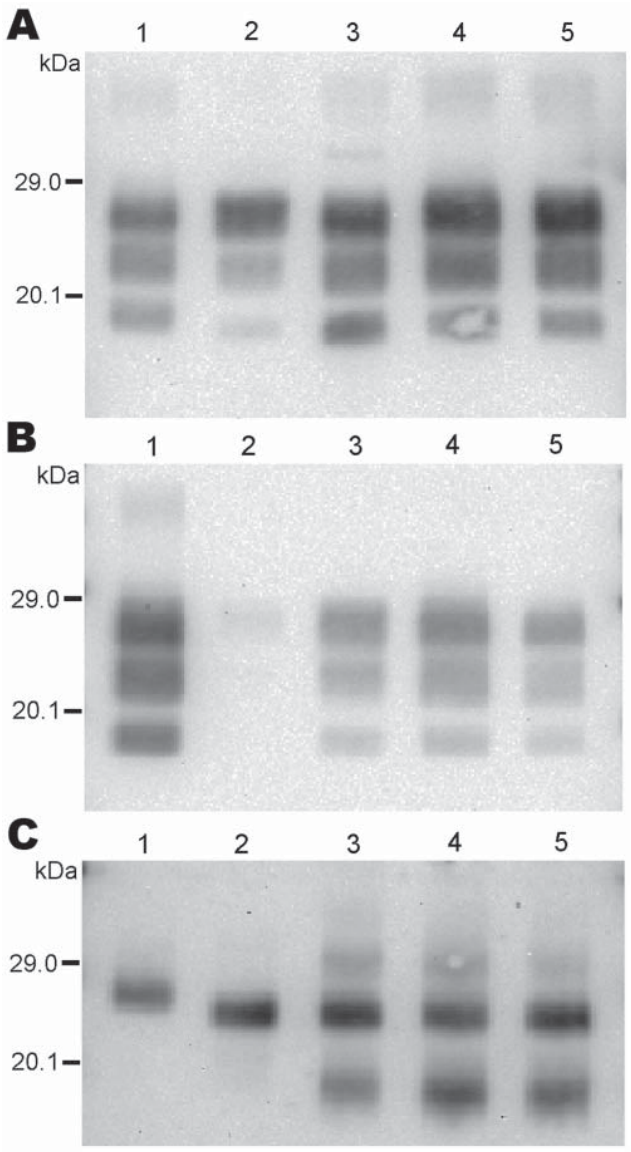

Figure 4. Western blot analysis of protease-resistant prion protein in 2 CH1641-like sheep isolates (06-017, lane 3; 06-287, lane 4) detected by Bar233 (A), P4 (B), and SAF84 (C) antibodies. These samples were compared with 2 sheep-passaged scrapie isolates (SSBP/1, lane 1; CH1641, lane 5) and an isolate from a sheep experimentally infected with classical spongiform encephalopathy (SB1, lane 2). Samples in panel C were deglycosylated with peptide N-glycosidase F before Western blot analysis.

goat case represents, to our knowledge, a spreading of the known species range for natural CH1641 infection. At least in sheep, for which 4.34 cases per million sheep tested were identified in this study, the frequency of CH1641-like scrapie was notably higher compared with other rare TSEs in ruminants such as atypical BSEs, which showed a frequency of 0.76 per million cattle tested during 2001–2007 (*40*). Thus, large-scale testing of animals would be required to identify these rare TSE isolates. Similar isolates were only identified in sheep in the other large series reported from 450 flocks in Great Britain (2 cases in 1 flock) (*23*) and in a previous study of 214 TSE-infected sheep in France (2 cases in 1 flock) (*27*). However, an underestimation of the frequency of such cases cannot be fully excluded. PrP^{res} features are assessed by analyzing a single homogenate prepared from a brain fragment from the animal. Stack et al. described a case in sheep that appeared as CH1641-like after repeated Western blot analysis of a brain stem sample, whereas previous analysis of the caudal medulla at the time of submission had shown the usual scrapie profile (*24*). Immunohistochemical test-

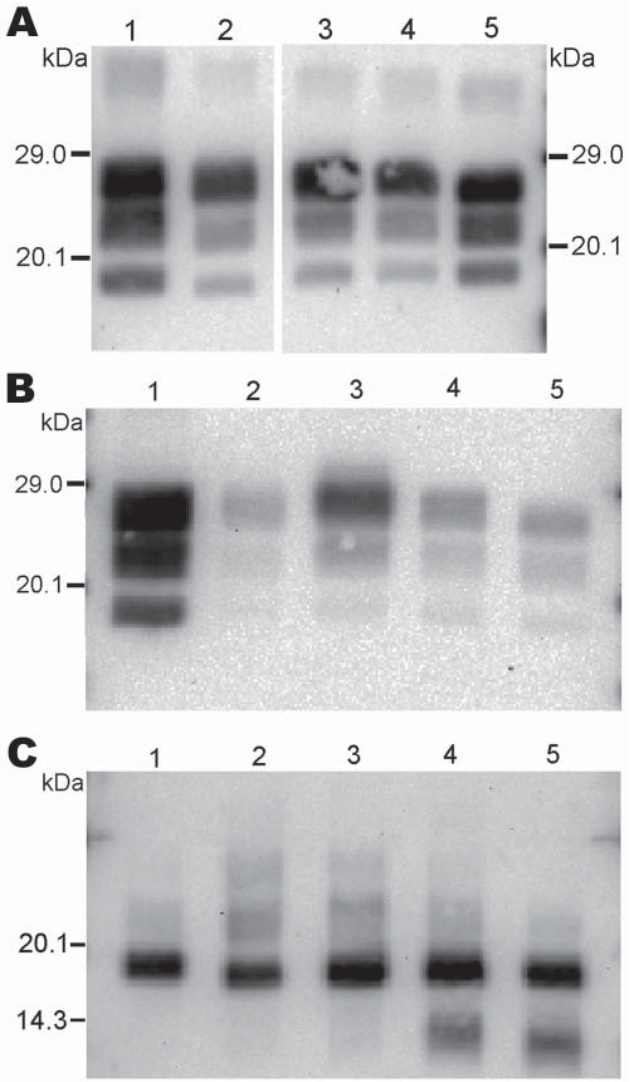

Figure 5. Western blot analysis of protease-resistant prion protein in 2 goat isolates (CH636, lane 3; 08-357, lane 4) detected by Bar233 (A), P4 (B), and SAF84 (C) antibodies. These samples were compared with an isolate from a goat naturally infected with scrapie (lane 1); an isolate from a goat experimentally infected with classical BSE (CH41x76, lane 2); and a sheep-passaged scrapie isolate (CH1641, lane 5). Samples in panel C were deglycosylated with peptide N-glycosidase F before Western blot analysis.

ing of 2 CH1641-like cases in sheep showed, that unlike BSE, PrP^{d} could be clearly identified by using P4 antibody in some of the brain stem nuclei and in lymphoid tissues (*27*). Finally, on the basis of identification of low levels of C-terminal PrP^{res} product in ovine transgenic mice infected with usual scrapie isolates, we hypothesized that a CH1641-like component might be present as a minor component in these scrapie cases that showed usual molecular features (*17*). All these data raise the question of the existence of possible mixtures of TSE agents in these particular CH1641-like isolates.

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Mr Vulin is a PhD candidate at the Agence Nationale de Sécurité Sanitaire, Lyon, France. His research focuses on studies of prion diseases of ruminants, including characterization of the disease-associated prion protein, with particular emphasis on unusual forms of these diseases.

References

1. Collinge J, Sidle KC, Meads J, Ironside J, Hill AF. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature. 1996;383:685–90. DOI: 10.1038/383685a0
2. Hill AF, Desbruslais M, Joiner S, Sidle KC, Gowland I, Collinge J, et al. The same prion strain causes vCJD and BSE. Nature. 1997;389:448–50, 526. DOI: 10.1038/38925
3. Prusiner SB. Prions. Proc Natl Acad Sci U S A. 1998;95:13363–83. DOI: 10.1073/pnas.95.23.13363
4. Caughey B, Raymond GJ. The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. J Biol Chem. 1991;266:18217–23.
5. Riek R, Hornemann S, Wider G, Glockshuber R, Wuthrich K. NMR characterization of the full-length recombinant murine prion protein, mPrP(23–231). FEBS Lett. 1997;413:282–8. DOI: 10.1016/S0014-5793(97)00920-4
6. Riesner D. Biochemistry and structure of PrP(C) and PrP(Sc). Br Med Bull. 2003;66:21–33. DOI: 10.1093/bmb/66.1.21
7. Gavier-Widen D, Stack MJ, Baron T, Balachandran A, Simmons M. Diagnosis of transmissible spongiform encephalopathies in animals: a review. J Vet Diagn Invest. 2005;17:509–27.
8. Bruce M, Chree A, McConnell I, Foster J, Pearson G, Fraser H. Transmission of bovine spongiform encephalopathy and scrapie to mice: strain variation and the species barrier. Philos Trans R Soc Lond B Biol Sci. 1994;343:405–11. DOI: 10.1098/rstb.1994.0036
9. Foster JD, Hope J, Fraser H. Transmission of bovine spongiform encephalopathy to sheep and goats. Vet Rec. 1993;133:339–41. DOI: 10.1136/vr.133.14.339
10. Opinion of the Scientific Steering Committee of the European Commission on Hypotheses on the Origin and Transmission of BSE. European Commission, adopted 29–30 Nov 2001. http://ec.europa.eu/food/fs/sc/ssc/out236_en.pdf
11. Opinion of the Scientific Steering Committee of the European Commission on Strategy to Investigate the Possible Presence of BSE in Sheep. European Commission, adopted on 4–5 Apr 2002. http://ec.europa.eu/food/fs/sc/ssc/out281_en.pdf
12. Hill AF, Sidle KC, Joiner S, Keyes P, Martin TC, Dawson M, et al. Molecular screening of sheep for bovine spongiform encephalopathy. Neurosci Lett. 1998;255:159–62. DOI: 10.1016/S0304-3940(98)00736-8
13. Stack MJ, Chaplin MJ, Clark J. Differentiation of prion protein glycoforms from naturally occurring sheep scrapie, sheep-passaged scrapie strains (CH1641 and SSBP1), bovine spongiform encephalopathy (BSE) cases, and Romney and Cheviot breed sheep experimentally inoculated with BSE using two monoclonal antibodies. Acta Neuropathol. 2002;104:279–86.
14. Thuring CM, Erkens JH, Jacobs JG, Bossers A, Van Keulen LJ, Garssen GJ, et al. Discrimination between scrapie and bovine spongiform encephalopathy in sheep by molecular size, immunoreactivity, and glycoprofile of prion protein. J Clin Microbiol. 2004;42:972–80. DOI: 10.1128/JCM.42.3.972-980.2004
15. Notari S, Strammiello R, Capellari S, Giese A, Cescatti M, Grassi J, et al. Characterization of truncated forms of abnormal prion protein in Creutzfeldt-Jakob disease. J Biol Chem. 2008;283:30557–65. DOI: 10.1074/jbc.M801877200
16. Biacabe AG, Jacobs JG, Bencsik A, Langeveld JP, Baron TG. H-type bovine spongiform encephalopathy: complex molecular features and similarities with human prion diseases. Prion. 2007;1:61–8. DOI: 10.4161/pri.1.1.3828
17. Baron T, Bencsik A, Vulin J, Biacabe AG, Morignat E, Verchere J, et al. A C-terminal protease-resistant prion fragment distinguishes ovine "CH1641-like" scrapie from bovine classical and L-Type BSE in ovine transgenic mice. PLoS Pathog. 2008;4:e1000137. DOI: 10.1371/journal.ppat.1000137
18. Jeffrey M, Martin S, Gonzalez L, Foster J, Langeveld JP, van Zijderveld FG, et al. Immunohistochemical features of PrP(d) accumulation in natural and experimental goat transmissible spongiform encephalopathies. J Comp Pathol. 2006;134:171–81. DOI: 10.1016/j.jcpa.2005.10.003
19. Simon S, Nugier J, Morel N, Boutal H, Creminon C, Benestad SL, et al. Rapid typing of transmissible spongiform encephalopathy strains with differential ELISA. Emerg Infect Dis. 2008;14:608–16. DOI: 10.3201/eid1404.071134
20. Baron TG, Madec JY, Calavas D. Similar signature of the prion protein in natural sheep scrapie and bovine spongiform encephalopathy-linked diseases. J Clin Microbiol. 1999;37:3701–4.
21. Baron TG, Madec JY, Calavas D, Richard Y, Barillet F. Comparison of French natural scrapie isolates with bovine spongiform encephalopathy and experimental scrapie infected sheep. Neurosci Lett. 2000;284:175–8. DOI: 10.1016/S0304-3940(00)01047-8
22. Baron TG, Biacabe AG. Molecular analysis of the abnormal prion protein during coinfection of mice by bovine spongiform encephalopathy and a scrapie agent. J Virol. 2001;75:107–14. DOI: 10.1128/JVI.75.1.107-114.2001
23. Nonno R, Esposito E, Vaccari G, Conte M, Marcon S, Di Bari M, et al. Molecular analysis of cases of Italian sheep scrapie and comparison with cases of bovine spongiform encephalopathy (BSE) and experimental BSE in sheep. J Clin Microbiol. 2003;41:4127–33. DOI: 10.1128/JCM.41.9.4127-4133.2003
24. Stack M, Jeffrey M, Gubbins S, Grimmer S, Gonzalez L, Martin S, et al. Monitoring for bovine spongiform encephalopathy in sheep in Great Britain, 1998–2004. J Gen Virol. 2006;87:2099–107. DOI: 10.1099/vir.0.81254-0
25. Hope J, Wood SC, Birkett CR, Chong A, Bruce ME, Cairns D, et al. Molecular analysis of ovine prion protein identifies similarities between BSE and an experimental isolate of natural scrapie, CH1641. J Gen Virol. 1999;80:1–4.
26. Foster JD, Dickinson AG. The unusual properties of CH1641, a sheep-passaged isolate of scrapie. Vet Rec. 1988;123:5–8. DOI: 10.1136/vr.123.1.5

27. Lezmi S, Martin S, Simon S, Comoy E, Bencsik A, Deslys JP, et al. Comparative molecular analysis of the abnormal prion protein in field scrapie cases and experimental bovine spongiform encephalopathy in sheep by use of Western blotting and immunohistochemical methods. J Virol. 2004;78:3654–62. DOI: 10.1128/JVI.78.7.3654-3662.2004
28. Foster J, Goldmann W, Parnham D, Chong A, Hunter N. Partial dissociation of PrP(Sc) deposition and vacuolation in the brains of scrapie and BSE experimentally affected goats. J Gen Virol. 2001;82:267–73.
29. Hunter N, Foster JD, Dickinson AG, Hope J. Linkage of the gene for the scrapie-associated fibril protein (PrP) to the *Sip* gene in Cheviot sheep. Vet Rec. 1989;124:364–6. DOI: 10.1136/vr.124.14.364
30. Morignat E, Cazeau G, Biacabe AG, Vinard JL, Bencsik A, Madec JY, et al. Estimates of the prevalence of transmissible spongiform encephalopathies in sheep and goats in France in 2002. Vet Rec. 2006;158:683–7. DOI: 10.1136/vr.158.20.683
31. Arsac JN, Biacabe AG, Nicollo J, Bencsik A, Baron T. Biochemical identification of bovine spongiform encephalopathies in cattle. Acta Neuropathol. 2007;114:509–16. DOI: 10.1007/s00401-007-0270-1
32. Arsac JN, Andreoletti O, Bilheude JM, Lacroux C, Benestad SL, Baron T. Similar biochemical signatures and prion protein genotypes in atypical scrapie and Nor98 cases, France and Norway. Emerg Infect Dis. 2007;13:58–65. DOI: 10.3201/eid1301.060393
33. Community Reference Laboratory of the European Union. Discriminatory testing handbook. TSE strain characterisation in small ruminants—a technical handbook for national reference laboratories in the EU. Version 3. 2009 March.
34. Gretzschel A, Buschmann A, Eiden M, Ziegler U, Luhken G, Erhardt G, et al. Strain typing of German transmissible spongiform encephalopathies field cases in small ruminants by biochemical methods. J Vet Med B Infect Dis Vet Public Health. 2005;52:55–63. DOI: 10.1111/j.1439-0450.2005.00827.x
35. Bruce ME, Boyle A, Cousens S, McConnell I, Foster J, Goldmann W, et al. Strain characterization of natural sheep scrapie and comparison with BSE. J Gen Virol. 2002;83:695–704.
36. Ronzon F, Bencsik A, Lezmi S, Vulin J, Kodjo A, Baron T. BSE inoculation to prion diseases–resistant sheep reveals tricky silent carriers. Biochem Biophys Res Commun. 2006;350:872–7. DOI: 10.1016/j.bbrc.2006.09.137
37. Eloit M, Adjou K, Coulpier M, Fontaine JJ, Hamel R, Lilin T, et al. BSE agent signatures in a goat. Vet Rec. 2005;156:523–4.
38. Jeffrey M, Gonzalez L, Chong A, Foster J, Goldmann W, Hunter N, et al. Ovine infection with the agents of scrapie (CH1641 isolate) and bovine spongiform encephalopathy: immunochemical similarities can be resolved by immunohistochemistry. J Comp Pathol. 2006;134:17–29. DOI: 10.1016/j.jcpa.2005.06.005
39. Stack M, Gonzalez L, Jeffrey M, Martin S, Macaldowie C, Chaplin M, et al. Three serial passages of bovine spongiform encephalopathy in sheep do not significantly affect discriminatory test results. J Gen Virol. 2009;90:764–8. DOI: 10.1099/vir.0.005983-0
40. Biacabe AG, Morignat E, Vulin J, Calavas D, Baron TG. Atypical bovine spongiform encephalopathies, France, 2001–2007. Emerg Infect Dis. 2008;14:298–300. DOI: 10.3201/eid1402.071141

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Endurance, Refuge, and Reemergence of Dengue Virus Type 2, Puerto Rico, 1986–2007

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To study the evolution of dengue virus (DENV) serotype 2 in Puerto Rico, we examined the genetic composition and diversity of 160 DENV-2 genomes obtained through 22 consecutive years of sampling. A clade replacement took place in 1994–1997 during a period of high incidence of autochthonous DENV-2 and frequent, short-lived reintroductions of foreign DENV-2. This unique clade replacement was complete just before DENV-3 emerged. By temporally and geographically defining DENV-2 lineages, we describe a refuge of this virus through 4 years of low genome diversity. Our analyses may explain the long-term endurance of DENV-2 despite great epidemiologic changes in disease incidence and serotype distribution.

Epidemic dengue fever (DF) and the emergence of dengue hemorrhagic fever (DHF) in the Americas are associated with increased endemicity and cocirculation of the 4 dengue virus (DENV) serotypes, 1–4 (*1*). These increases have been particularly evident in Puerto Rico, where transmission increased during the past 25 years (*2–4*). The first DHF epidemics in the Americas occurred in the 1980s and were caused by the Asian/American genotype of DENV-2, then new to the region, which rapidly replaced the American genotype (*5–7*). This replacement has been linked to a potential to cause higher viremia and severe illness (*8–10*). Introduction of DENV-3 in the mid 1990s and increased human population and travel further fostered larger and more frequent DF and DHF epidemics in the region (*11–13*).

Although all 4 DENV serotypes circulate on the island, DENV-2 circulated continuously for 25 years. Previously, a partial sequence analysis from 74 DENV-2 isolates collected in Puerto Rico during 7 years throughout a 14-year period (1987–2001) showed a DENV-2 lineage evolving through a series of turnover events (*14*). A lineage replacement in 1994 appeared to be associated with a foreign virus but only 3 other reintroductions were found, all linked to the 1998 epidemic, the largest in Puerto Rico history (*14*). This was a turning point in the epidemiology of dengue, with DENV-2 (and DENV-1 and -4) rapidly declining during the expansion of DENV-3. However, transmission of DENV-2 persisted at low levels during 1999–2003 and increased thereafter. This serotype turnover offers new opportunities to study the evolution of DENV-2. Our analysis illustrates the genetic composition and population diversity of DENV-2 throughout 22 consecutive years of sampling in Puerto Rico and may explain the evolutionary resilience and long-term establishment of this virus.

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Methods

Virus Isolates

We complied with the institutional review boards of the Centers for Disease Control and Prevention (CDC) (protocol 4797) and the Broad Institute of MIT and Harvard. DENV was obtained from human serum received through the passive surveillance system administered by CDC. Each sample was accompanied by a form that captured geographic and clinical information maintained for this study without patient identifiers. Primary or secondary status of infection was inferred by absence or presence of serum immunoglobulin G (*15*). Viruses were rescued into C6/36 cells (*16*). Selection of 3 isolates per year in the 5 municipalities with the highest reporting of DENV-2 cases resulted in 253 isolates, of which 140 were successfully se-

[1]These authors contributed equally to this article.

quenced and are representative of our virus repository with respect to patient age (27.7 vs. 22.6 years), sex (54.4% vs. 47.4% male), and history of infection (84.6% vs. 77% secondary infections). We also sequenced 20 regional isolates from neighboring countries.

Sequencing

We extracted RNA from tissue culture supernatant using the M48 or MDx BioRobot (QIAGEN, Valencia, CA, USA). cDNA was generated by using Sensiscript RT (QIAGEN) with random hexamers (Applied Biosciences, Foster City, CA, USA). Presence of cDNA was confirmed by PCR by using *Pfu*UltraII (Stratagene, La Jolla, CA, USA) or iTaq (Bio-Rad, Hercules, CA, USA) DNA polymerase and specific oligonucleotides (CDC, Atlanta, GA, USA). Fourteen pooled overlapping 2,000 nt amplicons were generated by reverse transcription–PCR at CDC (San Juan, PR) and sequenced at the Broad Institute (Cambridge, MA, USA) by bidirectional Sanger by using an ABI 3730 after PCR with 96 M13-tailed serotype-specific primers. Resulting reads were trimmed of the primer sequences, filtered for high quality, and assembled by using algorithms developed by the Broad Institute. All coding sequences for the poliproteins (10,173 nt) and parts of the 5′ and 3′ noncoding regions were deposited in GenBank.

Sequence Analyses

Coding sequences for the unprocessed polyprotein (5′ and 3′ noncoding regions excluded) were aligned by ClustalW software (www.ebi.ac.uk/Tools/clustalw/index.html) in MEGA 4 (www.megasoftware.net). Maximum-likelihood analysis and bootstrapping tests were performed in PAUP* (*16*) under the best-fit substitution model estimated by MODELTEST v3.07 (*14*) (parameters available on request). The 1983 Jamaican isolate JM_83_M20558 (*5*) served as outgroup. Mean rates of nucleotide substitution and relative genetic diversity (*Net*, where *t* is the generation time) were estimated by using Bayesian Markov Chain Monte Carlo (MCMC) from BEAST v1.4.7 (http://mbe.oxfordjournals.org/content/25/7/1459). General time reversible substitution model with strict and relaxed molecular clocks and constant population size or Bayesian Skyline coalescent analysis was used. All MCMC chains were run for sufficient length ensuring stationary parameters, with statistical error reflected in values of the 95% highest probability density. Amino acid differences were mapped by using parsimony methods in MacClade v4.08 (*17*). We determined d_N/d_S ratios with the single likelihood ancestor counting method using HYPHY and accessed through the Datamonkey server (*13*). Associations between phylogeny and geographic data were investigated by using Bayesian Tip-association Significance testing (http://evolve.zoo.ox.ac.uk/evolve/BaTS.html) with the posterior sample of trees calculated by BEAST. For the parsimony score, association index, and monophyletic clade size, we considered $p<0.05$ significant.

Results

During 1986–2007, dengue cases in Puerto Rico ranged from 2,000 to ≈16,000 per year (Figure 1, panel A), with major epidemics (≥8,000 cases) reported in 1986, 1992, 1994, 1998, and 2007 (*2*–*4*,*18*,*19*). Despite major fluctuations in serotype circulation, DENV-2 circulated predominantly for 10 years (Figure 1, panel B), alternating with DENV-1 through 2 periods of resurgence during the 1990s and cocirculation of DENV-4 (Figure 1, panel B). DENV-2 declined markedly after the 1998 epidemic and the dissemination of DENV-3 concomitant to the disappearance of DENV-1 and -4. However, DENV-2 continued to cause a low number of cases during 1999–2003 and reemerged in 2004–2007 (Figure 1, panel B). Samples from every year of the 22-year study period (Figure 1, panel C) comprised our analysis.

The Bayesian Skyline analysis (Figure 1, panel D) of the autochthonous viral sequences (Figure 2; clades IB and II in the online Appendix Figure, www.cdc.gov/EID/content/17/1/64-appF.htm) showed a gradual increase in the genetic diversity of DENV-2 during 1987–1991 that corresponds to a period of high transmission and dominance (Figure 1, panel B). This increase was followed by 9 years of high genetic diversity that coincided with a period of DENV-1 and DENV-4 cocirculation. The genetic diversity of DENV-2 declined sharply during 1999–2003, coinciding with a period of minimal DENV-2 transmission. Genetic diversity rebounded in 2005 to roughly pre-1999 levels as the virus reemerged.

The densely populated island of Puerto Rico (3,808,610 population; 3,508 square miles) is divided into 78 municipalities grouped in 8 regions. The 140 DENV-2 genomes from 37 municipalities represented all 8 regions and ranged from 2–20 isolates per year (Figure 1, panel C; Figure 2). We included 20 other sequences from Caribbean countries. The number of Puerto Rico sequences is proportional to the epidemic level or the relative proportion of DENV-2 identifications in the municipalities with highest DENV-2 reporting per year. The phylogeny of the 160 DENV-2 genomes showed 2 major clades (I and II) and a smaller clade (III) (Figure 2). Clade I contains 115 sequences and can be further subdivided in 2 subclades. Subclade IA (1998–2007) contains 13 Puerto Rico and 12 Caribbean sequences, including 1 from St. Thomas (SH 90 FJ898450) identified as the closest ancestor. Subclade IB (1994–2007) contains 90 sequences mostly of local origin, but the presence of 6 foreign and 1 local basal sequences confirms its foreign origin. Clade II (1986–1994) contains 39 local isolates. Basal to clades I and II is a St. John 1987 sequence (SJ 87 GQ868603), which suggests a possible origin. Clade III is formed by 4 local isolates

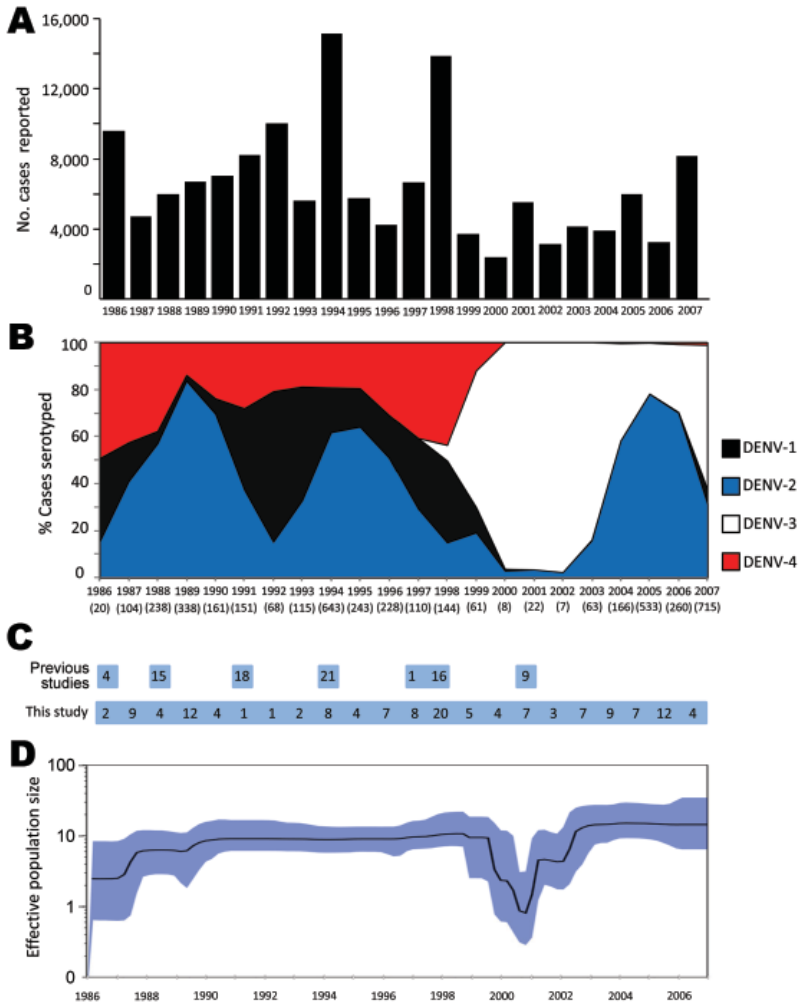

Figure 1. Historic overview of dengue, Puerto Rico, 1986–2007. A) Number of suspected, clinically defined cases of dengue fever/dengue hemorrhagic fever by year reported to the Centers for Disease Control and Prevention's Dengue Branch. B) Percentage of identifications of each serotype relative to the total of positive serotype identifications by using tissue culture isolation or reverse transcription–PCR per year. Numbers in parenthesis indicate numbers of dengue virus (DENV) serotype 2 identifications each year. Black, DENV-1; blue, DENV-2; white, DENV-3; red, DENV-4. C) Number of partially sequenced (E gene) autochthonous Puerto Rican isolates reported by previous studies (*14,20*) and whole genome sequences obtained in the present study by year of their corresponding case presentation. D) Bayesian coalescent inference of population dynamics and genetic diversity by using the Bayesian Skyline plot. Markov Chain Monte Carlo from BEAST version 1.4.7 (www.biomedcentral.com/1741-7007/8/114). Sampling procedures were used to estimate posterior distribution of DENV-2 genetic diversity in an effective population through the study period on the basis of full genome sequence data. x axis, time in years through the study period; y axis, product of the effective population size (relative genetic diversity) and generation length in years; black line, median estimate; blue shadow, 95% highest probability density.

during1987–1991. These genetically distinct isolates do not fit in clade I or II, but a separate analysis with publically available envelope gene sequences pointed to possible Caribbean origin (K.L. McElroy et al., unpub. data).

Four events merit recognition (Figure 2). First, a mixture of foreign and local strains at the base of subclades IA and IB provides evidence of multiple introductions. Eight Puerto Rico viruses associated with these foreign strains date from 1994 through 1999. These years also are associated with a distinct subgroup basal to subclade IB concomitant with the extinction of clade II in 1997. Second, subclade IB evolved mainly after the introduction of DENV-3 in 1998. Third, a period of limited circulation of DENV-2 reflected in low levels of genetic diversity (1999–2003) coincided with the expansion of DENV-3 and decline of DENV-1 and -4. Fourth, there was a resurgence of DENV-2 during 2004–2007.

Forty-nine amino acid differences mapped to the phylogeny were detected across the major internal branches of the tree. Twenty of these comprise major differences between clades I and II and between subclades IA and IB, as well as substitutions that arose during the continuous evolution of subclade IB (Figure 2). Only 1 aa substitution distinguished isolates in clades I/II from III: a hydrophilic glutamine to a hydrophobic leucine at position 131 in the E protein. Excluding PR79_1995_EU569708 as a possible foreign introduction, 18 aa differences distinguish isolates across clade I, 12 of which separate subclade IB from clade II and potentially could have been involved in the 1994–1997 lineage turnover (Figure 2). The remaining differences between isolates in subclades IB and II were present in nonstructural (NS) genes and are preponderantly conservative mutations, with the exception of position 31 in NS3, which was nonconservative. Among the additional changes, the only nonconservative mutation was a hydrophobic alanine to hydrophilic threonine at position 137 in NS4B that originated with PR40_1999 EU482730, and most changes were found in the NS genes.

Using Bayesian MCMC and d_N/d_S analyses, we estimated the mean substitution rates for the full genomes at 9

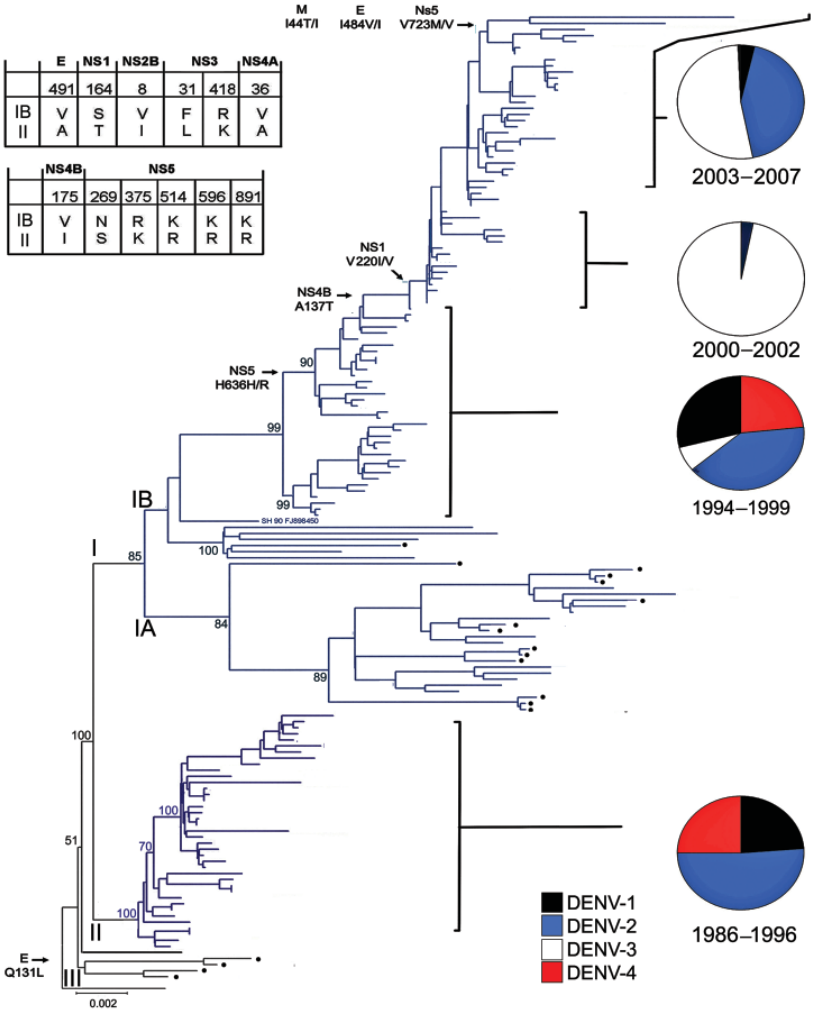

Figure 2. Evolution of dengue virus (DENV) serotype 2 in Puerto Rico. Maximum-likelihood phylogeny is shown for the 140 Puerto Rico and 20 international isolates of DENV-2 (see number of isolates by year below). Names of clades (I, II, and III) and subclades (IA, IB) are shown at the base of their respective branches on the phylogeny tree. Clade II (dark blue) circulated during 1986–1996 and clade I (light blue) during 1994–2007. Subclade IA and clade III represent foreign, transient reintroductions found throughout the 22-year study period. Black dots indicate 18 isolates from Puerto Rico with phylogenetic associations closer to foreign isolates than to other Puerto Rico viruses. Bootstrap values are shown for all clades (I, II, and II), subclades (IA, IB), and most immediate lineages. Twelve amino acid changes associated with IB/II differences are shown in the table, and 6 other selected changes across subclade IB are shown at the left at the base of the branches exhibiting the respective changes. Amino acid numbers refer to the position in each DENV protein. Relevant epidemiologic events are highlighted to the right with pie charts showing the relative levels of each serotype isolated for that period. Black, DENV-1; blue, DENV-2; white, DENV-3; red, DENV-4. GenBank accession numbers and geographic codes corresponding to all sequences are shown in an expanded online version of this figure (www.cdc.gov/EID/content/17/1/64-F2.htm). Scale bar indicates nucleotide substitutions per site.

$\times 10^{-4}$ to 1.1×10^{-3} for all clades, consistent with previously published rates (*20*,*21*). The low d_N/d_S ratios (0.07–0.08) provide evidence of a low percentage of substitutions that have been fixed along independent lineages, possibly indicating purifying, negative selection.

BaTS analysis shows that lineages often correlated with the corresponding region of origin of the isolates. Seven of the 8 regions had ≥4 isolates in subclade IB or clade II. This association was significant for 6 regions ($p \leq 0.05$) (Table). The most significant geographic correlation of lineages were found in the San Juan (1986–1990 and 1994–1996), Ponce (1987–1989), and Mayaguez (1989 and 1993) (Figure 3, panel A). In addition, isolates clustered geographically for San Juan (1997–1999 and 2001–2006), Caguas (1998–2001, 2004, and 2005), Ponce (1995–1997 and 2005), Mayaguez (1996–1998 and 2006), Aguadilla (1996–1998), and Arecibo (1994–1995, 2004, and 2006) (Figure 3, panel B). Considering the DENV-2 historical data, we recognize that high-reporting municipalities usually are located in regions where we identified significant phylogenetic clustering (online Appendix Figure). For example, in 1987, most DENV-2 cases originated from the Ponce and San Juan regions, where we identified lineages of clade I. For1994–1996, DENV-2 cases in San Juan, Ponce, Mayaguez, and Arecibo regions may reflect the coexistance of subclades IB and clade II.

We investigated other possible associations with the DENV-2 phylogeny, including age and DF/DHF status,

Table. Correlation between phylogeny and geographic location of dengue virus isolation, Puerto Rico 1986–2007*

Clade	Region	No. isolates	Estimated BaTs (95% HPD CIs)	p value
I B	Aguadilla	5	2 (2–2)	0.04
	Arecibo	12	3 (3–3)	0.03
	Caguas	17	2.75 (2–3)	0.04
II	Mayaguez	6	4 (4–4)	0.01
	Ponce	11	3 (3–3)	0.05
	San Juan	15	5 (5–5)	0.01

*Correlation estimated by using BaTs. Association index = 6.51 (95% CI 6.03–7.22). Parsimony score statistic = 52.27 (95% CI 51–54). BaTs, Bayesian Tip-association Significance testing; HPD, highest probability density; CI, confidence interval.

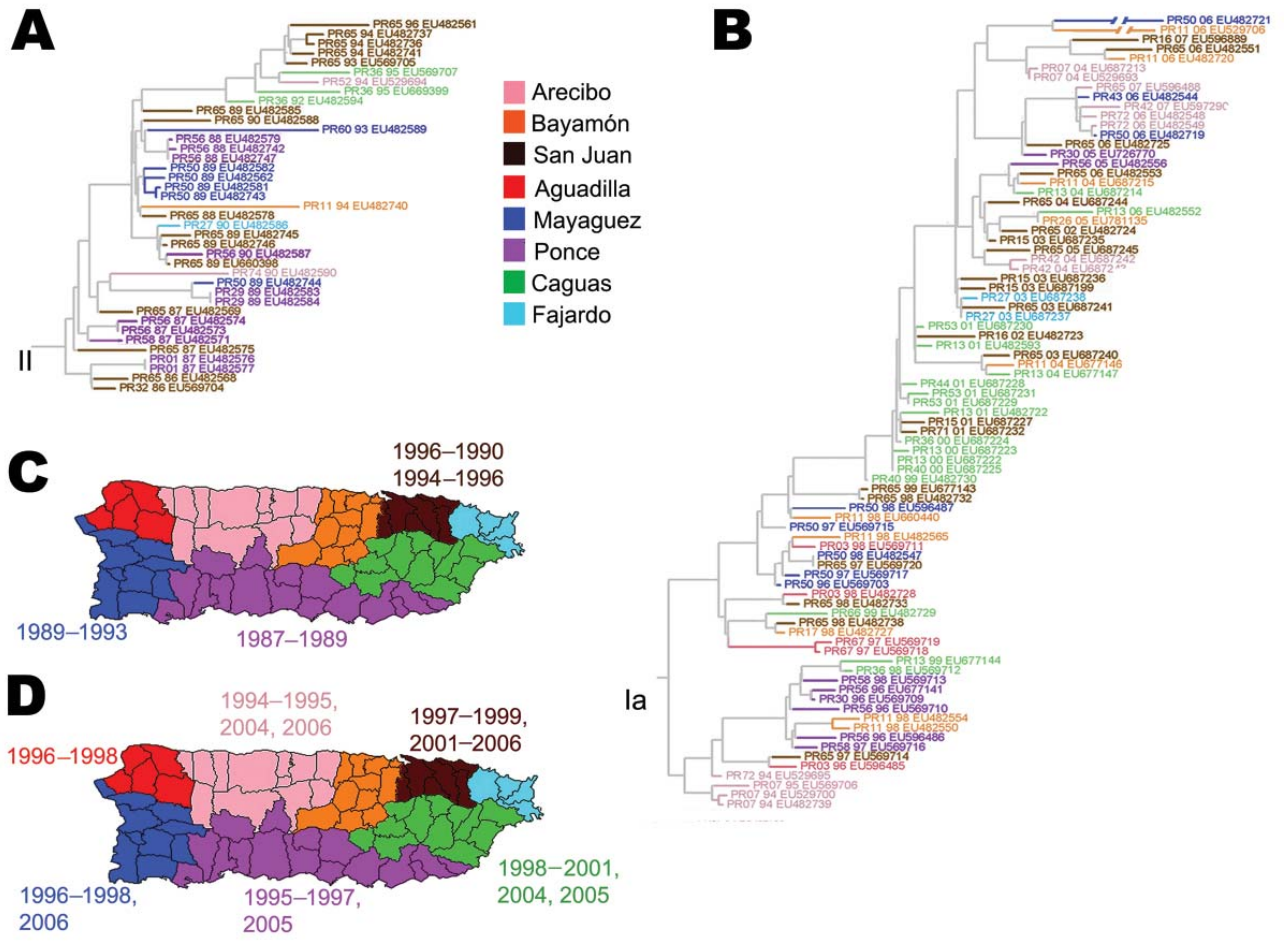

Figure 3. Geographic clustering of Puerto Rico dengue virus lineages. A) Maximum-likelihood phylogeny of clade II. All isolates indicate year of case presentation and GenBank accession numbers. B) Maximum-likelihood phylogeny of subclade IB shows isolates by year and GenBank accession numbers. Six regions had ≥5 isolates (San Juan, Caguas, Ponce, Mayaguez, Aguadilla, and Arecibo). C) Eight regions of Puerto Rico with colors corresponding to isolates in panel A and year for the 3 regions with more isolates of that clade: San Juan, Mayaguez, and Ponce. D) Eight regions of Puerto Rico, showing colors and years corresponding to isolates in panel B. Correlation between phylogeny and geographic location of isolation for the isolates in this study was estimated by using Bayesian Tip-association Significance testing. Association index 6.51 (95% confidence interval 6.03–7.22); parsimony score statistic 52.27 (95% confidence interval 51–54). Monophyletic clade size Bayesian Tip-association Significance estimates are shown for 6 regions of Puerto Rico with ≥5 isolates represented in at least 1 subclade and statistically representative geographic associations ($p \leq 0.05$).

but found none. Most DENV-2 infections were secondary (84.6% and 77% of DENV-2 infections in the CDC collection and this study, respectively). However, we found no relationship between phylogeny and incidence of primary or secondary infection in patients.

The year 1999 began a period of low circulation and low genetic diversity of the Caguas lineage of subclade IB (Figure 1, panel D; Figure 2; Figure 3, panel B) that lasted until 2003. During these 4 years, most DENV-2 cases originated from only 4 municipalities in eastern Puerto Rico (Figure 4, panel A); <20 additional DENV-2 cases were reported during that period in 12 other neighboring municipalities (Figure 4, panel A). Because phylogenetic lineages are geographically and temporally clustered, (Figure 2), we illustrated these associations on the map of Puerto Rico (Figure 4). This map shows that DENV-2 descendants from western Puerto Rico emerged in San Juan in 1997–1998 (Figure 4, panel B, top), then appeared and persisted within the refuge in 1999–2002 (Figure 4, panel C, middle) to then disseminate across the island in 2003–2005 (Figure 4, panel D). In the 4 municipalities with uninterrupted DENV-2 transmission, DENV-2 incidence increased 2 years after the islandwide increase (Figure 5). DENV-3 incidence within this DENV-2 refuge was minimal during the period of high DENV-2 incidence but peaked 2 years later concomitant with an increase across the rest of the island

Discussion

Puerto Rico is a model for fine-scale studies on DENV evolution in the Americas. The long-term persistence of

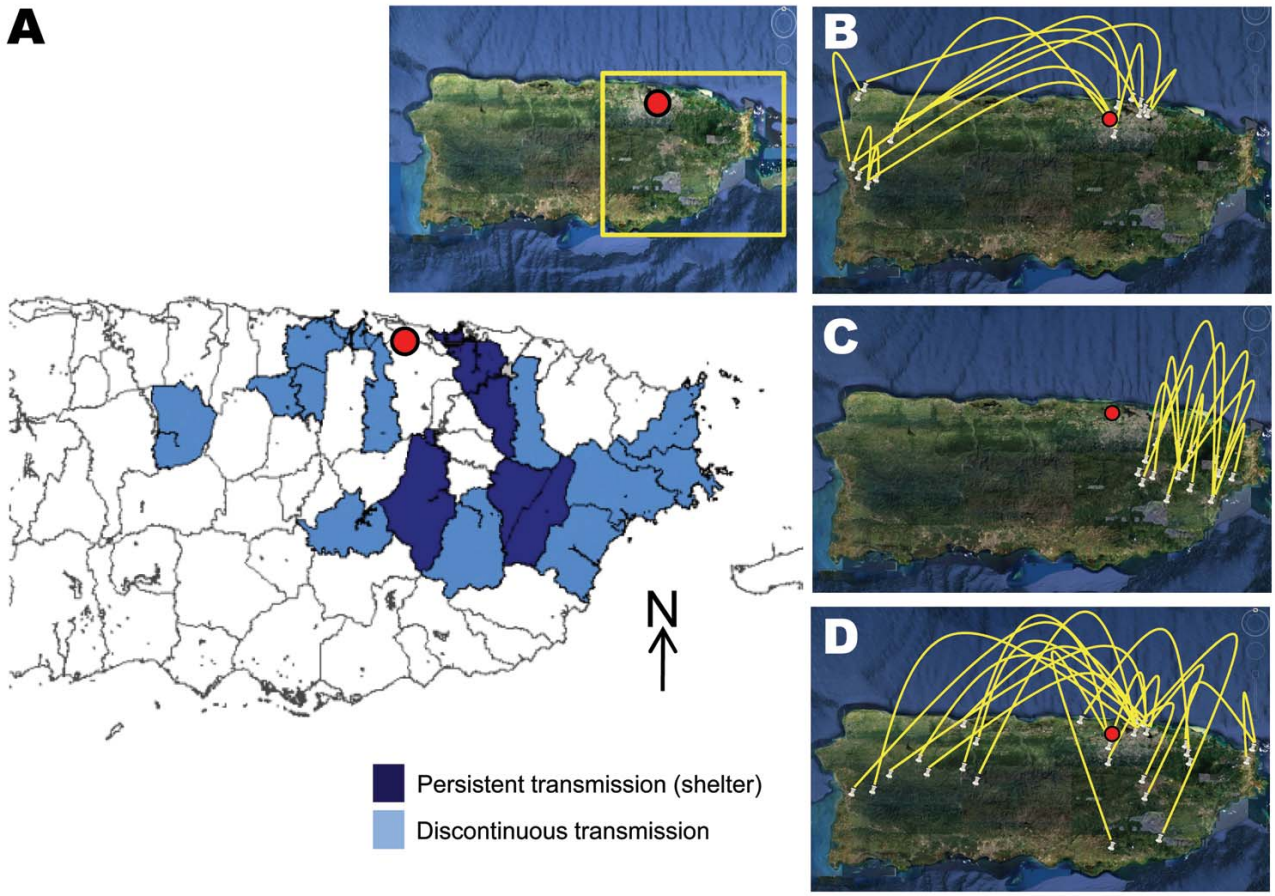

Figure 4. Epidemiology of dengue virus (DENV) serotype 2 in Puerto Rico, 1997–2006. A) Municipalities with persistent DENV-2 transmission (Caguas, Juncos, Las Piedras, Carolina) versus those with discontinuous transmission (Morovis, Toa Alta, Toa Baja, Cataño, Guaynabo, Cidra, San Lorenzo, Canóvanas, Humacao, Naguabo, Ceiba, Fajardo), 1998–2002. Inset shows satellite view; red dot indicates national capital (San Juan), and yellow box indicates region where DENV-2 took refuge during 2000–2002. B–D) Satellite view depicts virus transmission corridors. White pins point to specific geographic locations where DENV-2 isolates were collected during the specified time period. Yellow lines connect isolates by their phylogenetic affiliations suggesting migration of virus. B) DENV-2 traveled to the San Juan region from the west during 1997–1999; C) DENV-2 transmission retracted to the eastern, refuge region with restricted dispersion patterns during 2000–2002; D) DENV-2 reemerged focused on the San Juan region and later dispersed throughout the island during 2003–2006.

DENV-2 and its ability to reemerge after transient periods of low circulation is a remarkable aspect of the epidemiology of dengue in the region. The fact that 13% of DENV-2 isolates represent importations or close descendants from importations brings new insights to our understanding of DENV long-term circulation. Foreign viruses were identified in 8 years (1987, 1989, 1991, 1995, 1998, 1999, 2005, and 2007), of which only 1991 and 1998 had been previously sampled (*14*). Ten of the 18 introductions occurred during periods of high DENV-2 predominance: 1987–1991, 1995, and 2005–2007 (Figure 1, panel B; Figure 2). The other 8 introductions originated from the 1998 epidemic or shortly thereafter (1999). Therefore, DENV-2 seems to be introduced mainly during periods of favorable preponderance, not necessarily epidemic transmission of this serotype. Subclade IA viruses never established themselves, regardless of year of isolation or origin. These assessments showed a previously unknown feature of DENV-2 persistence: the endemic strain is recalcitrant to influences from frequent foreign introductions.

The relative inability of "foreign" DENV-2 to persist in the presence of the dominant subclade IB viruses is not well understood. The Puerto Rico strain might be highly adapted and thus have a fitness advantage, the frequently introduced strains might be simply underrepresented, or introduced strains may have disappeared through genetic drift. Isolate PR76_1995_EU569708, which lies basal to this subclade in the phylogeny (Figure 2), is more closely related to South American DENV-2 viruses than to other Puerto Rico viruses, and this lineage does not appear to have progressed, supporting the foreign origin of subclade IB. Our findings then show that subclade IB resulted from an introduced strain, as previously suggested by Bennett et al. (*14*), and successfully penetrated during a period of

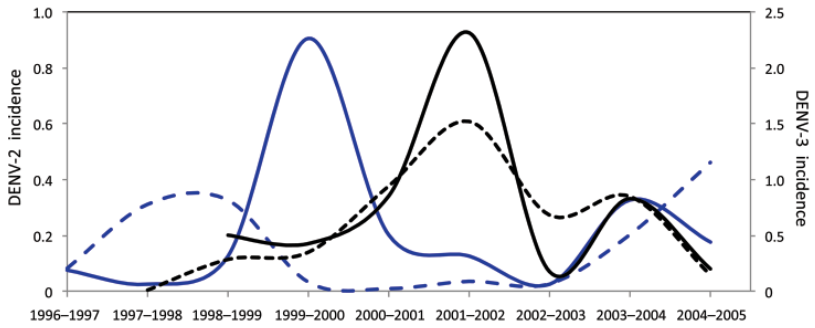

Figure 5. Incidence of dengue virus (DENV) serotypes 2 and 3 in Puerto Rico, 1996–2005. Solid blue line, incidence of DENV-2 within the refuge region; dashed blue line, incidence of DENV-2 in the rest of the island outside the refuge reason; solid black line, incidence of DENV-3 within the DENV-2 refuge region; dashed black line) incidence of DENV-3 in the rest of the island outside the refuge region. Incidence was calculated as number of confirmed, positive cases of each serotype per thousand residents.

proportionally high incidence of foreign introductions. Interestingly, this clade replacement was completed in 1997, less than a year before the finding of DENV-3 and the concomitant decline of DENV-1, -2, and -4. The early portion of subclade IB is seen as a period of short-lived lineages ending in 1997, therefore, the rise and expansion of this subclade mainly occurs in coexistence with DENV-3, a different epidemiologic scenario from that of the now extinct clade II a decade earlier.

The dominance of conservative amino acid changes that segregated the viruses by clade hinders the assessment of phenotypic changes. Compensatory mutations might have conferred replicative advantages that could have influenced the displacement of clade II or the persistence of subclade IB in Puerto Rico; however this hypothesis has not been tested. Positive selection was not identified, contrasting with previous analyses (*14*,*22*–*24*). Others have not detected positive selection and attribute lineage extinctions or clade replacements to stochastic events rather than natural selection (*25*). More analysis to detect site-specific selection is needed to corroborate whether positive selection is not at play in these populations of viruses.

The period 1999–2003 represents historically low rates of DENV-2 circulation (Figures 1, 2, 4), and the epidemiologic and phylogenetic aspects of this transient retrieval had not been studied previously. We show that the genetic variability of DENV-2 decreased during these 4 years when the virus was transmitted in only a subset of municipalities. DENV-2 represented 29% of the cases in this area but only 5% island-wide. The reason this region became a refuge of DENV-2 for 4 years remains unclear, but the low incidence of DENV-2 in prior years compared with the rest of the island suggests susceptibility for infection in this population (Figure 5). Studies in Thailand showed serotype displacement affecting population diversity and lineage turnover (*26*). Short-term serotype cross-protection has been suggested to contribute to serotype displacements (*27*–*29*), implying that as DENV-3 infected a large susceptible population, cross-protective antibodies momentarily impeded transmission of other serotypes and dissemination of DENV-2 outside the eastern refuge. Our study confirms the utility of systematic sampling and genome sequencing in large-scale surveillance systems as ways to understand the dynamics of dengue transmission and endemicity.

Acknowledgments

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References

1. Gubler DJ. Dengue/dengue haemorrhagic fever: history and current status. Novartis Found Symp. 2006;277:3–16; discussion 1622, 71–3, 251–3. DOI: 10.1002/0470058005.ch2
2. Dietz V, Gubler DJ, Ortiz S, Kuno G, Casta-Velez A, Sather GE, et al. The 1986 dengue and dengue hemorrhagic fever epidemic in Puerto Rico: epidemiologic and clinical observations. P R Health Sci J. 1996;15:201–10.
3. Rigau-Pérez JG, Vorndam AV, Clark GG. The dengue and dengue hemorrhagic fever epidemic in Puerto Rico, 1994–1995. Am J Trop Med Hyg. 2001;64:67–74.
4. Rigau-Pérez JG, Ayala-Lopez A, Garcia-Rivera EJ, Hudson SM, Vorndam V, Reiter P, et al. The reappearance of dengue-3 and a subsequent dengue-4 and dengue-1 epidemic in Puerto Rico in 1998. Am J Trop Med Hyg. 2002;67:355–62.
5. Lewis JA, Chang GJ, Lanciotti RS, Kinney RM, Mayer LW, Trent DW. Phylogenetic relationships of dengue-2 viruses. Virology. 1993;197:216–24. DOI: 10.1006/viro.1993.1582
6. Vorndam V, Kuno G, Rosado N. A PCR-restriction enzyme technique for determining dengue virus subgroups within serotypes. J Virol Methods. 1994;48:237–44. DOI: 10.1016/0166-0934(94)90122-8

7. Foster JE, Bennett SN, Vaughan H, Vorndam V, McMillan WO, Carrington CV. Molecular evolution and phylogeny of dengue type 4 virus in the Caribbean. Virology. 2003;306:126–34. DOI: 10.1016/S0042-6822(02)00033-8
8. Leitmeyer KC, Vaughn DW, Watts DM, Salas R, Villalobos I, de Chacon, et al. Dengue virus structural differences that correlate with pathogenesis. J Virol. 1999;73:4738–47.
9. Rico-Hesse R, Harrison LM, Salas RA, Tovar D, Nisalak A, Ramos C, et al. Origins of dengue type 2 viruses associated with increased pathogenicity in the Americas. Virology. 1997;230:244–51. DOI: 10.1006/viro.1997.8504
10. Guzman MG, Deubel V, Pelegrino JL, Rosario D, Marrero M, Sariol C, et al. Partial nucleotide and amino acid sequences of the envelope and the envelope/nonstructural protein-1 gene junction of four dengue-2 virus strains isolated during the 1981 Cuban epidemic. Am J Trop Med Hyg. 1995;52:241–6.
11. Gubler DJ. Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 2002;10:100–3. DOI: 10.1016/S0966-842X(01)02288-0
12. Rico-Hesse R. Dengue virus evolution and virulence models. Clin Infect Dis. 2007;44:1462–6. DOI: 10.1086/517587
13. Chaturvedi UC. The curse of dengue. Indian J Med Res. 2006;124:467–70.
14. Bennett SN, Holmes EC, Chirivella M, Rodriguez DM, Beltran M, Vorndam V, et al. Molecular evolution of dengue 2 virus in Puerto Rico: positive selection in the viral envelope accompanies clade reintroduction. J Gen Virol. 2006;87:885–93. DOI: 10.1099/vir.0.81309-0
15. Miagostovich MP, Nogueira RM, dos Santos FB, Schatzmayr HG, Araujo ES, Vorndam V. Evaluation of an IgG enzyme-linked immunosorbent assay for dengue diagnosis. J Clin Virol. 1999;14:183–9. DOI: 10.1016/S1386-6532(99)00059-1
16. Gubler DJ, Kuno G, Sather GE, Velez M, Oliver A. Mosquito cell cultures and specific monoclonal antibodies in surveillance for dengue viruses. Am J Trop Med Hyg. 1984;33:158–65.
17. Maddison WP, Maddison DR. Interactive analysis of phylogeny and character evolution using the computer program MacClade. Folia Primatol (Basel). 1989;53:190–202. DOI: 10.1159/000156416
18. Rigau-Perez JG, Ayuso-Lamadrid A, Wolff DR, Reiter P, Kuno G. Dengue severity throughout seasonal changes in incidence in Puerto Rico, 1989–1992. The Puerto Rico Association of Epidemiologists. Am J Trop Med Hyg. 1994;51:408–15.
19. Tomashek KM, Rivera A, Munoz-Jordan JL, Hunsperger E, Santiago L, Padro O, et al. Description of a large island-wide outbreak of dengue in Puerto Rico, 2007. Am J Trop Med Hyg. 2009;81:467–74.
20. Carrington CV, Foster JE, Pybus OG, Bennett SN, Holmes EC. Invasion and maintenance of dengue virus type 2 and type 4 in the Americas. J Virol. 2005;79:14680–7. DOI: 10.1128/JVI.79.23.14680-14687.2005
21. Twiddy SS, Holmes EC, Rambaut A. Inferring the rate and timescale of dengue virus evolution. Mol Biol Evol. 2003;20:122–9. DOI: 10.1093/molbev/msg010
22. Kumar SR, Patil JA, Cecilia D, Cherian SS, Barde PV, Walimbe AM, et al. Evolution, dispersal and replacement of American genotype dengue type 2 viruses in India (1956–2005). Selection pressure and molecular clock analyses. J Gen Virol. 2010;91:707–20. DOI: 10.1099/vir.0.017954-0
23. Twiddy SS, Woelk CH, Holmes EC. Phylogenetic evidence for adaptive evolution of dengue viruses in nature. J Gen Virol. 2002;83:1679–89.
24. Bennett SN, Holmes EC, Chirivella M, Rodriguez DM, Beltran M, Vorndam V, et al. Selection-driven evolution of emergent dengue virus. Mol Biol Evol. 2003;20:1650–8. DOI: 10.1093/molbev/msg182
25. Myat Thu H, Lowry K, Jiang L, Hlaing T, Holmes EC, Aaskov J. Lineage extinction and replacement in dengue type 1 virus populations are due to stochastic events rather than to natural selection. Virology. 2005;336:163–72. DOI: 10.1016/j.virol.2005.03.018
26. Zhang C, Mammen MP Jr, Chinnawirotpisan P, Klungthong C, Rodpradit P, Monkongdee P, et al. Clade replacements in dengue virus serotypes 1 and 3 are associated with changing serotype prevalence. J Virol. 2005;79:15123–30. DOI: 10.1128/JVI.79.24.15123-15130.2005
27. Adams B, Holmes EC, Zhang C, Mammen MP Jr, Nimmannitya S, Kalayanarooj S, et al. Cross-protective immunity can account for the alternating epidemic pattern of dengue virus serotypes circulating in Bangkok. Proc Natl Acad Sci U S A. 2006;103:14234–9. DOI: 10.1073/pnas.0602768103
28. Forshey BM, Morrison AC, Cruz C, Rocha C, Vilcarromero S, Guevara C, et al. Dengue virus serotype 4, northeastern Peru, 2008. Emerg Infect Dis. 2009;15:1815–8.
29. Vu TT, Holmes EC, Duong V, Nguyen TQ, Tran TH, Quail M, et al. Emergence of the Asian 1 genotype of dengue virus serotype 2 in Viet Nam: in vivo fitness advantage and lineage replacement in South-East Asia. PLoS Negl Trop Dis. 2010;4:e757. DOI: 10.1371/journal.pntd.0000757

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Tick-borne Encephalitis Virus in Wild Rodents in Winter, Finland, 2008–2009

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Rodents might maintain tick-borne encephalitis virus (TBEV) in nature through latent persistent infections. During 2 subsequent winters, 2008 and 2009, in Finland, we detected RNA of European and Siberian subtypes of TBEV in *Microtus agrestis* and *Myodes glareolus* voles, respectively. Persistence in rodent reservoirs may contribute to virus overwintering.

Tick-borne encephalitis (TBE) is a zoonotic disease endemic to a wide zone, from central and northern Europe to Siberia and Japan (*1*). The causative agent, tick-borne encephalitis virus (TBEV), is maintained in a cycle including ticks and their vertebrate hosts. Ticks serve as vectors and remain infected throughout their life cycle (transstadial transmission). Ticks may acquire the virus when they ingest blood from a viremic host. However, transmission of the virus from infected to uninfected ticks also occurs in the skin of vertebrate hosts, through migratory cells. This process, known as cofeeding, is considered to contribute to the natural cycle of the virus (*2*). Transovarial transmission of TBEV in ticks has also been reported (*3*). In addition to serving as hosts for cofeeding ticks, rodents have been considered to play a role in maintaining TBEV in nature through latent persistent infections, at least for the Siberian subtype (*4*), although strain and subtype differences may exist.

The 3 known subtypes of TBEV are European (TBEV-Eur), Siberian (TBEV-Sib), and Far-Eastern (TBEV-FE) (*5*). TBEV-Sib and TBEV-FE, carried by *Ixodes persulcatus* ticks, are monophyletic; TBEV-Eur and louping ill virus, spread mainly by *I. ricinus* ticks, are closely related to each other (*6*). Phylogenetic analyses suggest that TBEV-Sib and TBEV-FE subtypes evolved thousands of years ago, whereas TBEV-Eur has diversified more recently, ≈300 years ago (N.Y. Uzcátegui et al., unpub. data).

TBEV-Eur has a focal distribution, which is dependent on local climatic conditions (*7*). TBEV-Sib and TBEV-FE seem to be spread more widely throughout the *I. persulcatus* tick range (*1*). We studied the persistence of TBEV in wild rodents in natural TBE foci.

The Study

Small mammals were collected from 2 sites in Finland, representing TBEV-Eur– (Isosaari Island, Helsinki archipelago) and TBEV-Sib– (Kokkola archipelago) endemic areas (Figure 1). Clinical TBE cases and TBEV-positive ticks have been detected at each of these sites (*8*,*9*). The animals were trapped from February 19 through March 12 in 2008 and 2009, when daily maximum temperatures had been below the tick activity limit for months (Figure 2). Thus, TBEV RNA in rodent tissues would likely have persisted from the previous summer.

Animals were caught in snap traps set overnight and stored at –80°C until they were dissected. Brain, lungs, liver, and spleen were stored at –70°C. Pieces of lungs, liver, and spleen were also pooled for reverse transcription–PCR (RT-PCR). Blood from dissected heart was extracted and stored in phosphate-buffered saline for serologic analysis. A total of 50–100 mg of each tissue sample or organ pool was homogenized in 1 mL of TriPure Isolation Reagent (Roche Diagnostics Corp., Indianapolis, IN, USA) with glass beads and sand in a MagNA Lyser (Roche Diagnostics GmbH, Mannheim, Germany). RNA was extracted by using TriPure Isolation Reagent according to the manufacturer's instructions.

The RNA was dissolved in 25 μL of diethylpyrocarbonate-treated water and analyzed by using real-time RT-PCR as described by Schwaiger and Cassinotti (*10*), except we used 50 nmol/L forward primer, 300 nmol/L reverse primer, and 200 nmol/L probe. Samples positive by real-time RT-PCR were further analyzed by nested RT-PCR, amplifying a 252-nt sequence from the TBEV nonstructural protein (NS) 5 gene. Analysis was performed as described by Puchhammer-Stöckl et al. (*11*), using modified primer as described by Jääskeläinen et al. (*9*). Dissection of animals and RNA extraction were done in a laboratory in which no TBEV RNA or cDNA had been previously introduced. Diluted blood samples (≈1:10) were studied for antibodies against TBEV by using an immunofluorescence assay with TBEV-Eur–infected Vero E6 cells as antigen and polyclonal rabbit anti-mouse fluorescein isothiocyanate conjugate (Dako, Glostrup, Denmark). Sample and conjugate were incubated for 30 min at 37°C.

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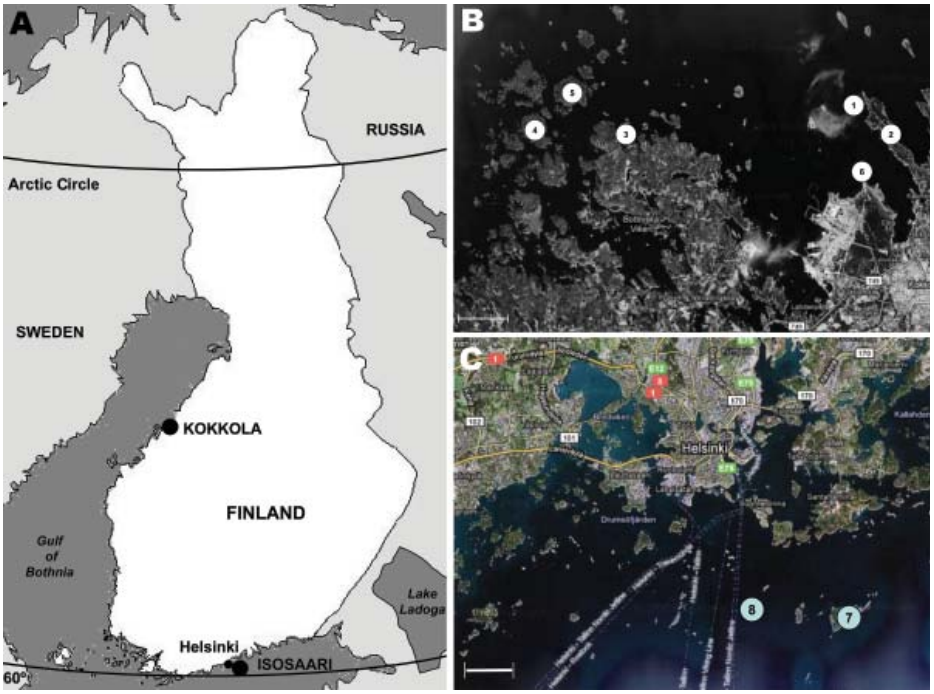

Figure 1. Sites at which rodents were trapped during winters of 2008 and 2009, Finland. A) Locations of trapping sites within Finland. B) Kokkola archipelago, where Siberian subtype of tick-borne encephalitis virus is endemic: 1,Trullevi, Kupu Island; 2,Trullevi; 3, Enträskholmen Island; 4, Börskär Island; 5, Norra Hamnskäret Island; 6, Harrbådan. C) Helsinki archipelago, Isosaari, where European subtype of tick-borne encephalitis virus is endemic: 7, Isosaari Island; 8, Harmaja Island. Scale bars indicate 2 km.

During the 2 subsequent years, 202 rodents and insectivores were trapped outside the tick-feeding season (Table). All rodents in the TBEV-Sib focus were bank voles (*Myodes glareolus*), and those in the TBEV-Eur focus were field voles (*Microtus agrestis*). Altogether 23 voles and 1 common shrew *(Sorex araneus)* were positive for TBEV RNA by real-time RT-PCR. Viral RNA was detected mostly in brain (or in brain and internal organs); for 2 voles, it was detected only in internal organs. Of the real-time RT-PCR–positive samples, 5 were also positive with the less sensitive (*10*,*11*) NS5 gene–targeting nested RT-PCR. The 165-nt stretch of the NS5 gene obtained from a bank vole (GenBank accession no. GU458800) from the TBEV-Sib–endemic area (Figure 1) differed 0–4 nt from published sequences from *I. persulcatus* ticks collected from the same area in 2004 (*9*). No sequence from the TBEV-Eur area could be recovered.

Serologic analysis showed that in the TBEV-Eur area, only 2 of 16 mammals whose brain tissue was positive for TBEV RNA had anti-TBEV antibodies; whereas, in the TBEV-Sib area, all 5 rodents whose brain tissue was positive for TBEV RNA had antibodies as well. The difference was significant (Fisher exact test, p = 0.001). One antibody-positive rodent did not have detectable levels of TBEV RNA in brain, lung, liver, or spleen.

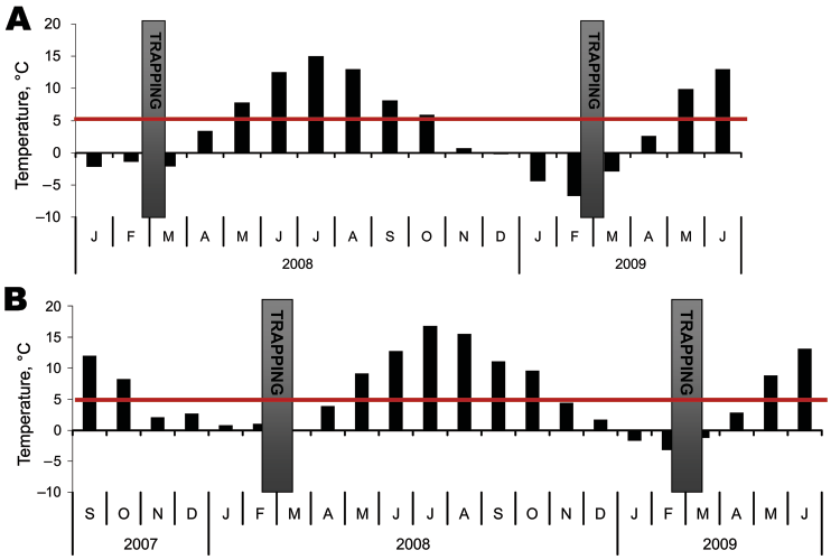

Figure 2. Monthly day and night mean temperatures at the trapping sites. Daily maximum temperatures had not reached 5°C for ≥50 days before trapping. Tick-feeding season is considered to begin when temperature in the ground reaches the tick activity limit and stays above it (*1*). A) Kokkola archipelago, where Siberian subtype of tick-borne encephalitis virus is endemic. B) Helsinki archipelago, where European subtype of tick-borne encephalitis virus is endemic. Although trapping was conducted on Isosaari, temperature data were unavailable and were instead collected on Harmaja, a nearby island (Figure 1). Gray bars indicate time of trapping; red line indicates tick activity limit. Data source: Finnish Meteorological Institute (http://ilmatieteenlaitos.fi/en/).

Table. Small mammals trapped during 2 subsequent winters (2008 and 2009) in TBEV-Eur– and TBEV-Sib–endemic areas, Finland*

Location (virus subtype), year, and mammal species	No. animals trapped	No. RNA positive by real-time PCR			No. (%) antibody positive†
		Brain	Organ pool/spleen‡	Total (%)	
Kokkola (TBEV-Sib)					
2008					
Myodes glareolus vole	63	1§	0	1 (1.6)	2 (3.2)
Sorex caecutiens shrew	4	0	0	0	0
S. araneus shrew	7	0	0	0	0
2009					
M. glareolus vole	17	4§	2	5 (29.4)	4 (23.5)
S. araneus shrew	3	0	1	1 (33.3)	0
Isosaari (TBEV-Eur)					
2008					
Microtus agrestis vole	71	3¶	2	4 (5.6)	0
S. araneus shrew	7	0	0	0	0
2009					
M. agrestis vole	24	13	0	13 (54.2)	2 (8.3)
S. araneus shrew	6	0	0	0	0

*TBEV, tick-borne encephalitis virus; TBEV-Eur, European subtype of TBEV; TVEV-Sib, Siberian subtype of TBEV.
†Blood samples, diluted ≈1:10 in phosphate-buffered saline, were screened by immunofluorescence assay with TBEV-infected Vero E6 cells as antigen.
‡For animals collected in 2008, organ pool of lungs, spleen, and liver were screened; for animals collected in 2009, only spleen was screened.
§Three brain samples positive for TBEV RNA by real-time reverse transcription–PCR (RT-PCR) (1 in 2008 and 2 in 2009) were also positive for the TBEV nonstructural protein (NS) 5 gene by nested RT-PCR.
¶Two brain samples positive for TBEV RNA by RT-PCR were also positive for the TBEV NS5 gene by nested RT-PCR.

Conclusions

The focal distribution of TBEV-Eur has generally been explained by climatic factors, which define the temporal occurrence of nymphs and larvae and, consequently, the frequency of cofeeding (*12*). TBEV-Sib is also transmitted vertically between generations of adapted reservoir rodents and nonadapted laboratory mice (*13*). Furthermore, virus persistence and latent infections in rodents outside the tick-feeding season may occur in the TBEV-Sib–endemic zone in Siberia. Thus, TBEV-Sib may be less dependent than TBEV-Eur on tick cofeeding, and thereby on the climate, and seems to occur less focally (*4,14*). However, TBEV has also been reported to persist over winter in western Slovakia, a TBEV-Eur–endemic area (*15*).

We detected TBEV RNA in brain and internal organ samples of rodents in TBEV-Eur– and TBEV-Sib–endemic areas (Figure 2) several months after tick-feeding season. Almost all TBEV RNA–positive rodents in the TBEV-Sib–endemic area had anti-TBEV antibodies, whereas in the TBEV-Eur area, most did not. This finding might indicate a difference in the infection process and host adaptation between the 2 subtypes. Persistent TBEV has been isolated from rodents in a TBEV-Eur–endemic area even when no antibodies for TBEV were detected (*15*).

The host species at the 2 trapping sites differed. To find bank voles as TBEV-Sib hosts was not unexpected, considering that the congeneric red vole (*Myodes rutilus*) is a common host for TBEV in Siberia (*14*). However, earlier studies have implicated yellow-necked mice (*Apodemus flavicollis*) and bank voles as major hosts for TBEV-Eur (*2*). In our TBEV-Eur focus, all TBEV-positive animals were field voles, which dominated Isosaari Island in the absence of *Myodes* and *Apodemus* spp. rodents.

Our results show that TBEV-Sib and TBEV-Eur may cause prolonged latent infections in host rodents. We detected TBEV RNA in brain and other tissues from rodents in some of the northernmost TBEV-endemic areas in Europe, where the daily maximum temperatures and the snow cover in winter do not enable nymphal or larval activity. Further comparative studies are needed to explain the type of latency and its possible role in the ecology of different subtypes of TBEV.

Acknowledgments

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in northern Europe, especially maintenance of the virus in nature and differences between the 3 subtypes of TBEV.

References

1. Lindquist L, Vapalahti O. Tick-borne encephalitis. Lancet. 2008;31:1861–71. DOI: 10.1016/S0140-6736(08)60800-4
2. Labuda M, Kozuch O, Zuffová E, Elecková E, Hails RS, Nuttall PA. Tick-borne encephalitis virus transmission between ticks cofeeding on specific immune natural rodent hosts. Virology. 1997;235:138–43. DOI: 10.1006/viro.1997.8622
3. Mansfield KL, Johnson N, Phipps LP, Stephenson JR, Fooks AR, Solomon T. Tick-borne encephalitis virus—a review of an emerging zoonosis. J Gen Virol. 2009;90:1781–94. DOI: 10.1099/vir.0.011437-0
4. Moshkin MP, Novikov EA, Tkachev SE, Vlasov VV. Epidemiology of a tick-borne viral infection: theoretical insights and practical implications for public health. Bioessays. 2009;31:620–8. DOI: 10.1002/bies.200800196
5. Ecker M, Allison SL, Meixner T, Heinz FX. Sequence analysis and genetic classification of tick-borne encephalitis viruses from Europe and Asia. J Gen Virol. 1999;80:179–85.
6. Grard G, Moureau G, Charrel RN, Lemasson J-J, Gonzalez J-P, Gallian P, et al. Genetic characterization of tick-borne flaviviruses: New insights into evolution, pathogenic determinants and taxonomy. Virology. 2007;361:80–92.
7. Randolph SE, Rogers DJ. Fragile trans mission cycles of tick-borne encephalitis may be disrupted by predicted climate change. Proc Biol Sci. 2000;267:1741–4. DOI: 10.1098/rspb.2000.1204
8. Han X, Aho M, Vene S, Peltomaa M, Vaheri A, Vapalahti O. Prevalence of tick-borne encephalitis virus in *Ixodes ricinus* ticks in Finland. J Med Virol. 2001;64:21–8. DOI: 10.1002/jmv.1012
9. Jääskeläinen AE, Tikkakoski T, Uzcátegui NY, Alekseev AN, Vaheri A, Vapalahti O. Siberian subtype tickborne encephalitis virus, Finland. Emerg Infect Dis. 2006;12:1568–71.
10. Schwaiger M, Cassinotti P. Development of a quantitative real-time RT-PCR assay with internal control for the laboratory detection of tick borne encephalitis virus (TBEV) RNA. J Clin Virol. 2003;27:136–45.
11. Puchhammer-Stöckl E, Kunz C, Mandl CW, Heinz FX. Identification of tick-borne encephalitis virus ribonucleic acid in tick suspensions and in clinical specimens by a reverse transcription–nested polymerase chain reaction assay. Clin Diagn Virol. 1995;4:321–6.
12. Randolph SE. The shifting landscape of tick-borne zoonoses: tick-borne encephalitis and Lyme borreliosis in Europe. Philos Trans R Soc Lond B Biol Sci. 2001;356:1045–56. DOI: 10.1098/rstb.2001.0893
13. Bakhvalova VN, Potapova OF, Panov VV, Morozova OV. Vertical transmission of tick-borne encephalitis virus between generations of adapted reservoir small rodents. Virus Res. 2009;140:172–8. DOI: 10.1016/j.virusres.2008.12.001
14. Bakhvalova VN, Dobrotvorsky AK, Panov VV, Matveeva VA, Tkachev SE, Morozova OV. Natural tick-borne encephalitis virus infection among wild small mammals in the southeastern part of western Siberia, Russia. Vector Borne Zoonotic Dis. 2006;6:32–41. DOI: 10.1089/vbz.2006.6.32
15. Kozuch O, Labuda M, Lysý J, Weismann P, Krippel E. Longitudinal study of natural foci of central European encephalitis virus in west Slovakia. Acta Virol. 1990;34:537–44.

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Serologic Status for Pandemic (H1N1) 2009 Virus, Taiwan

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We studied preexisting immunity to pandemic (H1N1) 2009 virus in persons in Taiwan. A total of 18 (36%) of 50 elderly adults in Taiwan born before 1935 had protective antibodies against currently circulating pandemic (H1N1) 2009 virus. Seasonal influenza vaccines induced antibodies that did not protect against pandemic (H1N1) 2009 virus.

As experts were predicting and warning of a new influenza pandemic (*1*), an influenza epidemic occurred in April 2009 in the United States and Mexico and resulted in a pandemic 2 months later. The etiologic agent was identified as pandemic (H1N1) 2009 virus. Worldwide, most patients infected with this virus were <25 years of age, and one third of serious cases were in persons <50 years of age (*2*,*3*).

The hemagglutinin gene of pandemic (H1N1) 2009 virus was shown to be derived from the 1918 swine influenza virus and contained other genes from human, avian, and swine influenza viruses from Eurasia (*2*). In this study, we evaluated levels of preexisting cross-reactive antibodies against pandemic (H1N1) 2009 virus produced after previous infection in children and adults in Taiwan. We also examined serologic changes after vaccination with seasonal nonadjuvanted influenza vaccine.

The Study

Serum samples were obtained during a nationwide influenza vaccine serologic study in Taiwan that started in 2006. Children (<5 years of age), adults (20–49 years of age), older adults (50–74 years of age), and elderly adults (≥75 years of age) were recruited. Serum samples were obtained immediately before and 3 weeks after intramuscular injection with 1 dose of nonadjuvanted, trivalent, inactivated influenza vaccine formulated for the 2008–09 Northern Hemisphere winter season (samples were obtained from some participants ≥75 years of age before and after receiving 1 dose of the vaccine formulated for the 2007–08 winter season).

Microneutralization (MN) and hemagglutination inhibition (HI) assays were performed according to the World Health Organization Manual on Animal Influenza Diagnosis and Surveillance (*4*). Using these assays with 0.75% guinea pig erythrocytes, we assayed samples for antibodies against A/California/07/2009 (H1N1) virus. Only prevaccination HI assays were conducted for children.

The seroprotection rate was defined as the percentage of serum titers ≥40 by HI or titers ≥160 by MN. The seroconversion rate was defined as the percentage of vaccine recipients whose serum HI titers or MN titers increased by at least 4-fold after vaccination. A p value <0.05 was considered significant. Stata software version 8.2 (StataCorp LP, College Station, TX, USA) was used for analysis.

A total of 176 participants (40 children, 36 adults, 50 older adults, and 50 elderly adults) were enrolled (Table). Few or no preexisting cross-reactive antibodies against pandemic (H1N1) 2009 virus were detected by HI assay in samples from children (prevaccination seroprotection rate 0%). As age increased, prevaccination seroprotection rates became higher for HI and MN assays. After vaccination, seroprotection rates and geometric mean titers measured by HI assay were essentially unchanged but increased significantly in the 3 adult groups when measured by MN assay ($p<0.05$). Seroconversion rates among all participants were low. Analyses of relationships between age and antibody titers are shown in the Figure.

We log-transformed MN and HI titers, and used multiple regression, controlling for age groups to analyze the correlation between age and titer. Doubling of HI titers corresponded to an estimated 75% ($p<0.01$) increment in MN titers adjusted by age. When adjusted for HI titers, MN titers in older adults and elderly adults were 1.74× ($p<0.01$) and 2× ($p<0.01$), respectively, those in adults. Older adults and elderly adults with the same HI titers were more likely to have higher MN titers than adults ($p<0.05$, by ordinal logistic regression analysis).

Conclusions

We found that children in Taiwan had few or no cross-reactive antibodies against pandemic (H1N1) 2009 virus. However, adults had some preexisting immunity to this virus. A major finding was that 18 (36%) of 50 elderly adults in Taiwan born before 1935 had protective antibodies against currently circulating pandemic (H1N1) 2009 virus. The seroprotection rate may be 50% in persons >80 years of age.

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Table. Geometric mean titers of antibodies and rates of seroprotection against pandemic (H1N1) 2009 virus before and after seasonal influenza vaccination, by age, Taiwan, 2007–2008*

Group, age, y	Prevaccination GMT (95% CI)	Prevaccination seroprotection rate, %	Postvaccination GMT (95% CI)	Postvaccination seroprotection rate, %	p value
Children <5, n = 40)					
HI	10.4 (9.9–10.9)	0	ND	ND	ND
Adults 20–49, n = 36					
HI	12.1 (10.7–13.7)	2.8	12.1 (10.7–13.7)	2.80	NS
MN	26.3 (20.9–32.8)	0	31.7 (25.0–40.3)	0	<0.05
Older adults 50–74, n = 50					
HI	16.7 (14.2–19.7)	16	16.7 (14.2–19.7)	16	NS
MN	59.0 (47.7–72.8)	20	74.6 (60.0–92.9)	32	<0.05
Elderly adults ≥75, n = 50					
HI	22.7 (19.5–26.4)	36	23.3 (19.8–27.4)	38	0.159
MN	85.7 (70.8–103.9)	32	107 (89.2–128.5)	44	<0.05

*GMT, geometric mean titer; CI, confidence interval; HI, hemagglutination inhibition; ND, not done; NS, not significant. MN, microneutralization. Mean ± SD ages for the 4 groups were 20.0 ± 11.3 mo for children, 34.5 ± 7.5 y for adults, 65 ± 6 y for older adults, and 79 ± 3.3 y for elderly adults.

The MN assay showed that seasonal influenza vaccines generated large increases in geometric mean titers in vaccinees in all age groups. We suggest that seasonal influenza vaccines are likely to elicit a certain degree of cross-reactive antibodies against pandemic (H1N1) 2009 virus and may provide some level of protection. In persons who had no preexisting seroprotective titers against pandemic (H1N1) 2009 virus, the cross-reactivity produced was not sufficient to prevent disease; however, it may protect against the severe forms of the disease.

Hancock et al. (*5*) reported that only 4% of persons in the United State born after 1980 had preexisting cross-reactive antibodies against pandemic (H1N1) 2009 virus, and that 34% of persons born before 1950 had neutralizing titers ≥80. However, Itoh et al. (*6*) reported that blood donors from Japan who were born after 1920 had almost no appreciable neutralizing antibodies against this virus. Because the hemagglutinin gene of pandemic (H1N1) 2009 virus is similar to that of viruses that circulated in humans during 1918–1957 (*7*), Itoh et al. suggested that pandemic (H1N1) 2009 virus is antigenically divergent from human influenza viruses (H1N1) that circulated during the 1920s–1950s.

Our results are consistent with those of Hancock et al. (*5*), who suggested that human influenza virus (H1N1) circulating in Taiwan after 1920 resembled the 1918 pandemic virus (H1N1) and pandemic (H1N1) 2009 virus and could lead to cross-protection against the current virus. Furthermore, unlike the situation in the United States, there was no program for vaccination against the 1976 swine influenza virus (A/NJ/76) in Taiwan. However, a similar virus was present in Taiwan before 1957. The results of our study also explain why only 7% of patients hospitalized for pandemic (H1N1) 2009 virus in Taiwan were ≥65 years of age (*8*). Similar epidemiologic observations have been reported in the United States (*3*) and New Zealand (*9*).

Whether elderly persons still have cross-reactivity several decades after exposure to 1918 (H1N1) virus is unknown. The concept of original antigenic sin is a probable explanation. Original antigenic sin has been described in relation to influenza virus, dengue virus, HIV, and several other viruses (*10–12*). For persons >65 years of age, the 1918 (H1N1) virus is likely the first influenza virus to which they were exposed, and their antibody response should have increased in subsequent years.

Regression analysis showed that older persons with high HI titers were more likely than younger adults to have higher MN titers. Because the HI assay detects only antibodies against hemagglutinin, the MN assay provides more information about the level of protective antibodies against influenza viruses. As a person ages, production of antibodies against hemagglutinin or other components of influenza virus should protect against potential infections. Although a titer of 40 by HI is accepted as a cutoff value for seroprotection, a consensus for protective titers by MN is lacking (*5*,*13*). We suggest that MN is probably more sensitive than HI for evaluating neutralizing antibodies against pandemic (H1N1) 2009 virus.

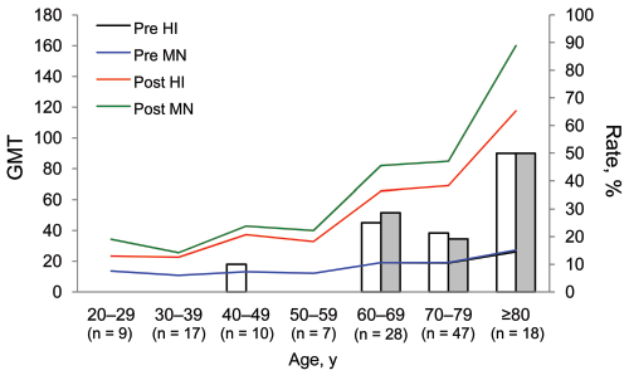

Figure. Seroprotection rates determined by hemagglutination inhibition (HI) assay (white bars) or microneutralization (MN) assay (gray bars) and geometric mean titer (GMT) of antibodies against pandemic (H1N1) 2009 virus in each 10-year age cohort, Taiwan, 2007–2008.

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References

1. Hsieh YC, Wu TZ, Liu DP, Shao PL, Chang LY, Lu CY, et al. Influenza pandemics: past, present and future. J Formos Med Assoc. 2006;105:1–6. DOI: 10.1016/S0929-6646(09)60102-9
2. Chang LY, Shih SR, Shao PL, Huang DT, Huang LM. Novel swine-origin influenza virus A (H1N1): the first pandemic of the 21st century. J Formos Med Assoc. 2009;108:526–32. DOI: 10.1016/S0929-6646(09)60369-7
3. Novel Swine-Origin Influenza (H1N1) Virus Investigation Team, Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009;360:2605–15. DOI: 10.1056/NEJMoa0903810
4. World Health Organization. WHO manual on animal influenza diagnosis and surveillance. Geneva: The Organization; 2002.
5. Hancock K, Veguilla V, Lu X, Zhong W, Butler EN, Sun H, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. N Engl J Med. 2009;361:1945–52. DOI: 10.1056/NEJMoa0906453
6. Itoh Y, Shinya K, Kiso M, Watanabe T, Sakoda Y, Hatta M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. Nature. 2009;460:1021–5.
7. Smith GJ, Vijaykrishna D, Bahl J, Lycett SJ, Worobey M, Pybus OG, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. Nature. 2009;459:1122–5. DOI: 10.1038/nature08182
8. Flu information. Central Epidemic Command Center. Taipei (Taiwan): Centers for Disease Control [cited 2009 Feb 23]. http://flu.cdc.gov.tw/public/Data/03214413671.pdf
9. Centers for Disease Control and Prevention. Surveillance for the 2009 pandemic influenza A (H1N1) virus and seasonal influenza viruses—New Zealand, 2009. MMWR Morb Mortal Wkly Rep. 2009;58:918–21.
10. Davenport FM, Hennessy AV, Francis T Jr. Epidemiologic and immunologic significance of age distribution of antibody to antigenic variants of influenza virus. J Exp Med. 1953;98:641–56. DOI: 10.1084/jem.98.6.641
11. Francis T Jr. Influenza: the new acquayantance. Ann Intern Med. 1953;39:203–21.
12. Kim JH, Skountzou I, Compans R, Jacob J. Original antigenic sin responses to influenza viruses. J Immunol. 2009;183:3294–301. DOI: 10.4049/jimmunol.0900398
13. Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. N Engl J Med. 2009;361:2424–35. DOI: 10.1056/NEJMoa0907650

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Serodiagnosis of Primary Infections with Human Parvovirus 4, Finland

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To determine the prevalence of parvovirus 4 infection and its clinical and sociodemographic correlations in Finland, we used virus-like particle–based serodiagnostic procedures (immunoglobulin [Ig] G, IgM, and IgG avidity) and PCR. We found 2 persons with parvovirus 4 primary infection who had mild or asymptomatic clinical features among hepatitis C virus–infected injection drug users.

A new member of family *Parvoviridae*, human parvovirus 4 (PARV4), was identified in plasma of an injection drug user (IDU) with unexplained fatigue, headaches, fever, night sweats, nausea, and diarrhea (*1*). In PCR studies of blood and postmortem tissues, virus was detected mainly in persons with histories of injection drug use (*2*–*5*). A recent PARV4 immunoglobulin (Ig) G study also showed higher prevalence of antibodies to PARV4 in IDUs and HIV-positive persons who had hemophilia than in HIV-positive men who have sex with men (*6*). The clinical role of this virus is unknown.

We report virus-like particle–based comprehensive serodiagnosis for PARV4 and determine its occurrence in Finland in 3 diverse population groups. In the highest prevalence group, we comparatively analyzed PARV4 IgG–positive and IgG–negative persons for sociodemographic and behavioral background factors and symptoms by using an HIV risk factor database (*7*).

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The Study

Group 1 (low risk) comprised 115 university students (1 serum sample/student). Group 2 (high risk) comprised 78 HIV IgG–positive IDUs from the Helsinki Cohort Study (*8*) (151 plasma samples, 1–7 samples/person). Group 3 (high risk) comprised 200 hepatitis C virus (HCV) IgG–positive patients (1 sample/person). Informed consent was obtained from persons in groups 1 and 2 and from 2 patients with primary infections in group 3. The study was reviewed and approved by the Helsinki University Central Hospital Ethics Committee (#281/13/03/01/09 and #469/2001).

Four genomic regions of open reading frame 2 of PARV4 genotype 1 (AY622943) were cloned for baculovirus expression by using PARV4 PCR–positive plasma (*1*) as initial template. The clone with nt region 3137–5122 (AY622943) and infectivity was constructed with primers PARV4*Eco*RI_3137: 5′-TATGAATT CATGATTGAGCATGGGG-3′ and PARV4EagI_5122: 5′-TACGGCCGTTACAGCAAATGAGAATAA-3′.

Protein expression and virus-like particle purification were conducted as for human bocavirus (HBoV) (*9*). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis identified a 73-kDa protein (Figure 1, panel A), which was immunoreactive by Western blotting (*10*) with 5 known PARV4 IgG–positive serum samples (*6*) but not with negative serum samples (Figure 1, panel B). Electron microscopy showed spherical particles ≈25 nm in diameter (Figure 1, panel C) that resembled those seen in vivo (*11*). The capsid protein region is 109 aa longer (N terminally) than that reported by Sharp et al. (*6*); both constructs assembled into capsids.

PARV4 IgG enzyme immunoassay (EIA) was conducted as for HBoV (*9*). Specific results were obtained by subtracting antigen-free background levels. For IgM EIA, a μ-capture format was used (*9*). IgG and IgM cutoff values, obtained from group 1 absorbances (mean + 4 × SD), were 0.141 and 0.205, respectively. IgG-avidity EIA was conducted as for HBoV (method A) (*12*); cutoff values for high and low avidity were 25% and 15%, respectively.

None of the 115 students (group 1) were PARV4 IgG positive, and 1 (0.9%) of 115 was weakly IgM positive (Figure 2, panel A). Sixty-one (78.2%) of 78 HIV-infected patients (group 2) were IgG positive, and 4 (5.1%) of 78 were IgM positive (Figure 2, panel B). Sixty-nine (34.5%) of 200 HCV-infected patients (group 3) were IgG positive, and 3 (1.5%) of 200 were IgM positive (Figure 2, panel C). Previous samples were available for 2 of the IgM-positive patients (A and B) in group 3. These samples showed seroconversion for IgG and an increase in IgG (Table 1).

PARV4 IgG avidity was determined in all persistently (>1 year) IgG-positive persons in group 2 (n = 29). Twenty-eight persons showed high IgG avidity, and 1 showed

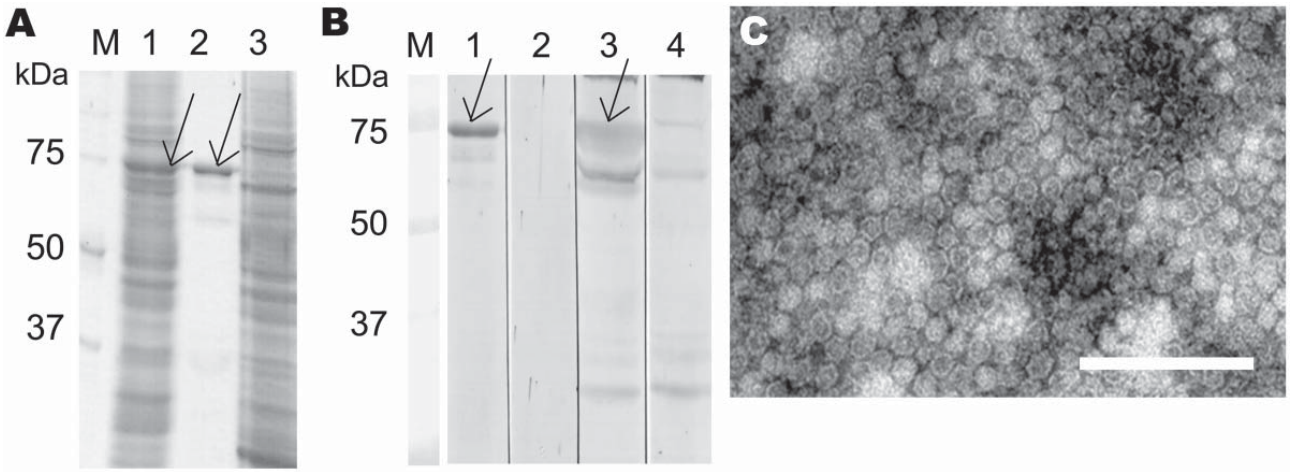

Figure 1. Parvovirus 4 (PARV4) virus-like particle (VLP) expression and immunoreactivity, Finland. A) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of PARV4-like particles in *Spodoptera frugiperda* armyworm (Sf)9 cells (lane 1), purified VLPs (lane 2), and uninfected Sf9 cells (lane 3). B) Western blotting with PARV4 immunoglobulin (Ig) G–positive serum (lanes 1, 3, and 4) or PARV4 IgG–negative serum (lane 2). Lanes 1 and 2, purified VLPs as antigen; lane 3, Sf9 cells expressing VLPs; lane 4, Sf9 cells expressing glutathione-S-transferase control antigen; lanes M, molecular mass marker. Arrows in panels A and B indicate the PARV4 capsid protein. C) Electron micrograph of purified VLPs. Scale bar = 200 nm.

borderline IgG avidity. All 4 IgG-positive persons had high-avidity IgG, which indicated previous immunity.

In group 3, a second sample from patient A, who showed seroconversion for IgG showed borderline IgG avidity. Patient B showed low IgG avidity in both samples (Table 1).

Groups 2 and 3 were also analyzed for PARV4 DNA by qualitative PCR (*13*) as modified (94°C for 10 min; 45 cycles at 94°C for 20s, 51°C or 56°C for 20s, and 72°C for 20s; and extension at 72°C for 7 min). Amplicons were subjected to electrophoresis and sequenced. In group 2, all 151 serum samples were PCR negative. In group 3, two patients (A and B) were PCR positive (Table 1).

PARV4 IgG–positive and IgG–negative IDUs (group 2) were compared for demographic and clinical characteristics. PARV4 IgG–positive persons reported more injection of drugs, persistent (>10 y) injection, and lending of injection equipment (Table 2). They also had a more frequent history of imprisonment and unemployment and were less educated. No differences were seen between PARV4 IgG–positive and IgG–negative persons with any symptoms (fever, tiredness, nocturnal sweating, cough, diarrhea, shortness of breath, swallowing complaints, muscle weakness, dizziness, skin abscesses or herpetic lesions, loss of eyesight, or headache) during 6 months before being interviewed.

Conclusions

We developed IgG-, IgM-, and IgG-avidity–based PARV4 serodiagnostic procedures; studied high-prevalence cohorts by PCR; and analyzed HIV-infected IDUs for demographic and clinical correlations with PARV4

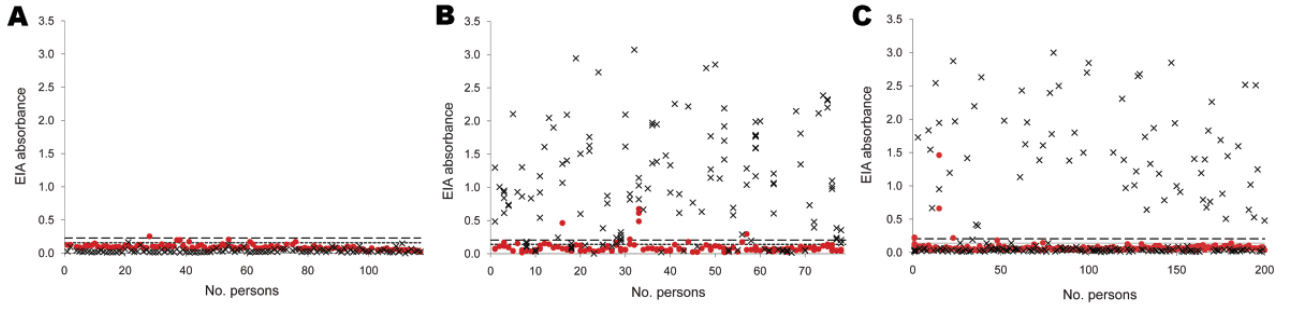

Figure 2. Parvovirus 4 (PARV4) enzyme immunoassay (EIA) results, Finland. Red dots, immunoglobulin (Ig) M; ×, IgG. Upper dashed line indicates IgM cutoff value (0.205), and lower dashed line indicates IgG cutoff value (0.141). A) Group 1: 115 university students (1 serum sample/person); none positive for PARV4 IgG, and 1 positive for PARV4 IgM. B) Group 2: 78 HIV-infected injection drug users (151 serum samples [1–7 samples/person]). Prevalences of PARV4 IgG and IgM were 78.2% (61/78) and 5.1% (4/78), respectively. C) Group 3: 200 hepatitis C virus–infected patients (1 sample/person). Prevalences of PARV4 IgG and IgM were 34.5% (69/200) and 1.5% (3/200), respectively.

Table 1. Virologic findings for PARV4 primary infections in 2 patients, Finland*

Patient	Date of sampling	IgG EIA absorbance	IgM EIA absorbance	PCR	IgG avidity, %
A	2004 Sep 8	0.016	0.051	–	ND
	2006 Mar 14	2.873	0.218	+	17.7
B	2006 Mar 13	0.950	1.461	+	8.9
	2006 Apr 6	1.946	0.661	+	10.1

*PARV4, parvovirus 4; Ig, immunoglobulin; EIA, enzyme inmmunoassay; –, negative; ND, not determined; +, positive.

IgG positivity. Among healthy university students, none had PARV4 IgG, which is consistent with low baseline IgG prevalences of 0% and 2.8% for another EIA (*6*). The PARV4 IgG seroprevalence of 78% among HIV-infected IDUs represents a high incidence of PARV4, which reflects the lengthy history of drug use among socially marginalized IDUs during an HIV outbreak in Finland (*7*).

Two HCV-infected patients had PARV4 primary infections, as shown by increasing IgG levels, detectable IgM, low or borderline IgG avidity, and viral DNA in serum. These 4 findings are presented as diagnostic criteria for PARV4 primary infection. As estimated by known kinetics of B19 virus diagnostics (*14*), these 2 PARV4 infections probably occurred in 2005. During that time, neither patient had contacted local healthcare providers. Conversely, these 2 patients used intravenous drugs daily, and might not have sought medical care unless they were severely ill.

Because PARV4 IgG seroprevalence in group 1 was 0% in this study, in contrast to prevalences of 60% for B19 (*12*) and 96% for HBoV (*9*) in the same students, serologic cross-reactivity between PARV4 and the other human parvoviruses appears highly unlikely. Amino acid sequence similarity is <30% between B19 and PARV4 and ≈40% between HBoV and PARV4.

PCR-negative results for group 2, including 4 patients who were IgM positive, are evidence against viremic primary, chronic, and recurrent PARV4 infections. However, because of the relatively low sensitivity of this PCR, the data do not rule out low levels of viral DNA in blood.

Analysis of HIV-infected IDUs supports the view that in northern Europe PARV4 is primarily a blood-borne virus. No differences were seen for factors related to sexual activity. However, our sample size was too small to make this conclusion. In a recent PCR study, PARV4 genotype

Table 2. Characteristics of PARV4 IgG–positive and IgG–negative HIV-infected injection drug users, Finland*

Characteristic	IgG positive, n = 61	IgG negative, n = 17	p value†
Age, y, median (range)	35 (17–61)	31 (21–55)	0.069
Male sex	44/61 (72)	12/17 (71)	1.000
Main drug was amphetamine‡	38/61 (62)	10/17 (59)	0.786
Duration of injection, y, median (range)	10 (0–36)	7 (0–30)	0.259
Duration of injection >10 y	45/59 (76)	7/17 (44)	**0.029**
History of imprisonment	49/61 (80)	9/17 (53)	**0.031**
Education ≤9 y	52/59 (88)	10/17 (59)	**0.011**
HCV antibody positive	59/60 (98)	14/15 (93)	0.362
HBsAg positive	3/61 (5)	3/17 (18)	0.114
HBc IgG positive	46/60 (77)	9/17 (56)	0.124
Used antiretroviral therapy§	29/61 (48)	4/17 (24)	0.099
CD4 cell count/μL, median§	303	323	0.168
Present situation			
Unstable living conditions (no permanent address)	35/59 (60)	5/15 (33)	0.088
Employed‡	0/53 (0)	3/16 (19)	**0.009**
Risk behavior			
Loaned needles or syringes	49/59 (83)	9/16 (56)	**0.040**
Borrowed needles or syringes	56/58 (97)	15/16 (94)	0.524
Had sexually transmitted diseases	37/57 (65)	7/17 (41)	0.097
Had commercial sex	18/61 (30)	8/15 (53)	0.127
Risk behavior past 6 mo			
Used drugs	48/61 (79)	8/17 (47)	**0.016**
Used injection drugs	46/61 (75)	8/17 (47)	**0.037**
Used condoms inconsistently	25/61 (41)	7/16 (44)	1.000
Had ≥2 sex partners	16/61 (26)	7/17 (41)	0.244

*Values are no. positive/no. tested (%) unless otherwise indicated. PARV4, parvovirus 4; Ig, immunoglobulin; HCV, hepatitis C virus; HBsAg, hepatitis B surface antigen; HBc, hepatitis B core antigen;
†By Fisher exact test or Mann-Whitney U test. Values in **boldface** are significant.
‡At the time of interview.
§Closest to the sampling for PARV4 tests.

3 was commonly found among infants in West Africa, and there was no evidence of parenteral exposure (*15*).

Using comprehensive serodiagnosis, we showed that PARV4 is ubiquitously present in IDUs in Finland and detected primary infections in 2 patients who had a full spectrum of diagnostic findings. Neither of these had sought medical help, which suggested that their primary infections may have been clinically mild or asymptomatic.

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Ms Lahtinen is a scientist at the Haartman Institute, Department of Virology of the University of Helsinki. Her research interests include clinical characteristics of parvoviruses.

References

1. Jones MS, Kapoor A, Lukashov VV, Simmonds P, Hecht F, Delwart E. New DNA viruses identified in patients with acute viral infection syndrome. J Virol. 2005;79:8230–6. DOI: 10.1128/JVI.79.13.8230-8236.2005
2. 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.
3. Lurcharchaiwong W, Chieochansin T, Payungporn S, Theamboonlers A, Poovorawan Y. Parvovirus 4 (PARV4) in serum of intravenous drug users and blood donors. Infection. 2008;36:488–91. DOI: 10.1007/s15010-008-7336-4
4. 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. DOI: 10.1097/QAD.0b013e3281e38558
5. Manning A, Willey SJ, Bell JE, Simmonds P. Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. J Infect Dis. 2007;195:1345–52. DOI: 10.1086/513280
6. 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 hemophiliacs and injection drug users, as detected by a serological assay for PARV4 antibodies. J Infect Dis. 2009;200:1119–25. DOI: 10.1086/605646
7. Kivelä P, Krol A, Simola S, Vaattovaara M, Tuomola P, Brummer-Korvenkontio H, et al. HIV outbreak among injecting drug users in the Helsinki region: social and geographical pockets. Eur J Public Health. 2007;17:381–6. DOI: 10.1093/eurpub/ckl252
8. Kivelä PS, Krol A, Partanen AL, Ristola MA. High prevalence of unprotected sex among Finnish HIV-positive and HIV-negative injecting drug users. Scand J Public Health. 2009;37:357–63. DOI: 10.1177/1403494809105290
9. Söderlund-Venermo M, Lahtinen A, Jartti T, Hedman L, Kemppainen K, Lehtinen P, et al. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. Emerg Infect Dis. 2009;15:1423–30. DOI: 10.3201/eid1509.090204
10. Kantola K, Hedman L, Allander T, Jartti T, Lehtinen P, Ruuskanen O, et al. Serodiagnosis of human bocavirus infection. Clin Infect Dis. 2008;46:540–6. DOI: 10.1086/526532
11. Tuke PW, Parry RP, Appleton H. Parvovirus PARV4 visualization and detection. J Gen Virol. 2010;91:541–4. DOI: 10.1099/vir.0.014852-0
12. Hedman L, Söderlund-Venermo M, Jartti T, Ruuskanen O, Hedman K. Dating of human bocavirus infection with protein-denaturing IgG-avidity assays-secondary immune activations are ubiquitous in immunocompetent adults. J Clin Virol. 2010;48:44–8. DOI: 10.1016/j.jcv.2010.02.003
13. Fryer JF, Delwart E, Hecht FM, Bernardin F, Jones MS, Shah N, et al. Frequent detection of the parvoviruses, PARV4 and PARV5, in plasma from blood donors and symptomatic individuals. Transfusion. 2007;47:1054–61. DOI: 10.1111/j.1537-2995.2007.01235.x
14. Enders M, Schalasta G, Baisch C, Weidner A, Pukkila L, Kaikkonen L, et al. Human parvovirus B19 infection during pregnancy–value of modern molecular and serological diagnostics. J Clin Virol. 2006;35:400–6. DOI: 10.1016/j.jcv.2005.11.002
15. 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. DOI: 10.3201/eid1607.100025

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Identification of Rickettsial Infections by Using Cutaneous Swab Specimens and PCR

Yassina Bechah, Cristina Socolovschi, and Didier Raoult

To determine the usefulness of noninvasive cutaneous swab specimens for detecting rickettsiae, we tested skin eschars from 6 guinea pigs and from 9 humans. Specimens from eschars in guinea pigs were positive for rickettsiae as long as lesions were present. Optimal storage temperature for specimens was 4°C for 3 days.

Rickettsiae are a group of obligate, intracellular, gram-negative bacteria. The family *Rickettsiaceae* includes the genera *Rickettsia* and *Orientia* (*1*). Rickettsiae are transmitted to humans by arthropods (*2*) and cause diseases characterized by fever, headache, rash, and vasculitis (*3*). An infection eschar is commonly found at the site of the arthropod bite because of local multiplication of the bacteria. Incidence of infection with rickettsiae is increasing worldwide (*4*) in certain disease-endemic foci, and seasonal, sporadic (*5*,*6*), and occasionally epidemic forms have been reported (*7*). Over the past 20 years, advances in molecular techniques and cell culture have facilitated identification of *Rickettsiales*, and new species and diseases have been described (*4*,*8*). Recently, a new *Rickettsia* species, 364D, was identified in patients from California (*9*).

Eschar biopsies are used for detection of *Rickettsia* spp., but this technique is invasive and painful for patients and is difficult to perform for certain areas of the body. Successful diagnosis in patients by using rapid, noninvasive, and painless techniques is beneficial. One study reported the usefulness of swabs of skin lesions in the diagnosis of 3 cases of Queensland tick typhus and 1 case of African tick bite fever (*10*). In addition, eschar crust samples were useful in the diagnosis of 1 case of infection with *Orientia tsutsugamushi*, the infectious agent of scrub typhus (*11*). To evaluate the potential usefulness of swabs of skin lesions for rickettsial diagnosis, we evaluated this procedure for eschars from 6 guinea pigs and 9 patients.

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The Study

The animal study was conducted beginning in February 2009, and the human study was conducted beginning in June 2009. *R. conorii, R. akari, R. rhipicephali*, *R. africae*, *R. parkeri*, and *O. tsutsugamushi* were grown in L929 cell monolayers, purified, and titrated as reported (*12*). A suspension of each rickettsial species (200 μL containing 1×10^5 rickettsia) was injected intradermally into 8 shaved areas on the backs of 6 Hartley guinea pigs (1 species/guinea pig) by using aseptic procedures (*12*). A negative control guinea pig was infected with 200 μL (1×10^6 cells/mL) of an L929 cell suspension. Infection sites were inspected daily for skin lesions. Animals were handled according to the regulations of Décret No. 887–848 du 10/19/1987, Paris. The experimental protocol was reviewed and approved by the Institutional Animal Care Committee, Université de la Méditerranée, Marseille.

Infection with each rickettsial species caused an eschar at the infection site (*12*). Eschars were observed at day 3 postinfection. A sterile cotton swab (Copan Italia S. p. A., Brescia, Italy) was rotated against the eschar (3 circular motions) and stored at 4°C for 24 h. Swabs were then placed in 400 μL of phosphate-buffered saline, and DNA was extracted by using the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Lesions were swabbed daily until the animal showed clinical recovery (day 20 postinfection for those infected with *R. akari*, *R. conorii*, and *R. rhipicephali* and day 13 postinfection for those infected with *R. africae*, *R. parkeri*, and *O. tsutsugamushi*).

Maximum number of DNA copies for *R. rhipicephali, R. akari*, and *R. conorii* was detected on day 4 postinfection (2.27×10^7, 2.96×10^7, and 9.28×10^7copies/5 μL of swab DNA extracts, respectively (Figure 1, panel A). Maximum number of DNA copies for *R. parkeri* was detected on day 3 postinfection (2.66×10^5 copies/5 μL), for *R. africae* on day 6 postinfection (6.73×10^5 copies/5 μL), and for *O. tsutsugamushi* on day 10 postinfection (2.7×10^7 copies/5 μL) (Figure 1, panel B).

Effects of temperature and storage time of cotton swabs on bacterial DNA were evaluated in 3 guinea pigs infected with *R. conorii.* Twelve swabs per animal were obtained daily for 5 days and stored in groups of 3 at 22°C, 4°C, –20°C, or –80°C. DNA was extracted after 1, 2, or 3 days of storage. Eschars appeared by day 3 postinfection and reached their maximum size by day 7. Storage at 4°C was the optimal temperature condition for isolation of DNA (7.53×10^6 copies/5 μL vs. 1.03×10^6, 3.77×10^6, or 4.49×10^6 copies/5 μL for swab storage at 22°C, –20°C, or –80°C respectively; $p = 0.0001$) (Figure 2, panel A). Storage time (24 h, 48 h, and 72 h) had no effect on DNA yield (Figure 2, panel B). Temperature had a significant effect ($p \leq 0.05$) on DNA yield and for the same extraction (Figure 2, panels C–E).

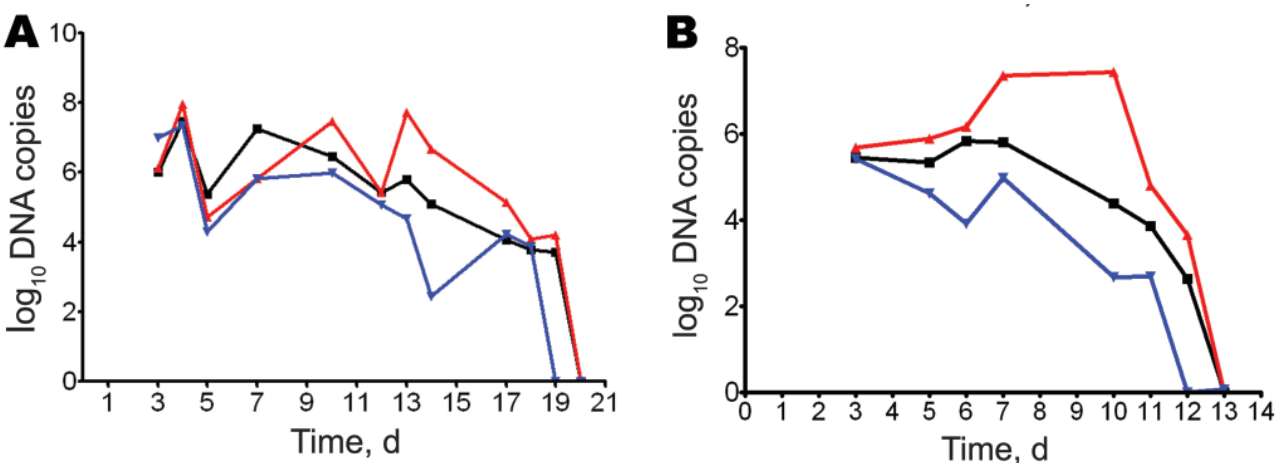

Figure 1. Molecular detection of *Rickettsia* spp. in swabs of skin lesions, Marseille, France. Guinea pigs were infected intradermally with different *Rickettsia* spp., and skin eschar swab specimens were obtained when lesions appeared. Samples (2 ± 1 mg) were tested, and DNA was extracted in a final volume of 100 μL. Number of rickettsial DNA copies was determined by quantitative PCR until day 20 postinfection for *R. akari* (black line), *R. conorii* (red line), and *R. rhipicephali* (blue line) (A) and until day 13 postinfection for *R. africae* (black line), *Orientia tsutsugamushi* (red line), and *R. parkeri* (blue line) (B). Values are copies of citrate synthase A gene/5 μL swab extract.

To demonstrate the usefulness of skin lesion swabs for detection of rickettsial infection, we used this technique with eschars from patients with suspected rickettsioses. Nine patients were included in this experiment after informed consent was obtained. This experiment was reviewed and approved by the local ethics committee (reference 09–016). DNA was extracted from swabs or skin biopsy specimens and tested by quantitative PCRs (qPCRs)

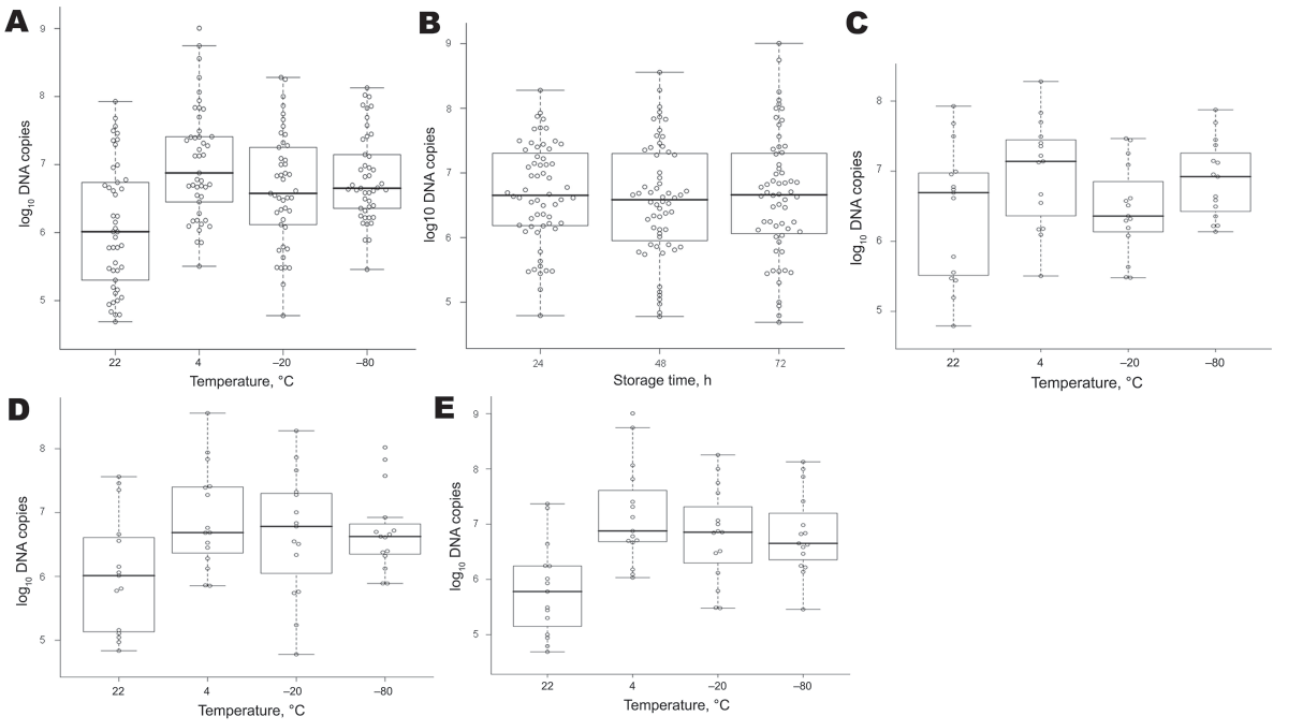

Figure 2. Effect of temperature (A), storage time (B), and temperature and storage times (C–E) on yield of rickettsial DNA, Marseille, France. Guinea pigs (n = 3) were infected with *Rickettsia conorii* and inspected daily for skin lesions. After lesions appeared, 12 swab specimens/animal were obtained daily for 5 days and stored in groups of 3 at 22°C, 4°C, −20°C, or −80°C. DNA was extracted after storage for 24 h, 48 h, or 72 h at each temperature in a final volume of 100 μL, and numbers of bacterial DNA copies were quantified in 5 μL of swab DNA extracts by using quantitative PCR. Box plots indicate 25th and 75th percentiles, horizontal lines indicate medians, and error bars indicate minimum and maximum values.

(*13*) specific for a fragment of the citrate synthase A gene, which is conserved among spotted fever group rickettsiae, or the gene coding periplasmic serine protease of *O. tsutsugamushi*; β-actin gene was used as a control (*14*).

When rickettsial DNA was amplified in samples, specific qPCR was performed by using specific primers and probes and on the basis of clinical and epidemiologic data (online Technical Appendix Table 1, www.cdc.gov/EID/content/17/1/83-Techapp.pdf) (*4*). If specific rickettsial DNA was not detected, PCR amplification and sequencing were performed to identify the causative agent (*4*,*15*). *R. montanensis* DNA was used as a positive control, and DNA from sterile biopsy samples and sterile water were used as a negative control.

The qPCR for the β-actin gene showed cycle threshold (C_t) values of 19–23 for skin biopsy samples and 22–37 for swab samples (Table). Spotted fever group rickettsial DNA was detected in biopsy samples from 5/5 patients and swab samples from 8/9 patients (online Technical Appendix Table 2). Specific qPCR showed a diagnostic result in 3/7 swabs samples and 4/5 skin biopsy samples.

We amplified *R. conorii* DNA from patients 1 and 2, *R. africae* DNA from patients 4 and 5, and *R. australis* DNA from patient 9. Rickettsial DNA from patients 3 and 7 showed 100% homology with the *R. sibirica mongolitimonae* citrate synthase A gene (GenBank accession nos. DQ097081 and DQ423370, respectively). Rickettsial DNA from patient 6 showed 99.1% homology with DNA from *R. slovaca*. Patient 9 was a technician who was accidentally infected by the aerosol route when handling *R. australis*. Only 2/11 swabs obtained from vesicular lesions of patient 9 were positive for rickettsial DNA and *R. australis* DNA after reamplification of primary PCR products. These samples showed 98% homology with *R. australis* 23S rRNA gene (GenBank accession no. AJ133711) (online Technical Appendix Table 2).

Conclusions

Our study showed the efficacy and reliability of skin lesion swabs for molecular detection of 6 *Rickettsia* species (Figure 1). Rickettsial DNAs were detected by using this technique as long as eschars persisted (≤19 days). For short-term storage of swabs, 4°C was the optimal temperature. Using swabs of eschars, we made a diagnosis of rickettsiosis for 8/9 patients. For patients 6, 7, and 8, for whom biopsy samples were not available, we confirmed the diagnosis by using swab samples. We also showed that for patient 9, who had a rickettsiosis but no eschar, swabbing

Table. Molecular results for 9 patients with rickettsioses for identification of *Rickettsia* spp., Marseille, France*

Patient no.	Swab no.	Skin swab specimens, C_t			Biopsy specimens, C_t			Final diagnosis
		Actin	Conserved sequence†	Specific sequence‡	Actin	Conserved sequence†	Specific sequence‡	
1	1	22.27	33.91	36.63	19.39	29.03	32.52	*R. conorii*
2	1	29.73	Neg	Neg	21.21	29.32	33.29	*R. conorii*
3	1	22.32	30.99	Neg	18.89	28.72	Neg	*R. sibirica mongolitimonae*
4	1	24.48	35.21	34.15	22.92	31.92	26.66	*R. africae*
5	1	31.13	34.67	Neg	20.68	33.66	31.9	*R. africae*
6§	1	35.49	35.29	Neg	–	–	–	*R. slovaca*
7§	1	24.78	30.63	ND	–	–	–	*R. sibirica mongolitimonae*
8§	1	24.19	Neg	Neg	–	–	–	*R. conorii*
	1	23.36	Neg	Neg	–	–	–	–
	1	21.94	37.97	Neg	–	–	–	–
	1	35.50	Neg	Neg	–	–	–	–
9§¶	1	32.50	Neg	Neg	–	–	–	*R. australis*
	1	32.05	Neg	Neg	–	–	–	–
	1	30.95	Pos	Neg	–	–	–	–
	1	24.99	Neg	Neg	–	–	–	–
	1	29.21	Neg	Neg	–	–	–	–
	1	31.78	Neg	Neg	–	–	–	–
	1	35.83	Neg	Neg	–	–	–	–
	1	24.98	Pos	Pos	–	–	–	–
	1	35.5	Neg	Pos	–	–	–	–
	1	36.96	Neg	Neg	–	–	–	–
	1	32.21	Neg	Neg	–	–	–	–

*C_t, cycle threshold; neg, negative; –, not applicable; ND, not done; pos, positive.
†Rickettsial DNA was identified by using a fragment of the citrate synthase A gene that is conserved among all spotted fever group rickettsiae.
‡Specific quantitative PCR was performed on the basis of epidemiologic data and tick bite history of each patient.
§No cutaneous biopsy samples were available.
¶Results for patient 9 correspond to results of re-amplification of products of the first PCRs.

of vesicular lesions may be useful for diagnosis, although these lesions were less sensitive than eschars.

Our results indicate that swabs of eschars can be used for molecular detection of rickettsial infections when biopsy samples are not available or biopsies are difficult to perform. We recommend that swabs be used for DNA extraction immediately after sampling or stored at 4°C until needed.

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References

1. Bechah Y, Capo C, Mege JL, Raoult D. Rickettsial diseases: from *Rickettsia*-arthropod relationships to pathophysiology and animal models. Future Microbiol. 2008;3:223–36. DOI: 10.2217/17460913.3.2.223
2. Raoult D, Roux V. Rickettsioses as paradigms of new or emerging infectious diseases. Clin Microbiol Rev. 1997;10:694–719.
3. Walker DH, Valbuena GA, Olano JP. Pathogenic mechanisms of diseases caused by *Rickettsia.* Ann N Y Acad Sci. 2003;990:1–11. DOI: 10.1111/j.1749-6632.2003.tb07331.x
4. Parola P, Paddock CD, Raoult D. Tick borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev. 2005;18:719–56. DOI: 10.1128/CMR.18.4.719-756.2005
5. Paddock CD, Holman RC, Krebs JW, Childs JE. Assessing the magnitude of fatal Rocky Mountain spotted fever in the United States: comparison of two national data sources. Am J Trop Med Hyg. 2002;67:349–54.
6. Hidalgo M, Orejuela L, Fuya P, Carrillo P, Hernandez J, Parra E, et al. Rocky Mountain spotted fever, Colombia. Emerg Infect Dis. 2007;13:1058–60.
7. Masters EJ, Olson GS, Weiner SJ, Paddock CD. Rocky Mountain spotted fever: a clinician's dilemma. Arch Intern Med. 2003;163:769–74. DOI: 10.1001/archinte.163.7.769
8. Raoult D. A new rickettsial disease in the United States. Clin Infect Dis. 2004;38:812–3. DOI: 10.1086/381896
9. Shapiro MR, Fritz CL, Tait K, Paddock CD, Nicholson WL, Abramowicz KF, et al. *Rickettsia* 364D: a newly recognized cause of eschar-associated illness in California. Clin Infect Dis. 2010;50:541–8. DOI: 10.1086/649926
10. Wang JM, Hudson BJ, Watts MR, Karagiannis T, Fisher NJ, Anderson C, et al. Diagnosis of Queensland tick typhus and African tick bite fever by PCR of lesion swabs. Emerg Infect Dis. 2009;15:963–5. DOI: 10.3201/eid1506.080855
11. Lee SH, Kim DM, Cho YS, Yoon SH, Shim SK. Usefulness of eschar PCR for diagnosis of scrub typhus. J Clin Microbiol. 2006;44:1169–71. DOI: 10.1128/JCM.44.3.1169-1171.2006
12. La Scola B, Bechah Y, Lepidi H, Raoult D. Prediction of rickettsial skin eschars in humans using an experimental guinea pig model. Microb Pathog. 2009;47:128–33. DOI: 10.1016/j.micpath.2009.06.004
13. Bechah Y, Capo C, Grau GE, Raoult D, Mege JL. A murine model of infection with *Rickettsia prowazekii*: implications for pathogenesis of epidemic typhus. Microbes Infect. 2007;9:898–906. DOI: 10.1016/j.micinf.2007.03.008
14. Mediannikov O, Fenollar F, Socolovschi C, Diatta G, Sokhna C, Bassene H, et al. *Coxiella burnetii* in humans and ticks in rural Senegal. PLoS Negl Trop Dis. 2010;4:e654. DOI: 10.1371/journal.pntd.0000654
15. Roux V, Rydkina E, Eremeeva M, Raoult D. Citrate synthase gene comparison, a new tool for phylogenetic analysis, and its application for the rickettsiae. Int J Syst Bacteriol. 1997;47:252–61. DOI: 10.1099/00207713-47-2-252

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Pandemic (H1N1) 2009 Outbreak at Camp for Children with Hematologic and Oncologic Conditions

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An outbreak of influenza A pandemic (H1N1) 2009 occurred among campers and staff at a summer camp attended by children with hematologic and oncologic conditions. The overall attack rate was 36% and was highest among children and adolescents (43%), persons with cancer (48%), and persons with sickle cell disease (82%).

Since it was first identified in April 2009 (*1*), the influenza A pandemic (H1N1) 2009 virus has sickened >1 million persons in the United States (www.cdc.gov/h1n1flu/surveillanceqa.htm). Because of the novelty of this virus, its transmissibility and severity are still under study.

We investigated an outbreak that occurred at a summer camp in northern rural Louisiana. Study approval was provided by the institutional review boards of Louisiana State University Health Sciences Center, Children's Hospital, and Tulane University Medical Center, New Orleans, Louisiana.

The Study

The camp opened July 26, 2009 (day 1), with 101 campers and 116 staff. Campers were children with hematologic or oncologic conditions and their nonaffected siblings. All participants were physically examined and questioned so that anyone with presence of or exposure to potentially communicable diseases could be excluded from attending camp. Campers (8–12/patrol) were grouped in age- and gender-specific patrols (B1–B4 and G1–G4) with assigned staff (4–5/patrol); they slept in bunkhouses and had common bathing facilities. All campers and staff dined together and shared various activities.

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On day 2, fever developed in a healthy camper in patrol G3 and promptly subsided. Fever also developed in a second camper (patrol B2) with sickle cell disease (SCD). This camper was seen at the local hospital, had a negative rapid influenza test (RIT) result, and was sent home. On day 3, fever and cough developed in 4 children (2 from B2); 1 was tested by RIT with a negative result. Evaluation of the entire B2 patrol found no one else symptomatic. On day 4, fever developed in1 child with cancer (B2) and 1 with SCD (G2 patrol); each had positive test results for influenza A virus.

The number of episodes of fever was considered excessive, and because additional campers reported having fever the camp was closed. At the time of dismissal, all campers and staff were provided masks and instructions about cough etiquette and handwashing; a dose of oseltamivir was also administered, and a prescription for oseltamivir was provided.

Ten days after the camp closed, all attendees were contacted (by email, telephone, and regular mail) to gather information about their outcomes. Clinical signs and symptoms of interest were fever (measured or subjective) or chills; cough or sore throat; muscle pain; and nausea, vomiting, or diarrhea. On the basis of an adaptation of the definition by the Centers for Disease Control and Prevention (www.cdc.gov/h1n1flu/clinicians), persons with fever or chills and symptoms in ≥2 other categories were classified as having influenza-like illness (ILI); persons with fever alone (without an explanation) and symptoms in 1 other category, or no documented fever but symptoms in ≥2 other categories, were classified as having probable ILI (P-ILI). A case-patient was defined as a person in whom ILI or P-ILI developed within 10 days of closing of the camp.

Questionnaires were returned by 88 (76%) and 77 (76%) of staff and campers, respectively. Mean age was 22.5 (range 14–69) years for staff and 10.5 (range 5–15) years for campers. Of 88 staff, 56 reported no chronic medical condition; a few reported cancer in remission (n = 7), SCD (n = 3), or other conditions (n = 22: 9 asthma, 2 inflammatory bowel disease, 2 unspecified, and 1 each with epilepsy, gall stones, chronic hepatitis C, immune thrombocytopenic purpura, Kartagener syndrome, mental retardation, porphyria, stroke, and type 1 diabetes mellitus). Of 77 campers, 45 (58%) reported no underlying illness, and a few reported cancer (n = 20: 12 leukemia, 2 lymphoma, 4 solid organ tumor, 2 unspecified; 2 were receiving maintenance therapy, the others had completed treatment), SCD (n = 8), or other (n = 4: 2 asthma, 1 immune thrombocytopenic purpura, and 1 cardiomyopathy).

Of 165 attendees who returned the questionnaire, 59 (38.5%) reported symptoms: fever (40, 68%), cough (30, 51%), sore throat (21, 36%), muscle pain (16, 27%), nau-

sea (10, 17%), diarrhea (9, 15%), vomiting (8, 14%), runny nose (6, 10%), and headache (4, 7%). Abdominal pain, weakness, earache, conjunctivitis, and joint stiffness were rare. Pain crisis developed in 2 patients with SCD. Twenty-five patients met the definition of ILI, and 34 met the definition of P-ILI (Figure). Symptoms were more common among campers than staff (46.8% vs. 26.1%; p = 0.009), persons of younger age (Table 1), and persons with cancer or SCD (Table 2). Proportions were compared with 2-tailed χ^2 or Fisher exact test by using Epi Info version 3.5 (www.cdc.gov/Epiinfo).

Probably because of small numbers, no difference was evident in the attack rate between staff assigned and unassigned to a patrol, between boy and girl campers, between patrols, or between those who recalled or did not recall being in contact with a case-patient. Twenty-six case-patients sought medical attention, and 12 (2 staff and 10 campers) were hospitalized; 8 had SCD and 4 were in active cancer treatment; all were regular-floor admissions; none required oxygen support or other intensive care; mean length of stay was 4.25 (range 3–6) days; and all recovered uneventfully.

Twenty-five persons (18 campers and 7 staff) were tested for influenza: 13 were tested at an outside facility, of whom 3 had positive results (method of testing unknown); 12 were tested at Children's Hospital by RIT (BinaxNOW; Invernass Medical, Waltham, MA, USA) and direct immunofluorescence assay (D^3 Ultra; Diagnostic Hybrids, Athens, OH, USA), and 5 (42%) had positive test results for influenza A virus. Of the 5 positive specimens obtained from patients, 4 were confirmed as pandemic (H1N1) 2009 virus by real-time reverse transcription–PCR.

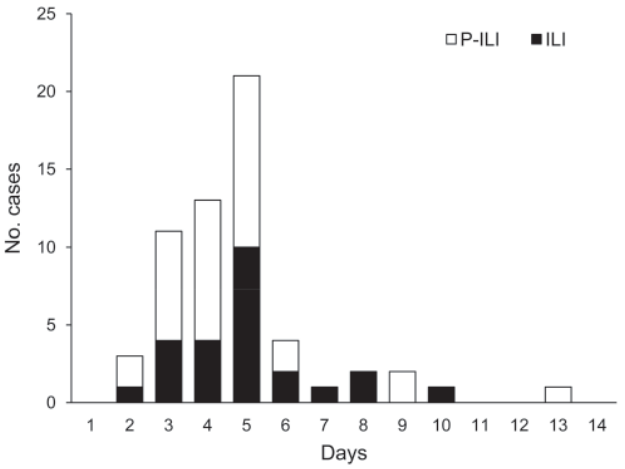

Figure. Outbreak curve of influenza-like illness (ILI) and probable ILI (P-ILI) cases during the 4 days of summer camp for children with hematologic and oncologic conditions (1–4) and the 10 days (5–14) after closing the camp, Louisiana, USA, 2009.

Conclusions

Limited preliminary information is available about summer camp outbreaks of pandemic (H1N1) 2009 (*2*). The closest scenarios (school outbreaks) reported attack rates of 3%–33% in the United States (*3*–*5*) and 2%–31% in the United Kingdom (*6*,*7*). Differences between studies and groups probably reflect different levels of exposure and surveillance definitions. In the outbreak reported here, the overall attack rate was 36%; infection was more common among campers (47%), children and adolescents (43%), those with cancer (48%), and those with SCD (82%). Because of their underlying condition, the threshold for evaluation and intervention is lower for children with hematologic or oncologic processes, which might account for some increased reporting of symptoms, hospitalization, and extended stay.

Children with SCD were disproportionately affected; 82% of them reported symptoms, and 89% of those symptomatic were hospitalized because of fever or pain crisis. For seasonal influenza, the hospitalization rate is 56× higher for children with SCD than those without SCD (*8*); the same trend seems true for pandemic (H1N1) 2009 infection. Among those with no underlying condition, the influenza attack rate was lower (28%), few (11%) persons sought medical attention, and none were hospitalized. For persons with no underlying condition, the reported symptoms suggest an illness no more severe than seasonal influenza.

A few limitations should be recognized. First, 25% of attendees did not return the questionnaire. Second, we used a clinical definition of ILI; however, although the limitations of the definition are well recognized, it is a well-accepted tool for outbreak investigation. Third, data are based on recall and report by participants. Fourth, only a few persons were tested to determine the causative agent. However, of the 12 patients we tested at Children's Hospital, 5 (42%) had influenza A virus and 4 were confirmed as having pandemic (H1N1) 2009 virus infection. This level of positivity is in accordance with sensitivity reported for available tests (*9*,*10*) and suggests that most, if not all, cases of ILI identified were caused by pandemic (H1N1) 2009 virus.

Table 1. Attack rates of influenza-illness according to age and underlying medical condition at summer camp for children with hematologic and oncologic conditions, Louisiana, USA, 2009*

	Underlying condition, no. positive/no. tested (%)		
Age group, y	No†	Yes‡	All§
5–15	19/54 (35.2)	18/33 (54.5)	37/87 (42.5)
16–30	10/41 (24.4)	11/28 (39.3)	21/69 (30.4)
31–69	0/6 (0)	1/3 (33.3)	1/9 (11.1)
Total	29/101 (28.7)	30/64 (46.9)	59/165 (35.8)

*By χ^2 test for trend for increasing age groups within each category.
†p = 0.049.
‡p = 0.255.
§p = 0.027.

Table 2. Risk for influenza and hospitalization according to category of underlying condition at summer camp for children with hematologic and oncologic conditions, Louisiana, USA, 2009*

	Risk for influenza symptoms			Risk for hospitalization, if symptomatic		
Underlying condition	Staff	Campers	All	Staff	Campers	All
None	10/56 (17.9)	19/45 (42.2)	29/101 (28.7)	0/10 (0)	0/19 (0)	0/29 (0)
Other than cancer or sickle cell disease	7/22 (31.8)	1/4 (25.0)	8/26 (30.8)	0/7 (0)	0/1 (0)	0/8 (0)
Cancer	**4/7 (57.1)†**	9/20 (45.0)	**13/27 (48.1)†**	0/4 (0)	**4/9 (44.4)‡**	**4/13 (30.8)‡**
Sickle cell disease	**2/3 (66.6)†**	**7/8 (87.5)†**	**9/11 (81.8)‡**	**2/2 (100.0)†**	**6/7 (85.7)§**	**8/9 (88.9)§**
Total	23/88 (26.1)	36/77 (46.8)	59/165 (35.8)	2/23 (8.7)	10/36 (27.8)	12/59 (20.3)

*Values given as no. in category/total no. with condition (%). **Boldface** indicates significance. Data were analyzed by using 2-tailed Fisher exact test for each category compared with persons with no underlying condition.
†p = 0.01–0.05.
‡p = 0.0001–0.01.
§p<0.0001.

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References

1. Centers for Disease Control and Prevention. Swine influenza A (H1N1) infection in two children—southern California, March–April 2009. MMWR Morb Mortal Wkly Rep. 2009;58:400–2.
2. Centers for Disease Control and Prevention. Oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infection in two summer campers receiving prophylaxis—North Carolina, 2009. MMWR Morb Mortal Wkly Rep. 2009;58:969–72.
3. Iuliano AD, Reed C, Guh A, Desai M, Dee DL, Kutty P, et al. Notes from the field: outbreak of 2009 pandemic influenza A (H1N1) virus at a large public university in Delaware, April–May 2009. Clin Infect Dis. 2009;49:1811–20. DOI: 10.1086/649555
4. Centers for Disease Control and Prevention. Outbreak of 2009 pandemic influenza A (H1N1) at a school—Hawaii, May 2009. MMWR Morb Mortal Wkly Rep. 2010;58:1440–4.
5. France AM, Jackson M, Schrag S, Lynch M, Zimmerman C, Biggerstaff M, et al. Household transmission of 2009 influenza A (H1N1) virus after a school-based outbreak in New York City, April–May 2009. J Infect Dis. 2010;201:984–92. DOI: 10.1086/651145
6. Health Protection Agency West Midlands H1N1 Investigation Team. Preliminary descriptive epidemiology of a large school outbreak of influenza A (H1N1)v in the West Midlands, United Kingdom, May 2009. Euro Surveill. 2009;14(27):pii=19264.
7. Smith A, Johnson S, Saldana L, Ihekweazu C, O'Moore E. An outbreak of influenza A (H1N1) in a boarding school in South East England, May–June 2009. Euro Surveill. 2009;14:pii=19263.
8. Bundy DG, Strouse JJ, Casella JF. Burden of influenza-related hospitalization among children with sickle cell disease. Pediatrics. 2010;125:234–43. DOI: 10.1542/peds.2009-1465
9. Centers for Disease Control and Prevention. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) virus—United States, 2009. MMWR Morb Mortal Wkly Rep. 2009;58:826–9.
10. Vasoo S, Stevens J, Singh K. Rapid antigen tests for diagnosis of pandemic (swine) influenza A/H1N1. Clin Infect Dis. 2009;49:1090–3. DOI: 10.1086/644743

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Reducing *Baylisascaris procyonis* Roundworm Larvae in Raccoon Latrines

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Baylisascaris procyonis roundworms, a parasite of raccoons, can infect humans, sometimes fatally. Parasite eggs can remain viable in raccoon latrines for years. To develop a management technique for parasite eggs, we tested anthelmintic baiting. The prevalence of eggs decreased at latrines, and larval infections decreased among intermediate hosts, indicating that baiting is effective.

The emergence of zoonotic diseases, which account for ≈58% of all infectious diseases in humans, is linked to changing land use and resource consumption patterns (*1*). Ecosystem disturbances from human population growth and globalization result in rapid spread of zoonotic pathogens (*2*). Recent integrated approaches to solving global health issues acknowledge that wildlife reservoirs facilitate zoonotic pathogen emergence and emphasize the need for increased collaboration between the ecology and infectious disease communities (*2*). We describe a multidisciplinary collaboration that used an experimental approach to lower the prevalence, and possibly break the life cycle, of a zoonotic parasite, the *Baylisascaris procyonis* roundworm.

The Study

Raccoons (*Procyon lotor*) are the host of *B. procyonis* roundworms, intestinal parasites (*3*). Up to 82% of adult raccoons and 90% of juvenile raccoons are infected (*3*). Mature worms produce thousands of eggs daily (*3*). These eggs are eliminated through raccoon feces and accumulate at raccoon latrines (*4*). *B. procyonis* roundworm eggs remain infective for many years and can infect juvenile raccoons and intermediate hosts such as rodents and birds that ingest them (*3*). Transmission often occurs at raccoon latrines when eggs are ingested with seeds found in fecal material (*4*). Larvae migrate through intermediate host tissues and can enter the central nervous system, resulting in death (*3*). Adult raccoons become infected when they prey on infected intermediate hosts (*3*). Raccoon population densities have increased in response to increased anthropogenic resources that are available in agricultural and urban ecosystems (*5*). Thus, raccoon latrines often exist near human habitats, increasing the risk for zoonoses (*4*).

Reported cases of human *B. procyonis* roundworm infections are rare (n = 18), and all have occurred in North America; however, prevention is a public health priority because of the severity of the resulting neurologic disease (*6–10*). Our objective was to develop a management technique that could interrupt transmission of *B. procyonis* roundworm eggs between raccoons and intermediate hosts, ultimately decreasing the environmental levels of eggs and potential for reinfection. We examined the effects of latrine removal and treatment of raccoons by using randomly distributed anthelmintic baits on the basis of *B. procyonis* roundworm prevalence at latrines and among intermediate hosts. By implementing a specific, protocol-based approach to disease prevention, supported by experimentally derived data, we hope to provide public health officials with an effective, spatially explicit, prophylactic method for reducing infection risk.

We conducted this study in Grant, Miami, and Wabash counties in north-central Indiana in portions of the Upper Wabash Basin. This area is 88% agricultural; only 8% of the landscape remains forested (*11*). Some contiguous riparian forest remains; however, most patches are <5 hectares (ha; 1 ha = 10,000 m^2) (*11*). Our experiment was conducted in 16 forest patches (1.91–8.80 ha). Eight treatment patches received anthelmintic baits, and 8 control patches did not. The range of patch sizes, levels of patch isolation, and raccoon densities in treatment and control patches were representative of the landscape (Figure).

In March 2007 (spring 07), we removed all visible latrines (n = 559) in the treatment patches. We located latrines by systematically searching all appropriate horizontal substrate and area at the bases of large trees throughout each forest patch (*3*). After manual removal, we used a torch to sterilize the substrate and surrounding soil associated with each latrine (online Technical Appendix, www.cdc.gov/EID/content/17/1/90-Techapp.pdf). At control sites, we sampled a minimum of 20 latrines (n = 198) by removing ≈2 g fecal material per fecal deposit at each latrine (*12*). We returned to our study sites 3 additional times for fecal sampling in October and November 2007 (fall 07), June 2008 (summer 08), and November 2008 (fall 08). During these subsequent visits, we sampled ≈2 g of fecal material per fecal deposit at a minimum of 20

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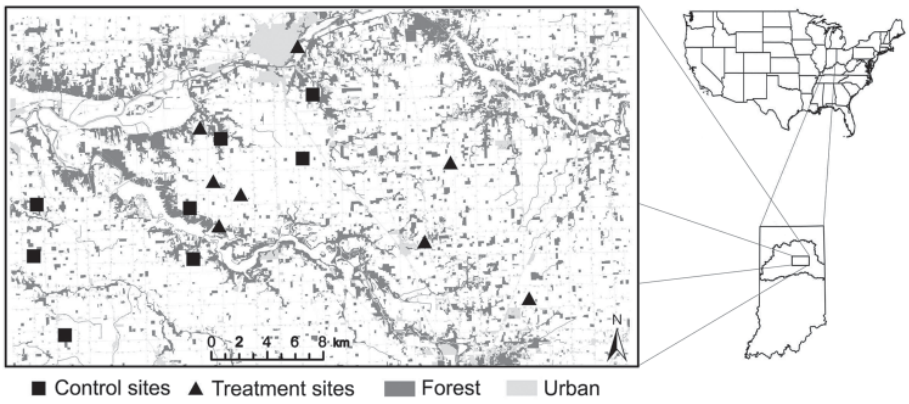

Figure. Study area of raccoon latrines showing locations of treatment and control patches, Upper Wabash Basin, north-central Indiana, 2007–2008. Dominant land use is represented by degree of shading.

latrines in all treatment and control patches. All samples were stored at –20°C until they were examined for *B. procyonis* roundworm eggs. Eggs were identified by microscopic examination following centrifugal fecal flotation in Sheather sugar solution (*3*). We identified *B. procyonis* roundworm eggs on the basis of size and morphologic appearance and designated each sample as positive or negative. Prevalence was measured as the proportion of positive samples at each study patch during each sampling period. Differences between pretreatment and posttreatment prevalence and between treatment and control patches were determined by using log linear analyses performed with PROC CATMOD SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) (goodness-of-fit tests).

In spring 07, after the initial latrine removal from treatment patches, baits were distributed throughout treatment patches once a month for the duration of the study. Baiting densities were determined on the basis of average abundance of raccoons in each study patch (online Technical Appendix).

Prevalence of *B. procyonis* roundworm larvae within an intermediate host, white-footed mice (*Peromyscus leucopus*), was determined. A minimum of 10 mice were captured from each of the 16 study patches during each of 3 sampling periods: 1 pretreatment (summer 07), and 2 posttreatment (fall 07 and summer 08). After capture, mice were euthanized with carbon dioxide and refrigerated until examination for *B. procyonis* roundworm larvae. Brains were removed and examined separately by pressing them between glass plates, and larvae were examined under a dissecting microscope. We recovered larvae from tissues digested in acid–pepsin solution (*3*). Larvae were counted and identified (*3*). Prevalence of infection was determined for mice within each study patch for each sampling period. Differences between treatment and control patches were determined by Fisher exact test (*13*).

We collected 1,797 fecal samples. Pretreatment sampling of latrines in spring 07 detected *B. procyonis* roundworm eggs at 757 (33%) of latrines sampled across all patches (Table). However, prevalence of eggs in treatment patches declined by ≥3-fold after baiting in all sampling periods ($p<0.04$). Our baseline pretreatment estimate of prevalence of infection among intermediate hosts did not differ ($p = 0.426$) between treatment patches (32%) and control patches (37%). Approximately 1 year after baiting activities began, we detected a significant decline in the prevalence of *B. procyonis* roundworm larvae in mice between treatment and control patches (27% vs. 38%; $p = 0.05$; Table).

Conclusions

Current public health initiatives to prevent human infections with *B. procyonis* roundworms focus on education of human health care and veterinary professionals (*6*). Our practical approach decreased prevalence of the parasite, suggesting decreased transmission and possibly reduced risk for humans. Baiting strategies have effectively controlled rabies (*14*) and decreased prevalence of zoonotic parasites, including *Echinococcus multilocularis* tapeworms (*15*). Our baiting strategy combined with latrine removal effectively decreased egg levels at latrines and ultimately decreased prevalence among mice. Hegglin and Deplazes (*15*) demonstrated a long-term decrease in prevalence of *E. multilocularis* tapeworms among foxes (definitive hosts) after monthly baiting for ≈4 years and conjectured that this decrease was caused by decreased infections among intermediate hosts. Our study supports their hypothesis because we measured decreases in prevalence among intermediate hosts after baiting. The reduction of prevalence at latrines and among intermediate hosts suggests that our low-cost approach (online Technical Appendix) could have a lasting effect on transmission dynamics; however, further study to assess frequency of distribution and type and dose of baits for sustained prevalence is needed. Raccoon latrines are commonly found near homes (*4*), and implementation of baiting strategies, in conjunction with traditional raccoon management on public lands, could reduce the risk for transmission on nearby private properties.

Table. Findings from baited patches in study of prevalence of *Baylisascaris procyonis* roundworms at raccoon latrines and among intermediate hosts, Upper Wabash Basin, north-central Indiana, 2007–2008*

Patch	1	2	3	4	5	6	7	8
Control patches								
Size, ha	8.19	6.67	2.95	1.91	6.67	6.43	4.28	4.39
Raccoons/ha	1.22	1.35	1.69	3.66	2.25	1.56	1.17	3.42
Baits/ha	0	0	0	0	0	0	0	0
Prevalence at latrines								
Spring 07	0.36	0.17	0.43	0.06	0.45	0.27	0	0.38
Fall 07	0.65	0.05	0	0.13	0.13	0.24	0	0.15
Summer 08	0.19	0	0.08	0.05	0	0.05	0	0
Fall 08	0.40	0.13	0.03	0.10	0.40	0.50	0.07	0.47
Total change	0.11	–0.24	–0.93	0.67	–0.11	0.85	NC	0.24
Prevalence among intermediate hosts								
Summer 07	0.25	0.42	0.35	0.38	0.09	0.27	0.56	0.54
Fall 07	0.27	0.28	0.50	0.22	0.18	0.38	0.45	0.25
Summer 08	0.44	0.33	0.53	0.55	0.56	0.25	0.05	0.37
Total change	0.76	–0.21	0.51	0.44	5.22	–0.07	–0.91	–0.32
Treatment patches								
Size, ha	9.66	6.72	4.72	2.50	2.46	3.66	6.00	8.80
Raccoons/ha	1.40	1.19	1.91	2.00	4.47	3.83	4.00	1.70
Baits/ha	7.00	5.95	9.53	10.00	22.36	19.12	20.00	8.52
Prevalence at latrines								
Spring 07	0.12	0.64	0.08	0.38	0.38	0.29	0.05	0.52
Fall 07	0	0.05	0	0.18	0.15	0.17	0.18	0
Summer 08	0	0	0	0	0.05	0	0	0
Fall 08	0.17	0	0.03	0.03	0.30	0.17	0	0.03
Total change	0.42	–1.00	–0.63	–0.92	–0.21	–0.41	–1.00	–0.94
Prevalence among intermediate hosts								
Summer 07	0.28	0.50	0.25	0.30	0.25	0.33	0.18	0.33
Fall 07	0.47	0.47	0.58	0.50	0.36	0.45	0.25	0.40
Summer 08	0.25	0.10	0	0.35	0.40	0.45	0.22	0.33
Total change	–0.11	–0.80	–1.00	0.17	0.60	0.36	0.22	0

*Prevalence, proportion of positive samples (parasites present). The effect of treatment on prevalence by patch is represented by the proportional change between pretreatment and final sampling. ha, hectare (10,000 m^2); spring 07, March 2007; fall 07, October and November 2007; summer 08, June 2008; fall 08, November 2008; NC, no change.

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References

1. Woolhouse ME, Gowtage-Sequeria S. Host range and emerging and reemerging pathogens. Emerg Infect Dis. 2005;11:1842–7.
2. Kahn LH. Confronting zoonoses, linking human and veterinary medicine. Emerg Infect Dis. 2006;12:556–61.
3. Kazacos KR. *Baylisascaris procyonis* and related species. In: Samuel WM, Pybus MJ, Kocan AA, editors. Parasitic diseases of wild mammals. Ames (IA): Iowa State University Press; 2001. p. 301–41.
4. Page LK, Anchor C, Luy E, Kron S, Larson G, Madsen L, et al. Backyard raccoon latrines and risk for *Baylisascaris procyonis* transmission to humans. Emerg Infect Dis. 2009;15:1530–1. DOI: 10.3201/eid1509.090128
5. Beasley JC, Rhodes OE Jr. Relationship between raccoon abundance and crop damage. Human-Wildlife Interactions. 2008;2:248–59 [cited 2010 Nov 29]. http://www.berrymaninstitute.org/journal/fall2008/Beasley_Rhodes.pdf
6. Wise ME, Sorvillo FJ, Shafir SC, Ash LR, Berlin OG. *Baylisascaris procyonis*, the common roundworm of raccoons: a review of current literature. Microbes Infect. 2005;7:317–23. DOI: 10.1016/j.micinf.2004.12.005
7. Chun CS, Kazacos KR, Glaser C, Bardo D, Dangoudoubiyam S, Nash R. Global neurological deficits with *Baylisascaris* encephalitis in a previously healthy teenager. Pediatr Infect Dis J. 2009;28:925–7. DOI: 10.1097/INF.0b013e3181a648f1
8. Hajek J, Yau Y, Kertes P, Soman T, Laughlin S, Kanani R, et al. A child with raccoon roundworm meningoencephalitis: a pathogen emerging in your own backyard? Can J Infect Dis Med Microbiol. 2009;20:e177–80.

9. Pai PJ, Blackburn BG, Kazacos KR, Warrier RP, Begue RE. Full recovery from *Baylisascaris procyonis* eosinophilic meningitis. Emerg Infect Dis. 2007;13:928–30.
10. Perlman JE, Kazacos KR, Imperato GH, Desai RU, Schulman SK, Edwards J, et al. *Baylisascaris procyonis* neural larva migrans in an infant in New York City. J Neuroparasitology. 2010;1:1–5 [cited 2010 Nov 29]. http://www.ashdin.com/journals/jnp/N100502.pdf
11. Moore JE, Swihart RK. Modeling patch occupancy by forest rodents: incorporating detectability and spatial autocorrelation with hierarchically structured data. J Wildl Manage. 2005;69:933–49. DOI: 10.2193/0022-541X(2005)069[0933:MPOBFR]2.0.CO;2
12. Smyser TJ, Page LK, Rhodes OE Jr. Optimization of raccoon latrine surveys for quantifying exposure to *Baylisascaris procyonis.* J Wildl Dis. 2010;46:929–33.
13. Sidwa TJ, Wilson PJ, Moore GM, Oertli EH, Hicks BN, Rohde RE, et al. Evaluation of oral rabies vaccination programs for control of rabies epizootics in coyotes and gray foxes. J Am Vet Med Assoc. 2005;227:785–92. DOI: 10.2460/javma.2005.227.785
14. Hegglin D, Deplazes P. Control strategy for *Echinococcus multilocularis.* Emerg Infect Dis. 2008;14:1626–8. DOI: 10.3201/eid1410.080522

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Serotype Distribution and Drug Resistance in *Streptococcus pneumoniae*, Palestinian Territories

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To determine antimicrobial drug resistance of *Streptococcus pneumoniae* serotypes, we analyzed isolates from blood cultures of sick children residing in the West Bank before initiation of pneumococcal vaccination. Of 120 serotypes isolated, 50.8%, 73.3%, and 80.8% of the bacteremia cases could have been prevented by pneumococcal conjugate vaccines. Serotype 14 was the most drug-resistant serotype isolated.

Streptococcus pneumoniae infection is a common cause of illness and death worldwide, responsible for an estimated 1.2 million infant deaths each year (*1*). The polysaccharide capsule is one of the primary virulence factors that contributes to *S. pneumoniae* pathogenicity (*2*). Management of *S. pneumoniae* infections has been complicated by the emergence of multiple antimicrobial drug–resistant strains (*3*).

Before the introduction of the 7-valent polysaccharide-protein conjugate vaccine (PCV7), serotypes included in this vaccine were responsible for ≈90% of pneumococcal infections in children living in industrialized countries; in developing countries, coverage has been reported as low as 26% (*4,5*). The use of PCV7 reduced the incidence of invasive pneumococcal disease (IPD) in children <5 years of age in the United States by 76% (*6*). However, nonvaccine serotypes (i.e., 19A, 6C) have emerged as primary pathogens in IPD (*7*). More recently, the American Advisory Committee on Immunization Practices recommended using the new Food and Drug Administration–licensed 13-valent pneumococcal conjugate vaccine (PCV13), which contains the 7 serotypes present in PCV7 and 6 additional serotypes (1, 3, 5, 6A, 7F, and 19A).

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The Study

In the Palestinian Territories, West Bank, the epidemiology of IPD is not well defined. Our study characterized the serotypes and antimicrobial drug resistance patterns of 120 consecutive *S. pneumoniae* isolates collected from blood cultures of patients admitted to Caritas Baby Hospital (n = 113) during January 2001–April 2010 or Maqassed Islamic Hospital (n = 7) during January 2009–April 2010. Both hospitals are well-equipped and are the major hospitals that perform blood cultures in the West Bank. Blood cultures are overused at both institutions; thus, isolated *S. pneumoniae* are representative of circulating serotypes. Because *S. pneumoniae* meningitis and invasive pneumonia are rare, these IPDs were not included in the study.

Blood cultures were collected from patients suspected of having sepsis or endocarditis. Criteria for collecting blood cultures included leukocytosis with left shift, elevated C-reactive protein level, fever, signs of toxicity, hypotension, and hemodynamic instability. One isolate per patient was included in the study. Patients' ages ranged from 1 day to 11 years; most (71.7%) were <2 years of age.

All BACTEC (Becton Dickinson, Sparks, MD, USA)–positive blood culture samples were placed on 5% sheep blood agar, chocolate agar, and MacConkey agar obtained from Hy-Laboratories Ltd. (Rehovot, Israel). Suspected *S. pneumoniae* colonies were identified on the basis of colony morphologic appearance, α-hemolysis on 5% sheep blood agar, Gram stain appearance, bile solubility, and optochin susceptibility. All strains were stored at –80°C until further testing.

S. pneumoniae isolates were serotyped by performing a series of PCRs using primers described by Pai et al (*8*). Because the serotype 6B primer cross-reacts with the common 6A *cps* operon sequence, we did not differentiate serotypes 6A/B.

Of the 120 *S. pneumoniae* isolates, 117 (97.5%) were serotyped; 3 (2.5%) could not be typed. These results are similar to those of Pai et al., who successfully typed 95.5% of the *S. pneumoniae* stains by using the same PCR technique (*8*). In the West Bank, 20 *S. pneumoniae* serotypes were identified; serotypes 6A/B, 14, 1, and 9V were the predominant strains (online Appendix Table, www.cdc.gov/EID/content/17/1/94-appT.htm). Overall, 97 (80.8%)

of the serotypes are included in PCV13, and ≥61 (50.8%) are covered by PCV7 (6A/B not differentiated).

After serotypes were stratified by age groups, 86 (71.7%) *S. pneumoniae* strains were isolated from children ≤2 years of age; 34 (28.3%) were isolated from children >2 years of age. High *S. pneumoniae* serotype coverage by PCV13 was noted for each age group, 77.9% and 76.5%, respectively. Conversely, serotype coverage provided by PCV7 for each age group was 60.5% and 26.5%, respectively. Of the 6 additional serotypes included in PCV13 and not in PCV7, serotypes 1, 3, and 5 appeared to be common in our study population. Notably, *S. pneumoniae* serotype 1 was not seen in children <2 years of age.

Distribution of the *S. pneumoniae* serotypes was similar to that in a study by Fraser et al. in Israeli and in Bedouin children residing in southern Israel (*9*). However, unlike the study from southern Israel in which serotypes 1 and 5 predominated, in this study, serotypes 6A/B and 14 predominated. Different *S. pneumoniae* serotype distribution was also noted when serotypes isolated from the West Bank were compared with those from other regional countries such as Kuwait and Saudi Arabia (*10*).

Drug susceptibility testing of the different *S. pneumoniae* serotypes was performed by using disk diffusion on Mueller-Hinton agar supplemented with 5% sheep blood agar for penicillin (oxacillin) (1 μg), erythromycin (15 μg), ofloxacin (5 μg), co-trimoxazole (25 μg), and vancomycin (30 μg) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (*11*). In addition, penicillin and cefotaxime minimal MICs were determined for all isolates by Etest according to the manufacturer's recommendations (AB Biodisk, Solna, Sweden). Interpretation of penicillin and cefotaxime MIC results was performed by using the CLSI nonmeningitis guidelines, (parenteral penicillin susceptible ≤2 μg/mL, intermediate 4 μg/mL, resistant ≥8 μg/mL, and for cefotaxime susceptible ≤1 μg/mL, intermediate 2 μg/mL, resistant ≥4 μg/mL), and the meningitis guidelines were applied for penicillin (parenteral penicillin susceptible ≤0.06 μg/mL, resistant ≥0.12 μg/mL) (*11*).

Overall, of the 120 *S. pneumoniae* blood isolates evaluated, 50 (41.7%) were penicillin susceptible; 70 (58.3%) were resistant based on the 1-μg oxacillin disk-diffusion results. However, MICs for penicillin showed that 118 (98.3%) isolates were susceptible and only 2 (1.7%) *S. pneumoniae* isolates were intermediately resistant (online Appendix Table). These results are similar to those from Germany and the United States, which reported low penicillin intermediate rates of 0.2% and 5.6%, respectively (*12,13*). Unlike the absence of resistant isolates in this study, both countries reported 1.2% resistance rates.

The small sample size is a limitation in this study and mandates caution when comparing the results with those of other studies. The use of broth microdilution to determine penicillin MIC, as reported in the Germany study or Etest as performed in this study, has been documented to produce comparable results (*14*). The penicillin resistance rate of the *S. pneumoniae* isolates was higher after applying the CLSI meningitis guidelines (45.8% sensitive and 54.2% resistant).

Unlike the low penicillin resistance rate, higher resistance rates were noted for erythromycin and co-trimoxazole. Of the 120 *S. pneumoniae* isolates evaluated, 32 (26.7%) were resistant to erythromycin, and 53 (44.2%) were resistant to co-trimoxazole (online Appendix Table). After stratifying erythromycin and co-trimoxazole resistance rates by serotype, we showed that serotype 14 had the highest resistance rates for both antimicrobial drugs (75%). Resistance to >1 agent was noted for 53.3% of the serotype 14 isolates (online Appendix Table). *S. pneumoniae* erythromycin resistance in the West Bank differed from that in Israel (10%); however, each location had similar co-trimoxazole (51%) resistance patterns. The spread of the co-trimoxazole–resistant clones is alarming because the World Health Organization has recommended use of this antimicrobial drug for treatment of nonsevere pneumoniae in children >2 months of age. No resistance was noted for cefotaxime, ofloxacin, and vancomycin (online Appendix Table).

Conclusions

Our study reports the distribution of *S. pneumoniae* serotypes in blood cultures of children residing in the West Bank. This study favors use of PCV13 because 80.8% vaccine coverage of the causative serotypes can be achieved compared with 50.8% with PCV7. In the West Bank, the increased serotype coverage of PCV13 in part results from inclusion of *S. pneumoniae* serotypes 1, 5, and 19A, which account for 23.4% of the serotypes not in PCV7. The introduction of PCV13 in the vaccination program will not only help reduce the incidence of IPD but will also help reduce infections caused by drug-resistant *S. pneumoniae* serotypes, such as serotype 14. Continuous monitoring of serotype distribution in this population will ensure that available vaccines can provide adequate coverage of circulating pneumococcal serotypes.

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References

1. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet. 2009;374:893–902. DOI: 10.1016/S0140-6736(09)61204-6
2. Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. Nat Rev Microbiol. 2008;6:288–301. DOI: 10.1038/nrmicro1871
3. Beekmann SE, Heilmann KP, Richter SS, Garcia-de-Lomas J, Doern GV. Antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and group A beta-haemolytic streptococci in 2002–2003. Results of the multinational GRASP Surveillance Program. Int J Antimicrob Agents. 2005;25:148–56. DOI: 10.1016/j.ijantimicag.2004.09.016
4. Ispahani P, Slack RC, Donald FE, Weston VC, Rutter N. Twenty year surveillance of invasive pneumococcal disease in Nottingham: serogroups responsible and implications for immunisation. Arch Dis Child. 2004;89:757–62. DOI: 10.1136/adc.2003.036921
5. Saha SK, Baqui AH, Darmstadt GL, Ruhulamin M, Hanif M, El Arifeen S, et al. Comparison of antibiotic resistance and serotype composition of carriage and invasive pneumococci among Bangladeshi children: implications for treatment policy and vaccine formulation. J Clin Microbiol. 2003;41:5582–7. DOI: 10.1128/JCM.41.12.5582-5587.2003
6. Centers for Disease Control and Prevention. Invasive pneumococcal disease in young children before licensure of 13-valent pneumococcal conjugate vaccine—United States, 2007. MMWR Morb Mortal Wkly Rep. 2010;59:253–7.
7. Jacobs MR, Good CE, Bajaksouzian S, Windau AR. Emergence of *Streptococcus pneumoniae* serotypes 19A, 6C, and 22F and serogroup 15 in Cleveland, Ohio, in relation to introduction of the protein-conjugated pneumococcal vaccine. Clin Infect Dis. 2008;47:1388–95. DOI: 10.1086/592972
8. Pai R, Gertz RE, Beall B. Sequential multiplex PCR approach for determining capsular serotypes of *Streptococcus pneumoniae* isolates. J Clin Microbiol. 2006;44:124–31. DOI: 10.1128/JCM.44.1.124-131.2006
9. Fraser D, Givon-Lavi N, Bilenko N, Dagan R. A decade (1989–1998) of pediatric invasive pneumococcal disease in 2 populations residing in 1 geographic location: implications for vaccine choice. Clin Infect Dis. 2001;33:421–7. DOI: 10.1086/321874
10. Mokaddas EM, Rotimi VO, Albert MJ. Implications of *Streptococcus pneumoniae* penicillin resistance and serotype distribution in Kuwait for disease treatment and prevention. Clin Vaccine Immunol. 2008;15:203–7. DOI: 10.1128/CVI.00277-07
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 29th infomational supplement; CLSI document M100–S19. Wayne (PA): The Institute; 2009.
12. Imohl M, Reinert RR, van der Linden M. Serotype-specific penicillin resistance of *Streptococcus pneumoniae* in Germany from 1992 to 2008. Int J Med Microbiol. 2010;300:324–30. DOI: 10.1016/j.ijmm.2009.11.004
13. Centers for Disease Control and Prevention. Effects of new penicillin susceptibility breakpoints for *Streptococcus pneumoniae*—United States, 2006–2007. MMWR Morb Mortal Wkly Rep. 2008;57:1353–5.
14. Rowe AK, Schwartz B, Wasas A, Klugman KP. Evaluation of the Etest as a means of determining the antibiotic susceptibilities of isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* from children in the Central African Republic. J Antimicrob Chemother. 2000;45:132–3. DOI: 10.1093/jac/45.1.132

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CTX-M–producing Non-Typhi *Salmonella* spp. Isolated from Humans, United States

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CTX-M–type β-lactamases are increasing among US *Enterobacteriaceae* isolates. Of 2,165 non-Typhi *Salmonella* isolates submitted in 2007 to the National Antimicrobial Resistance Monitoring System, 100 (4.6%) displayed elevated MICs (≥2 mg/L) of ceftriaxone or ceftiofur. Three isolates (serotypes Typhimurium, Concord, and I 4,5,12:i:–) contained $bla_{CTX\text{-}M\text{–}5}$, $bla_{CTX\text{-}M\text{–}15}$, and $bla_{CTX\text{-}M\text{–}55/57}$, respectively.

Severe non-Typhi *Salmonella* (NTS) infections are commonly treated with fluoroquinolones such as ciprofloxacin or, in children, with extended-spectrum cephalosporins such as ceftriaxone. The emergence of *Salmonella* spp. isolates that display resistance to extended-spectrum cephalosporins is of increasing public health concern. In the United States, almost all resistance to extended-spectrum cephalosporins among *Salmonella* spp. isolates is caused by AmpC-type β-lactamases; extended-spectrum β-lactamases (ESBLs), including cefotaximases (CTX-M), rarely have been reported. Likewise, among other *Enterobacteriaceae* isolates in the United States, CTX-M enzymes have been considered rare until recently.

In 2007, Lewis et al. reported that CTX-M was the predominant ESBL among *Enterobacteriaceae* isolates in a US health care system (San Antonio, TX) (*1*). Among the ESBL-producing isolates collected, CTX-M enzymes increased in prevalence from 25% in 2002 to 70% in 2006. The emergence was observed mainly in urinary tract isolates of *Escherichia coli*, and the predominating enzyme was CTX-M-15 (*1*). Similarly, a US study investigating clinical samples of *Enterobacteriaceae* submitted to The Hospital of the University of Pennsylvania (Philadelphia, PA) in 2007 reported that 48% of cephalosporin-resistant *E. coli* isolates were CTX-M positive (*2*). Furthermore, CTX-M enzymes, and CTX-M-15 in particular, were common among ESBL-producing *Enterobacteriaceae* isolates collected at 15 US medical centers participating in the Meropenem Yearly Susceptibility Test Information Collection Program during 2007 (*3*).

In the United States, the National Antimicrobial Resistance Monitoring System (NARMS) has systematically monitored antimicrobial susceptibility of NTS since 1996. This program is a collaborative effort between the Centers for Disease Control and Prevention (CDC), the Food and Drug Administration, and the US Department of Agriculture. Reports on increased prevalence of CTX-M enzymes among *Enterobacteriaceae* isolates in the United States prompted us to investigate CTX-M enzymes among NARMS NTS isolates collected from humans in 2007.

The Study

In 2007, public health laboratories in all US state health departments submitted every twentieth NTS isolated from humans to CDC for susceptibility testing by NARMS. MICs were determined by using broth microdilution (Sensititer, Trek Diagnostics, Westlake, OH, USA) and interpreted according to Clinical Laboratory Standards Institute criteria, where available.

Of the 2,165 human NTS isolates submitted to NARMS in 2007, a total of 100 (4.6%) displayed elevated MICs (≥2 mg/L) of ceftriaxone or ceftiofur, extended-spectrum cephalosporins used in human and veterinary medicine, respectively. Genomic DNA prepared from the 100 isolates and a PCR screen obtained by using degenerate primers capable of detecting all CTX-M enzymes identified 3 positive isolates, including serotypes Typhimurium; I 4,5,12:i:–; and Concord (*4*). Most (66%) of the remaining 97 isolates harbored a bla_{CMY} gene.

The 3 CTX-M–producing *Salmonella* spp. infections occurred in 2 female patients (8 months of age and 72 years of age) and 1 male patient (1 year of age). Interviews were available for 2 patients, the 8-month-old (her parents) and the 72-year-old; in both instances, gastrointestinal symptoms with diarrhea were reported, and medical care was sought. Both patients received antimicrobial drug treatment (azithromycin and levofloxacin, respectively). The 72-year-old patient had traveled internationally before illness onset; the 8-month-old patient, who was infected with *S. enterica* serovar Concord, was an adoptee from Ethiopia.

All 3 isolates displayed resistance to β-lactams and extended-spectrum cephalosporins (Table). The *S. enterica* serovar Typhimurium and Concord isolates displayed additional multiresistance phenotypes. In addition, the serovar Typhimurium isolate displayed resistance to the quinolone nalidixic acid, a resistance phenotype associated with

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Table. Characteristics of non-Typhi *Salmonella* isolates harboring bla_{CTX-M} genes reported to the National Antimicrobial Resistance Monitoring System, United States, 2007*

Isolate no.	Serotype	Submitting state	Resistance pattern	CTX MIC, mg/L	*bla* genes	*qnr* gene	Foreign travel association	Transferable
AM33608	Typhimurium	SC	AMP, AUG, CHL, CTX, NAL, SUL, SXT, XNL	64	CTX-M-5, OXA-1	–	ND	No
AM32667	I 4,5,12:i:–	CT	AMP, CTX, XNL	64	CTX-M-55/57	–	No	Yes
AM32764	Concord	OR	AMP, CHL, CTX, GEN, STR, SUL, SXT, TET, XNL	64	CTX-M-15, SHV-12	*qnrA1*	Ethiopia	No

*CTX, ceftriaxone; AMP, ampicillin; AUG, amoxicillin-clavulanic acid; CHL, chloramphenicol; NAL, nalidixic acid; SUL, sulfamethoxazole or sulfisoxazole; SXT, trimethoprim–sulfamethoxazole; XNL, ceftiofur; –, none detected; ND, not determined; GEN, gentamicin; STR, streptomycin; TET, tetracycline.

decreased susceptibility to fluoroquinolones. The serovar Typhimurium and Concord isolates showed decreased susceptibility to ciprofloxacin (MIC 0.25 mg/L and 0.125 mg/L, respectively). PCR for the plasmid-mediated mechanisms *qnrA,B,S* and *aac(6')Ib-cr* showed a *qnrA* gene in the serovar Concord isolate. Sequencing confirmed *qnrA1*.

Group-specific PCR primers were used to characterize the presumed bla_{CTX-M} genes (*5*). *S. enterica* serovar Concord and I 4,5,12:i:– harbored group I enzymes, whereas the *S. enterica* serovar Typhimurium isolate harbored a group II enzyme. Sequencing showed $bla_{CTX-M-15}$ in the serovar Concord isolate, $bla_{CTX-M-5}$ in the serovar Typhimurium isolate, and $bla_{CTX-M-55/57}$ in the serovar I 4,5,12:i:– isolate. Presence of other β-lactamase–encoding genes (bla_{TEM}, bla_{SHV}, bla_{CMY}, bla_{PSE}, and bla_{OXA}) was investigated by using PCR (*6*–*9*). Amplification and sequencing confirmed a bla_{OXA-1} and a bla_{SHV-12} gene in the serovar Typhimurium and Concord isolates, respectively (*7*,*8*).

The genetic environment of each bla_{CTX-M} gene was investigated by using PCR aimed at identifying insertion elements IS*Ecp1*, IS*26*, and CR1 (formerly known as orf513) (*10*). Amplification and sequencing of the PCR products confirmed the IS*Ecp1* element upstream of each bla_{CTX-M} gene (*10*). In addition to IS*Ecp1*, an IS*26* element was detected upstream of the $bla_{CTX-M-5}$ and $bla_{CTX-M-15}$ genes.

To determine whether the CTX-M enzymes were plasmid borne, we extracted and transformed plasmids into electrocompetent *E. coli* DH10B. The $bla_{CTX-M-55/57}$ gene transferred to *E. coli*; repeated attempts to transfer the $bla_{CTX-M-5}$ and $bla_{CTX-M-15}$ genes were unsuccessful. PCR amplification and plasmid pulsed-field gel electrophoresis confirmed the presence of the $bla_{CTX-M-55/57}$ gene on a 70-kb plasmid in the transformant. The plasmid was not typeable by PCR-based incompatibility/replicon typing.

Conclusions

We describe 3 CTX-M–producing isolates of NTS collected from humans in the United States during 2007. CTX-M–producing *Salmonella* spp. previously have been reported among the NARMS collection of human isolates. The first isolate was *S. enterica* serovar Typhimurium from a 3-month-old child in Georgia in 2003 (*11*). This infection was considered domestically acquired because the child's family did not report a history of international travel. In addition, a CTX-M-15–producing isolate of *S. enterica* serovar Concord was identified among NARMS NTS collected in 2006 (*12*). However, in contrast to the previous case, this infection most likely was acquired abroad because the patient reported travel to Ethiopia in conjunction with illness onset.

At least 1 of the infections described in the present study probably was acquired abroad; the CTX-M-15–producing *S. enterica* serovar Concord isolate was isolated from an adopted child from Ethiopia. Thus, both instances of CTX-M–producing serovar Concord isolates identified in NARMS thus far have been associated with travel to Ethiopia. The emergence of CTX-M-15–producing serovar Concord infections among Ethiopian adoptees has been described previously (*13*). In addition, the emergence of serovar Concord isolates that produced CTX-M-15, SHV-12, and QnrA1 was recently described (*14*).

The fact that bla_{CTX-M} genes commonly are located on plasmids and in conjunction with mobile genetic elements such as IS*Ecp1* most likely has contributed to their dissemination. The $bla_{CTX-M-55/57}$ gene that was transferable to *E. coli* in the present study was located on a 70-kb plasmid. The fact that the $bla_{CTX-M-5}$ and $bla_{CTX-M-15}$ gene did not transfer might suggest chromosomal locations. In fact, Fabre et al. found that most CTX-M–15–producing *S. enterica* serovar Concord isolates studied harbored the bla_{CTX-M} gene on the chromosome (*13*).

The recently reported increase in CTX-M–producing *Enterobacteriaceae* in the United States raises concern. First, a reservoir of ESBLs and CTX-M genes among *E. coli* and *Klebsiella* spp. constitutes a risk factor for increased spread of resistance to other pathogenic bacteria, including *Salmonella* spp. Second, use of cephalosporins to treat serious ESBL-producing bacterial infections has been associated with high rates of treatment failure (*15*). Thus, an increase in CTX-M–producing *Salmonella* spp. strains is likely to directly affect treatment, especially among children for whom use of fluoroquinolones is contraindicated.

Moreover, the decreased susceptibility to ciprofloxacin of the serovar Typhimurium and Concord isolates in the present study raises concern about the emergence of isolates showing concurrent resistance to both extended-spectrum cephalosporins and fluoroquinolones. Continued surveillance of resistant bacteria, in combination with prudent use of antimicrobial agents in animals and humans, is crucial for limiting further spread of CTX-M–producing isolates of *Enterobacteriaceae* in the United States.

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References

1. 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 U.S. health care system. Antimicrob Agents Chemother. 2007;51:4015–21. DOI: 10.1128/AAC.00576-07
2. McGettigan SE, Hu B, Andreacchio K, Nachamkin I, Edelstein PH. Prevalence of CTX-M beta-lactamases in Philadelphia. J Clin Microbiol. 2009;47:2970–4. DOI: 10.1128/JCM.00319-09
3. Castanheira M, Mendes RE, Rhomberg PR, Jones RN. Rapid emergence of bla_{CTX-M} among *Enterobacteriaceae* in U.S. medical centers: molecular evaluation from the MYSTIC Program (2007). Microb Drug Resist. 2008;14:211–6. DOI: 10.1089/mdr.2008.0827
4. Bonnet R, Recule C, Baraduc R, Chanal C, Sirot D, De Champs C, et al. Effect of D240G substitution in a novel ESBL CTX-M-27. J Antimicrob Chemother. 2003;52:29–35. DOI: 10.1093/jac/dkg256
5. Pitout JD, Hossain A, Hanson ND. Phenotypic and molecular detection of CTX-M-beta-lactamases produced by *Escherichia coli* and *Klebsiella* spp. J Clin Microbiol. 2004;42:5715–21. DOI: 10.1128/JCM.42.12.5715-5721.2004
6. Briñas L, Zarazaga M, Sáenz Y, Ruiz-Larrea F, Torres C. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. Antimicrob Agents Chemother. 2002;46:3156–63. DOI: 10.1128/AAC.46.10.3156-3163.2002
7. Chen S, Zhao S, White DG, Schroeder CM, Lu R, Yang H, et al. Characterization of multiple-antimicrobial-resistant salmonella serovars isolated from retail meats. Appl Environ Microbiol. 2004;70:1–7. DOI: 10.1128/AEM.70.1.1-7.2004
8. Rasheed JK, Jay C, Metchock B, Berkowitz F, Weigel L, Crellin J, et al. Evolution of extended-spectrum beta-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob Agents Chemother. 1997;41:647–53.
9. Winokur PL, Vonstein DL, Hoffman LJ, Uhlenhopp EK, Doern GV. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and *Salmonella* isolates from food animals and humans. Antimicrob Agents Chemother. 2001;45:2716–22. DOI: 10.1128/AAC.45.10.2716-2722.2001
10. Eckert C, Gautier V, Saladin-Allard M, Hidri N, Verdet C, Ould-Hocine Z, et al. Dissemination of CTX-M–type beta-lactamases among clinical isolates of *Enterobacteriaceae* in Paris, France. Antimicrob Agents Chemother. 2004;48:1249–55. DOI: 10.1128/AAC.48.4.1249-1255.2004
11. Sjölund M, Yam J, Schwenk J, Joyce K, Medalla F, Barzilay E, et al. Human *Salmonella* infection yielding CTX-M beta-lactamase, United States. Emerg Infect Dis. 2008;14:1957–9. DOI: 10.3201/eid1412.080494
12. Huang S, Rickert R, Matar C, Pecic G, Howie RL, Joyce K, et al. *Salmonella* isolates with decreased susceptibility to extended-spectrum cephalosporins in the United States. Foodborne Pathog Dis. 2005;2:12–23. DOI: 10.1089/fpd.2005.2.12
13. Fabre L, Delaune A, Espie E, Nygard K, Pardos M, Polomack L, et al. Chromosomal integration of the extended-spectrum beta-lactamase gene $bla_{CTX-M-15}$ in *Salmonella enterica* serotype Concord isolates from internationally adopted children. Antimicrob Agents Chemother. 2009;53:1808–16. DOI: 10.1128/AAC.00451-08
14. Morosini MI, Valverde A, Garcia-Castillo M, Nordmann P, Canton R. Persistent isolation of *Salmonella* Concord harbouring CTX-M-15, SHV-12 and QnrA1 in an asymptomatic adopted Ethiopian child in Spain also colonized with CTX-M-14- and QnrB-producing Enterobacteriaceae. J Antimicrob Chemother. 2010;65:1545–6. DOI: 10.1093/jac/dkq168
15. Paterson DL, Ko WC, Von Gottberg A, Casellas JM, Mulazimoglu L, Klugman KP, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory. J Clin Microbiol. 2001;39:2206–12. DOI: 10.1128/JCM.39.6.2206-2212.2001

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Emergence of *Rickettsia africae*, Oceania

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We detected *Rickettsia africae*, the agent of African tick-bite fever (ATBF), by amplification of fragments of *gltA*, *ompA*, and *ompB* genes from 3 specimens of *Amblyomma loculosum* ticks collected from humans and birds in New Caledonia. Clinicians who treat persons in this region should be on alert for ATBF.

Spotted fever group (SFG) rickettsioses are caused by obligate intracellular gram-negative bacteria of the genus *Rickettsia* and are transmitted by hematophagous arthropods, mainly ticks. These zoonoses are important emerging vector-borne infections of humans worldwide. They share characteristic clinical features, including fever, rash, and sometimes an inoculation eschar at the bite site, depending on the rickettsial agent that is involved (*1*).

In Oceania, tick-borne rickettsioses have been reported primarily in Australia. They include Queensland tick typhus (*R. australis*) along the east coast of Australia (*2*), Flinders Island spotted fever (*R. honei*) in southeast Australia (*2*), and variant Flinders Island spotted fever (*R. honei* strain "marmionii") in eastern Australia (*2*). Furthermore, the DNA of at least 8 incompletely described SFG rickettsiae have been detected in ticks, and the pathogenicity of these rickettsiae remains unknown (*2*). Additionally, *R. felis*, the agent of flea-borne SFG rickettsiosis, has been found in Western Australia (*3*), New Zealand (*4*), and recently in New Caledonia (*5*). However, little is known about rickettsioses in the rest of Oceania.

The Study

From February 2001 to November 2007, tick samples were obtained as part of other, ongoing studies in Oceania. A total of 92 ticks were collected: 14 *Amblyomma loculosum* (13 nymphs, 1 female), including 2 from humans on Chesterfield Island, New Caledonia, and 12 from birds on Walpole Island, New Caledonia; 9 female *A. breviscutatum* from swine on Santo Island, Vanuatu; 2 female *A. laticaudae* from snakes on Pindai Island, New Caledonia; 60 female *Haemaphysalis longicornis*, including 38 from cattle on Santo Island, Vanuatu, 10 from cattle in Port-Laguerre, New Caledonia, and 12 from horses in New Caledonia; and 7 female *Rhipicephalus sanguineus* from dogs in Païta, New Caledonia. Tick species were identified by using taxonomic keys and stored in 70% ethanol before being tested. DNA was extracted from each tick with the QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's instructions and stored at 4°C until it was used in PCR amplifications. Each sample was tested by quantitative PCR (qPCR) in a LightCycler instrument (Roche Diagnostics GmbH, Mannheim, Germany) for the presence of *Rickettsia* spp. DNA by using primers and Taqman probes (Eurogentec, Seraing, Belgium) that targeted a partial sequence of the citrate synthase (*gltA*) gene (*6*). Tick DNA samples positive by using qPCR were also subjected to standard PCR using CS2d and CS.1258n, which amplify an 1,178-bp fragment of *Rickettsia gltA*. An additional PCR amplification was performed by using primers 190.70, 190.180, and 190.701, which amplify a 629–632-bp fragment of rickettsial *ompA*. Positive controls (*R. montanensis* DNA) and negative controls (sterile water and DNA extracted from uninfected ticks from laboratory colonies) were included in each test. We also amplified and sequenced the 2,113–4,346-bp portion of rickettsial *ompB* (*7*). All amplified products were purified, directly sequenced, and sequences were compared with sequences in the GenBank database.

Positive and negative controls gave expected results in all tests. qPCR and subsequent standard PCR for *ompA* and *gltA* were positive for 3 specimens of *A. loculosum*: 2 nymphs collected from humans on Chesterfield Island, New Caledonia, and 1 female collected from birds on Walpole Island, New Caledonia. The 590-bp amplicons of *ompA* and 1,135-bp amplicons of *gltA* obtained from all samples showed 100% identity with the relevant genes of strain ESF-5 of *R. africae* (GenBank accession no. CP001612.1). A 2,153-bp amplicon of a portion of *ompB* showed 99.8% identity with *R. africae* (strain ESF-5).

Multispacer typing of the detected rickettsiae was performed by using the *dksA-xerC*, *mppA-purC,* and *rpmE-tRNA-fMet* intergenic spacers (*8*). The sequence of the *dksA-xerC* intergenic spacer was found to be 242 bp instead of the 177 bp described for the ESF-5 strain of *R. africae* (GenBank accession no. DQ008280), due to the 65-bp repeat of portion 1–65. A single-nucleotide polymorphism was also found. The *rpmE-tRNA-fMet* (GenBank accession no. DQ008246) spacer showed 100% identity with the *rpmE-tRNA-fMet* spacer of *R. africae*. The *mppA-purC* amplification attempt was negative. Whether this intergenic spacer has been lost or substantially modified remains unknown. Finally, qPCR with primers 1267F/1267R specific

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for a sequence of the *R. africae* plasmid (*pRA*) produced positive results in 3 ticks infected by *R. africae* (*9*).

Conclusions

By using molecular criteria (*10*), we identified *R. africae* in an unexpected place and in unexpected tick species. Indeed, *R. africae* is the typical agent of African tick-bite fever. This SFG rickettsia is prevalent mostly in sub-Saharan Africa, where vectors are ticks of the genus *Amblyomma* (mainly *A. hebraeum* and *A. variegatum*) (*11*). The rates of *R. africae* infection in ticks in ATBF-endemic areas are typically high and may reach 100% (*11*). These ticks have also been shown to act as reservoirs for *R. africae* (*12*). ATBF occurs 5 to 10 days after the bite of a tick infected with *R. africae* (*1*). Clinical features include an inoculation eschar, frequently more than one, predominant on the lower limbs; fever; regional lymphadenopathy; and a rash that is sometimes vesicular (*1*).

In our study, 2 of 3 amplified and sequenced genes showed 100% identity with *R. africae*, and a third gene (*ompB*) showed a degree of difference less than that needed to determine a new species (*10*). Thus, our findings demonstrate the existence of a variant *R. africae,* despite the previously identified clonality of its strains in Africa (*9*) and the West Indies, where it was carried by *A. variegatum* ticks from western Africa in the 18th century (*1*).

New Caledonia is a French territory (249,000 inhabitants; 18,575.5 km^2 of land area) located in the subregion of Melanesia in the southwest Pacific. Chesterfield Island and Walpole Island belong to the archipelago and are uninhabited coral sand cays colonized by marine birds. We detected *R. africae* in *A. loculosum* ticks in Oceania. This tick is known to infest marine birds that are distributed on numerous tropical islands of the southern oceans (*13*). *A. loculosum* ticks have been found in the Indian Ocean (Tanzania, Seychelles Islands, Mauritius, Cocos [Keeling] Island group, Madagascar), on the Coral Sea Islands (Queensland, numerous islands and reefs), near New Caledonia (Surprise Island), and in the Caroline Group in the Pacific Ocean (*13*). Other hosts are goats (Australia) and lizards (Seychelles) (*13*). Aride virus, an ungrouped arbovirus, has been isolated from *A. loculosum* ticks collected from dead birds in the Seychelles, which suggests that this tick may be a vector of bird infections (*14*). *A. loculosum* ticks are also known to readily feed on humans (Seychelles, Australia, New Caledonia, Caroline Islands) (*13*).

Two of the 3 specimens of *A. loculosum* ticks that tested positive for rickettsiae were collected from humans who worked with birds on Chesterfield Island, but those persons only remained on the island for a short period. The duration of tick attachment was not known, but to the best of our knowledge, no illness developed after these tick bites. Further investigations are necessary to evaluate the prevalence and distribution of *R. africae* in New Caledonia and in other islands of Oceania, as well as its interactions with *A. loculosum* ticks. However, clinicians in Oceania and around the world should be aware of the presence of *R. africae* in New Caledonia, an island increasingly visited by tourists from all over the world, and in other islands where *A. loculosum* ticks are prevalent.

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References

1. Parola P, Paddock CD, Raoult D. Tick-borne rickettsioses around the world: emerging diseases challenging old concepts. Clin Microbiol Rev. 2005;18:719–56. DOI: 10.1128/CMR.18.4.719-756.2005
2. Graves S, Stenos J. Rickettsioses in Australia. Ann N Y Acad Sci. 2009;1166:151–5. DOI: 10.1111/j.1749-6632.2009.04530.x
3. Schloderer D, Owen H, Clark P, Stenos J, Fenwick SG. *Rickettsia felis* in fleas, Western Australia. Emerg Infect Dis. 2006;12:841–3.
4. Kelly PJ, Meads N, Theobald A, Fournier PE, Raoult D. *Rickettsia felis, Bartonella henselae,* and *B.clarridgeiae*, New Zealand. Emerg Infect Dis. 2004;10:967–8.
5. Mediannikov O, Cabre O, Qu F, Socolovschi C, Davoust B, Marié JL, et al. *Rickettsia felis* and *Bartonella clarridgeiae* in fleas from New Caledonia. Vector Borne Zoonotic Dis. Epub 2010 Jun 23.
6. Rolain JM, Sthul L, Maurin M, Raoult D. Evaluation of antibiotic susceptibilities of three rickettsial species including *Rickettsia felis* by a quantitative PCR DNA assay. Antimicrob Agents Chemother. 2002;46:2747–51. DOI: 10.1128/AAC.46.9.2747-2751.2002
7. Roux V, Raoult D. Phylogenetic analysis of members of the genus *Rickettsia* using the gene encoding the outer-membrane protein rOmpB (*ompB*). Int J Syst Evol Microbiol. 2000;50:1449–55.
8. Fournier PE, Zhu Y, Ogata H, Raoult D. Use of highly variable intergenic spacer sequences for multispacer typing of *Rickettsia conorii* strains. J Clin Microbiol. 2004;42:5757–66. DOI: 10.1128/JCM.42.12.5757-5766.2004
9. Fournier PE, El Karkouri K, Leroy Q, Robert C, Giumelli B, Renesto P, et al. Analysis of *Rickettsia africae* genome reveals that virulence acquisition in *Rickettsia* species may be explained by genome reduction. BMC Genomics. 2009;10:166. DOI: 10.1186/1471-2164-10-166
10. Fournier PE, Dumler JS, Greub G, Zhang J, Wu Y, Raoult D. Gene sequence-based criteria for identification of new *Rickettsia* isolates and description of *Rickettsia heilongjiangensis* sp. nov. J Clin Microbiol. 2003;41:5456–65. Medline DOI: 10.1128/JCM.41.12.5456-5465.2003
11. Parola P, Inokuma H, Camicas JL, Brouqui P, Raoult D. Detection and identification of spotted fever group *Rickettsiae* and *Ehrlichiae* in African ticks. Emerg Infect Dis. 2001;7:1014–7. DOI: 10.3201/eid0706.010616
12. Socolovschi C, Huynh TP, Davoust B, Gomez J, Raoult D, Parola P. Transovarial and trans-stadial transmission of *Rickettsiae africae* in *Amblyomma variegatum* ticks. Clin Microbiol Infect. 2009;15(S2):317–8.
13. Hoogstraal H, Wassef HY, Converse JD, Keirans JE, Clifford CM, Feare CJ. *Amblyomma loculosum* (*Ixodoidae*): identity, marine bird and human hosts, virus infection, and distribution in the southern oceans. Ann Entomol Soc Am. 1976;69:3–14.

14. Converse JD, Hoogstraal H, Moussa MI, Kaiser MN, Casals J, Feare CJ. Aride virus, a new ungrouped arbovirus infecting *Amblyomma loculosum* ticks from roseate terns in the Seychelles. Arch Virol. 1976;50:237–40. DOI: 10.1007/BF01320577

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New Delhi Metallo-β-Lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada

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Multidrug-resistant *Klebsiella pneumoniae* and *Escherichia coli* isolates harboring New Delhi metallo-β-lactamase (NDM-1) were isolated from a patient who had returned to Canada from India. The NDM-1 gene was found on closely related incompatibility group A/C type plasmids. The occurrence of NDM-1 in North America is a major public health concern.

Carbapenem-hydrolyzing β-lactamases, i.e., carbapenemases, in bacterial clinical isolates are an increasing concern because they often also confer resistance to most other β-lactam antimicrobial agents. Among *Enterobacteriaceae*, carbapenemases are mainly found in the Ambler class A penicillinase or class B metallo-enzyme groups. VIM and IMP are the most frequently acquired class B enzymes and are commonly found in southern Europe and the Far East, rarely in North America. *Klebsiella pneumoniae* carbapenemase (KPC) class A has been found worldwide, although it emerged in the eastern United States in the mid 1990s and subsequently has successfully established itself in multiple states (*1*).

Recently, a new class B enzyme, New Delhi metallo-β-lactamase (NDM-1), was characterized from a *K. pneumoniae* isolate from Sweden; the bacteria seem to have been imported from India (*2*). *Enterobacteriaceae* isolates harboring NDM-1 have now been found in multiple areas of India and Pakistan and in the United Kingdom (*3–5*); such isolates were recently reported from 3 US states (*6*). In Canada, carbapenemase-harboring isolates are rare, reported only for a small outbreak of clonal *Pseudomonas aeruginosa* isolates harboring IMP-7, *P. aeruginosa* isolates harboring VIM-2, a *Serratia marcescens* isolate harboring a SME-2 class A β-lactamase, and 3 isolates of *K. pneumoniae* harboring KPC-3 (*7–10*). We characterized NDM-harboring clinical isolates from a patient who had recently traveled to India.

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The Study

A 76-year-old woman returned to Vancouver, British Columbia, Canada, in early 2010 after spending 3.5 months in northern India. Before her trip, she had been in good health. In India, persistent nonbloody diarrhea developed, for which she did not seek medical attention. One month later, she was treated in the hospital for hypertension and congestive heart failure. She was discharged 3 days after admission but readmitted 3 days later because of ongoing diarrhea and decreased consciousness. Unspecified encephalitis and a urinary tract infection were diagnosed, but despite antibacterial drug therapy, her neurologic status did not improve over the next 3 weeks. She was discharged from the hospital in India and transferred to Canada.

When she arrived at the hospital in Vancouver on February 14, 2010, her vital signs reflected distributive shock: temperature 38.3°C, blood pressure 100/80 mm Hg, and heart rate 126 beats/minute. Sepsis was suspected and she was given imipenem and vancomycin. Within 24 hours, her level of consciousness had deteriorated, and she was admitted to the intensive care unit and intubated for airway protection. Blood cultures were negative, but urine culture (>1 × 10^8 CFU/mL) grew highly drug-resistant *K. pneumoniae* N10–0469 (February 15, 2010) with intermediate resistance to chloramphenicol and susceptibility to colistin. The urine was packed with leukocytes, and no other source for sepsis was found. A perirectal sample, screened for resistant gram-negative rods, grew *K. pneumoniae* N10–0506 and *E. coli* N10–0505 (February 16, 2010). A stool specimen was negative for ova and parasites but positive for *Clostridium difficile* toxin.

The patient was given vancomycin and metronidazole for the *C. difficile* infection and colistin for the *K. pneumoniae* infection. Treatment with colistin was discontinued shortly after its initiation because of the onset of renal complications; treatment with chloramphenicol was successful. Test results for infectious causes of encephalitis (e.g., malaria smears, cerebrospinal fluid culture for bacteria, and staining for acid-fast bacteria and fungi) were negative, as were test results for cryptococcal antigen, herpes simplex viruses 1 and 2, Japanese encephalitis virus, and rabies virus. Results of computed tomographic scans, magnetic resonance imaging of the head, and an electroencephalogram were suggestive of global metabolic encephalopathy. Neurologic symptoms did not improve, despite the successful treatment of the urinary tract infection. Subsequently, *E. coli* N10–0705 isolate with an extended-spectrum

β-lactamase phenotype was obtained from urine on March 9, 2010, for which the patient was treated with imipenem.

Ventilator and vasopressor support were eventually removed, and the patient was transferred to the general medicine ward. Several days later, her condition worsened and she died; the final diagnosis was toxic metabolic leukoencephalopathy, probably related to sepsis. The patient's family refused to allow an autopsy.

Macrorestriction analysis of the 4 isolates showed that *K. pneumoniae* N10–0469 and N10–0506 were closely related, although *E. coli* N10–0505 and N10–0705 were not (Figure 1). All 4 isolates contained multiple plasmids (data not shown). β-lactamase PCR and sequencing were conducted (primers listed in Table 1). *K. pneumoniae* N10–0469 and N10–0506 harbored the genes for SHV-1, CTX-M-15, OXA-1, CMY-6, and NDM-1; *E. coli* N10–0505 harbored TEM-1, CTX-M-15, CMY-6, and NDM-1; and *E. coli* N10–0705 harbored TEM-1, CTX-M-15, OXA-1, and CMY-42. The first characterized isolate from Sweden that harbored $bla_{\text{NDM-1}}$ also harbored CMY-4, a Trp221Arg variant of the widespread CMY-2 β-lactamase (*3*). CMY-6 is a Trp221Leu variant of CMY-2 (GenBank accession no. AJ011293). CMY-42 is a Val231Ser variant of CMY-2.

Antimicrobial drug susceptibility testing by Sensititer panels ESB1F and CMV1AGNF (Trek Diagnostic Systems, Cleveland, OH, USA) found that all 4 isolates were multidrug resistant and that 3 of the 4 isolates, not *E. coli* N10–0705, were nonsusceptible to carbapenems (Table 2). Nonsusceptibility to carbapenems was also shown by Etest (bioMérieux, St. Laurent, Quebec, Canada) and disk diffusion (Table 2). *K. pneumoniae* N10–0469 and *E. coli*

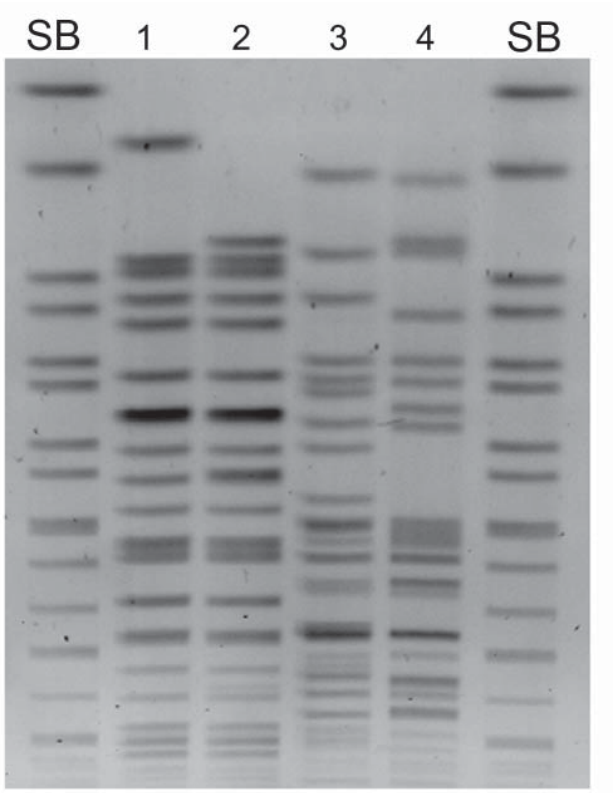

Figure 1. Macrorestriction analysis (*Xba*I) by pulsed-field gel electrophoresis of *Klebsiella pneumoniae* N100469 (lane 1), *K. pneumoniae* N10–0506 (lane 2), *Escherichia coli* N10–0505 (lane 3), *E. coli* N10–0705 (lane 4), *Salmonella enterica* serovar Branderup molecular mass marker (*Xba*I) (lanes SB).

Table 1. Primers used in study of New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada, 2010

Name	Sequence, 5′ → 3′*	Product size, bp
SHV-UP	CGCCGGGTTATTCTTATTTGTCGC	1,016
SHV-LO	TCTTTCCGATGCCGCCGCCAGTCA	
TEM-G	TTGCTCACCCAGAAACGCTGGTG	708
TEM-H	TACGATACGGGAGGGCTTACC	
CTX-U1	ATGTGCAGYACCAGTAARGTKATGGC	593
CTX-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG	
OXA1-F	CGCAAATGGCACCAGATTCAAC	464
OXA1-R	TCCTGCACCAGTTTTCCCATACAG	
CMY2-A	TGATGCAGGAGCAGGCTATTCC	323
CMY2-B	CTAACGTCATCGGGGATCTGC	
KPC1	ATGTCACTGTATCGCCGTC	863
KPC2	AATCCCTCGAGCGCGAGT	
VIM1	GTTTGGTCGCATATCGCAAC	382
VIM2	AATGCGCAGCACCAGGATAGAA	
IMP1	CCWGATTTAAAAATYGARAAGCTTG	522
IMP2	TGGCCAHGCTTCWAHATTTGCRTC	
NDM-F	GGTGCATGCCCGGTGAAATC	660
NDM-R	ATGCTGGCCTTGGGGAACG	
NDM-A	CACCTCATGTTTGAATTCGCC	984
NDM-B	CTCTGTCACATCGAAATCGC	

*H is A, C, or T; K is G or T; R is A or G; S is G or C; and W is A or T.

Table 2. Antimicrobial drug susceptibilities of the isolates from study of New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada, 2010*

Test/antimicrobial agent	*K. pneumoniae* N10–0469	*E. coli* DH10B (pNDM-Kp10469)	*E. coli* N10–0505	*E. coli* DH10B (pNDM-Ec10505)	*E. coli* DH10B
Broth microdilution (MIC, μg/mL)					
Amoxicillin/clavulanic acid	>32	>32	32	>32	4
Piercillin/tazobactam	>64	>64	>64	>64	≤4
Ampicillin	>16	>16	>16	>16	2
Cefazolin	>16	>16	>16	>16	≤8
Cefotaxime	>64	>64	>64	>64	≤0.25
Cefotaxime/clavulanic acid	>64	>64	>64	>64	≤0.12
Ceftazidime	>128	>128	>128	>128	≤0.25
Ceftazidime/clavulanic acid	>128	>128	>128	>128	0.25
Cefepime	>16	16	>16	16	≤1
Cefoxitin	>64	>64	>64	>64	8
Imipenem	>16	4	2	2	≤0.5
Meropenem	>8	2	8	2	≤1
Chloramphenicol	16	4	32	4	≤2
Tetracycline	>32	≤4	32	≤4	≤4
Amikacin	>64	>64	>64	>64	2
Gentamicin	>16	>16	>16	>16	≤0.25
Ciprofloxacin	>2	≤0.015	>2	≤0.015	≤0.015
Trimethoprim/sulfamethoxazole	>4	≤0.12	>4	≤0.12	≤0.12
Etest (MIC, μg/mL)					
Aztreonam	>256	4	>256	4	0.094
Imipenem	>32	4	>32	4	0.19
Meropenem	>32	8	32	6	0.032
Ertapenem	>32	16	>32	16	0.008
Colistin	0.38	ND	0.38	ND	ND
Polymyxin B	0.19	ND	0.25	ND	ND
Tigecycline	1.5	ND	0.125	ND	ND
Disk diffusion (zone diameter, mm)					
Imipenem	6	14	11	13	33
Meropenem	6	15	10	14	35
Ertapenem	6	13	6	13	35

*NDM, New Delhi metallo-β-lactamase; ND, not done.

N10–0505 were susceptible to colistin, polymixin B, and tigecycline.

Multilocus sequence typing of *K. pneumoniae* N10–0469 showed that it was sequence type (ST) 16 (*11*). The first characterized strain of *K. pneumoniae* that harbored NDM-1 was ST14, unrelated to ST16 (*3*). Mutilocus sequence typing of *E. coli* N10–0505 showed it to be ST405 (*12*). The worldwide spread of $bla_{\text{CTX-M-15}}$ has been partly attributed to 2 epidemic strains, ST131 and ST405 (*13*).

E. coli DH10B harboring NDM-1 plasmids was obtained by electrotransformation with whole plasmid DNA with selection on 0.25 μg/mL meropenem. Agarose gel analysis of transformants showed that they carried only a single, large plasmid. PCR and sequencing showed that CMY-6 and NDM-1 were located on an ≈102-kb plasmid (pNDM-Kp10469), originating from *K. pneumoniae* N10–0469, and on an ≈129-kb plasmid (pNDM-Ec10505), originating from *E. coli* N10–0505 (Figure 2). Incompatibility (Inc) group PCR (*14*) and fingerprinting (*15*) showed that both plasmids were related Inc A/C types, indicating possible horizontal transfer in vivo (Figure 2).

Transformant susceptibility to aztreonam is consistent with the inability of class B enzymes to efficiently hydrolyze this drug. Transformants exhibited lower carbapenem MICs than did clinical isolates, likely reflecting total β-lactamase content and additional resistance mechanisms such as porin mutations in the clinical isolates (Table 2). The recent emergence of $bla_{\text{NDM-1}}$ in India has been linked to its spread on the Inc A/C-type, IncF1/FII-type, or unknown plasmid types (*3*). Analysis for class 1 integron cassettes found that both plasmids contained a 0.8-kb cassette that coded for *aac(6')-Ib*–type aminoglycoside 6′-N-acetyl transferase. PCR for the ISCR1 element associated with some class 1 integrons was negative. Plasmids harboring NDM-1 plasmids were successfully transferred by conjugation from *K. pneumoniae* N10–0469 and *E. coli* N10–0505 to recipient strain *E. coli* RG192 (rifampin resistant).

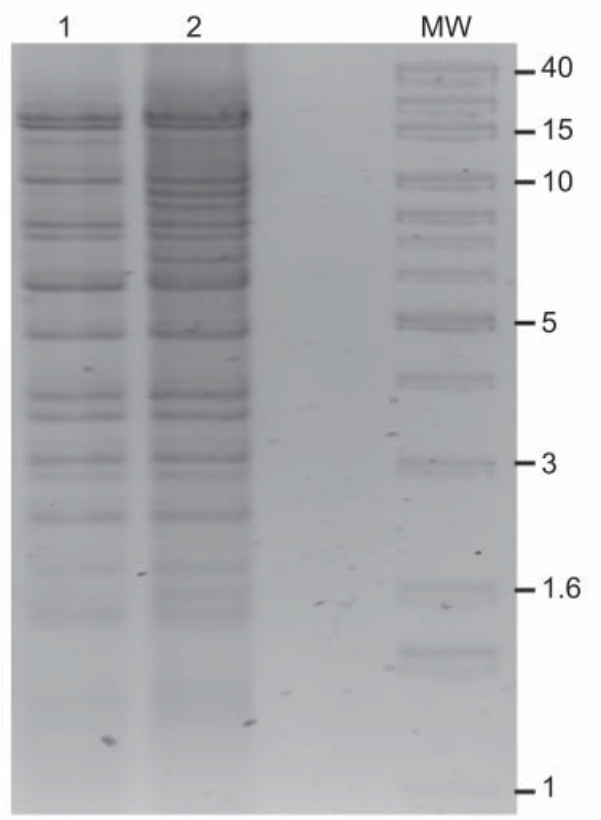

Figure 2. *Bgl*II restriction digests of New Delhi metallo-β-lactamase (pNDM)–Kp10469 (lane 1) and pNDM-Ec10505 (lane 2). MW, 1-kb extension ladder molecular mass marker (Invitrogen, Carlsbad, CA, USA). Sizes (kb) are indicated for some bands.

Conclusions

North America has thus far escaped the widespread establishment of metallo-β-lactamase–harboring organisms. Therefore, the emergence of bla_{NDM-1}-harboring *Enterobacteriaceae* in North America is of concern because such isolates exhibit resistance to drugs commonly used to treat gram-negative infections (β-lactams, fluoroquinolones, and aminoglycosides) and have shown a propensity to spread rapidly (*4*–*6*).

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References

1. Kitchel B, Rasheed J, Patel J, Srinivasan A, Navon-Venzia S, Carmeli Y, et al. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. Antimicrob Agents Chemother. 2009;53:3365–70. DOI: 10.1128/AAC.00126-09
2. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-beta-lactamase gene, bla_{NDM-1}, and a novel esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;50:5046–54. DOI: 10.1128/AAC.00774-09
3. Kumarasamy K, Toleman M, Walsh T, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10:597–602. DOI: 10.1016/S1473-3099(10)70143-2
4. Kumarasamy K, Thirunarayan MA, Krishnan P. Coexistence of bla_{OXA-23} with bla_{NDM-1} and *armA* in clinical isolates of *Acinetobacter baumanii* from India. J Antimicrob Chemother. 2010;65:2253–4. DOI: 10.1093/jac/dkq273
5. Health Protection Agency. Multi-resistant hospital bacteria linked to India and Pakistan. Health Protection Report 2009;3:3–4 [cited 2010 Jul 14]. http://www.hpa.org.uk/hpr/archives/2009/hpr2609.pdf
6. Centers for Disease Control and Prevention. Detection of *Enterobacteriaceae* isolates carrying metallo-beta-lactamase—United States, 2010. MMWR Morb Mortal Wkly Rep. 2010;59:750.
7. Gibb PA, Tribuddharat C, Moore RA, Louie TJ, Krulicki W, Livermore DM, et al. Nosocomial outbreak of carbapenem-resistant *Pseudomonas aeruginosa* with a new bla_{IMP} allele, bla_{IMP7}. Antimicrob Agents Chemother. 2010;202:255–8.
8. Pitout J, Chow BL, Gregson DB, Laupland KB, Elsayed S, Church DL. Molecular epidemiology of metallo-beta-lactamase–producing *Pseudomonas aeruginosa* in the Calgary Health Region: emergence of VIM-2-producing isolates. J Clin Microbiol. 2007;45:294–8. DOI: 10.1128/JCM.01694-06
9. Carrer A, Poirel L, Pitout JD, Church D. Occurrence of an SME-2-producing *Serratia marcescens* isolate in Canada. Int J Antimicrob Agents. 2008;31:175–87. DOI: 10.1016/j.ijantimicag.2007.10.007
10. Goldfarb D, Harvey S-B, Jessamine K, Jessamine P, Toye B, Desjardins M. Detection of plasmid-mediated KPC-producing *Klebsiella pneumoniae* in Ottawa, Canada: evidence of intrahospital transmission. J Clin Microbiol. 2009;47:1920–2. DOI: 10.1128/JCM.00098-09
11. Diancourt L, Passet V, Verhoef J, Grimont P, Brisse S. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J Clin Microbiol. 2005;43:4178–82. DOI: 10.1128/JCM.43.8.4178-4182.2005
12. 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. DOI: 10.1111/j.1365-2958.2006.05172.x
13. Coque T, Novais A, 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. DOI: 10.3201/eid1402.070350
14. Caratolli A, Bertini A, Villa L, Falbo V, Hopkins K, Threlfall E. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods. 2005;63:219–28. DOI: 10.1016/j.mimet.2005.03.018
15. Mulvey MR, Susky E, McCracken M, Morck DW, Read RR. Similar cefoxitin-resistance plasmids circulating in *Escherichia coli* from human and animal sources. Vet Microbiol. 2009;134:279–87. DOI: 10.1016/j.vetmic.2008.08.018

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Seasonal Influenza A (H1N1) Infection in Early Pregnancy and Second Trimester Fetal Demise

Richard W. Lieberman, Natasha Bagdasarian, Dafydd Thomas, and Cosmas Van De Ven

A second trimester fetal demise followed influenza-like illness in early pregnancy. Influenza A virus (H1N1) was identified in maternal and fetal tissue, confirming transplacental passage. These findings suggested a causal relationship between early exposure and fetal demise. Management of future influenza outbreaks should include evaluation of products of conception associated with fetal loss.

Increased maternal illness and death appeared in early reports of influenza A pandemic (H1N1) 2009. Three pregnancy-related complications associated with pandemic (H1N1) 2009 were reported in May 2009: 1 postpartum maternal death, 1 preterm birth, and 1 early second trimester (weeks 14–20) loss (*1*). In April 2010, a summary of pandemic (H1N1) 2009 among 788 pregnant women demonstrated a disproportionately high risk for death; the rate of spontaneous miscarriage was 1.4%, but details were not provided (*2*). Correlating influenza exposure to pregnancy loss is not straightforward because first trimester miscarriage is common, second trimester loss before 24 weeks is not well studied (*3*), and viral identification in products of conception has rarely been attempted. We report a second trimester fetal demise that occurred after exposure to seasonal influenza A virus (H1N1) early in pregnancy.

The Case

In 2008, a 30-year-old primigravida physician experienced intrauterine fetal demise (IUFD) at 20 weeks' gestation, as established by last menstrual period and confirmed by ultrasound at 8 weeks. The patient spent most of her pregnancy providing direct patient care. In October 2007, during gestational weeks 2–6, she was exposed to at least 2 patients with presumed influenza. She reported symptoms of fever, myalgia, headache, and cough during gestational week 4. She had no testing for influenza, did not receive antiviral therapy, and took acetaminophen; her symptoms resolved. She received the killed seasonal influenza vaccine at gestational week 6. An ultrasound at 18 weeks demonstrated early growth restriction and oligohydramnios with normal appearing fetal kidneys and head. IUFD was noted at 20 weeks.

The patient had an unremarkable medical history, did not smoke, and was normotensive throughout pregnancy. Antibodies to cytomegalovirus and toxoplasmosis were not detected.

The immature placenta showed no visible abnormalities. Microscopically, histiocytes were abundant in the maternal space (chronic intervillositis) and were noted within the fetal chorionic villi (Hofbauer cells; Figure 1, panel A). Histologic analysis of fetal autopsy specimens showed scattered luminal histiocytes of the lung and gut. No other

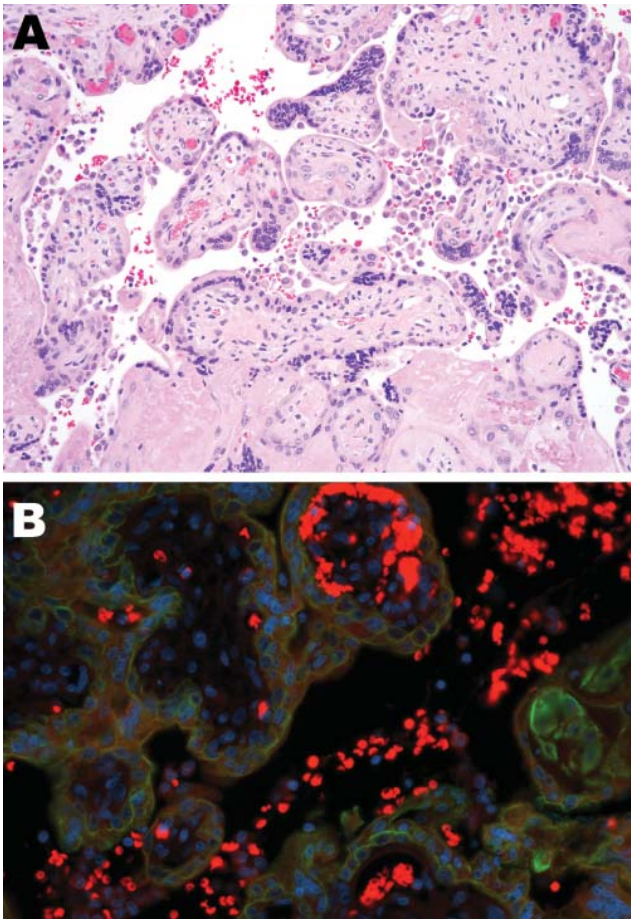

Figure 1. Tissue sample from 30-year-old primigravida patient exposed to seasonal influenza (H1N1). A) Intervillous (maternal) spaces with clusters/sheets of histiocytes (chronic intervillositis) and fibrotic fetal chorionic villi with Hofbauer cells–histiocytic inflammation (hematoxylin and eosin stain, original magnification ×200). B) Dual-stained immunofluorescent assay showing antibodies to influenza A virus (H1N1) (tetramethylrhodamine isothiocyanate, red) and cytokeratin (fluorescein isothiocyanate, green) in intravillous (fetal) and intervillous (maternal) space.

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inflammatory changes or viral inclusions were identified in the placenta or fetal organs.

Chronic intervillositis was evident in the placental histology and has been previously described with nonspecific changes in miscarriage and recurrent pregnancy loss (*4*). Formalin-fixed placental tissue was imaged with electron microscopy. Histiocytes identified from the maternal intervillous space and fetal chorionic villi demonstrated characteristics of viral production (Figure 2). Several well-formed viral capsids were noted within the cytoplasm, each containing regularly spaced projections along the surface of the virion corresponding to the hemagglutinin and neuraminidase spikes.

PCR for M1 capsid protein confirmed influenza A. Immunohistochemical testing was performed on tissue from the placenta and on a sampling of fetal organs by using influenza A virus (H1N1)–specific antibody. In the placenta, stippled immunofluorescence (Figure 1, panel B) was noted in the histiocytes of the intervillous space (maternal) and fetal intravillous histiocytes. In the fetal organs, immunofluorescence for subtype H1N1 was notable in the surface epithelial cells and scattered luminal histiocytes of the fetal respiratory and gastrointestinal tracts. No immunofluorescence was noted within the fetal organs, which implied transamnionic passage of virus (amnionic fluid infection). The fetus was XY karyotype. Y-specific immunofluorescent stain with histiocyte counterstaining was performed. Because only the intravillous (fetal) histiocytes stained positively, maternal and fetal inflammatory responses were confirmed.

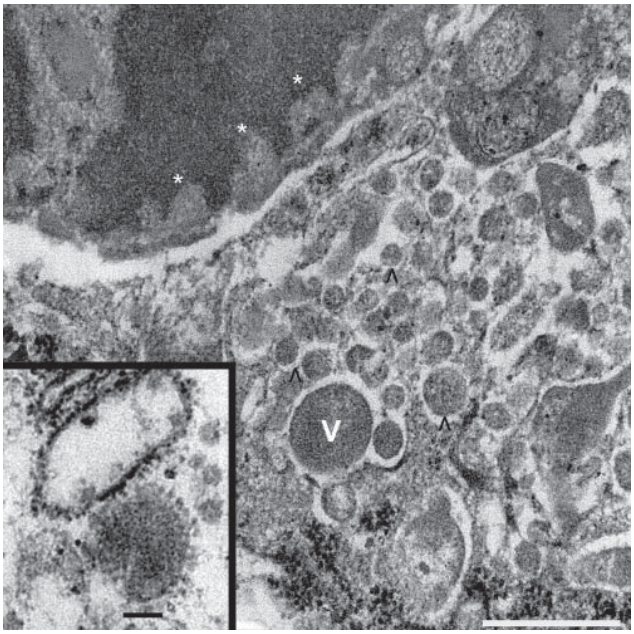

Figure 2. Tissue sample from 30-year-old primigravida patient exposed to seasonal influenza (H1N1). Electron microscopy (original magnification ×70,000) of maternal intervillous space and fetal chorionic villi, showing intranuclear viral transcription aligning along the nuclear envelope–electron hypodensities (asterisks), and intracytoplasmic viral production in varying stages shown by numerous electron densities (V and ^). Scale bar = 500 nm. Inset: enlarged image of mature virion with capsular projections. Ultrastructural features are characteristic of influenza viruses. Scale bar = 100 nm.

Conclusions

Harris reported higher rates of miscarriage after exposure and infection in the earliest months of pregnancy in the Spanish influenza outbreak of 1918 (subtype H1N1) (*5*). Hardy et al. reported a similar observation following the Asian influenza outbreak of 1957–58 (*6*), as did investigators in the United Kingdom during the 1985–86 influenza season (*7*).

Several case reports document transplacental transfer of influenza virus. Yawn et al. documented influenza A transplacental transfer from the mother to amniotic fluid and fetal heart (*8*). Mel'nikova et al. summarized placental findings associated with influenza infections in publications from 1987 and 1994, describing perivillous and villous inflammation with concurrent amniotic fluid immunofluorescence for influenza virus (*9*). In another study (*10*), researchers from Beijing, People's Republic of China, reported autopsy findings for 2 patients (1 of whom was 4 months pregnant) who died of avian influenza A (H5N1). Viral antigens were present in the maternal pulmonary system, and virus was detected in placental Hofbauer cells (histiocytes), cytotrophoblast, fetal lung, circulating mononuclear cells, and liver macrophages (*10*). Their documentation of tropism of the influenza virus to placental tissue is similar to the histopathologic features in our report. Our unexpected discovery of viral production in placental, maternal, and fetal histiocytes is another example of transplacental passage of influenza.

Miscarriage or completion of pregnancy may follow nonfatal maternal influenza infection. Adverse fetal outcomes have reportedly included congenital malformation and schizophrenia, but factors that determine these effects remain unclear (*11*,*12*). Fetal consequences of influenza exposure may be an effect of systemic inflammatory response to the infection, which could represent direct action of the virus on the placenta, the maternal–fetal interface, or the fetus. Uchide et al. reported the inflammatory response of decidual and fetal tissue in the presence of influenza virus infection and demonstrated expression of mRNA for a set of proinflammatory cytokines such as interleukin-6, tumor necrosis factor-α, and granulocyte–macrophage colony-stimulating factor, secreted in substantial amounts in response to exposure to influenza virus in vitro (*13*). As our case suggests, a complex interaction of maternal and fetal responses at the interface is a more likely explanation.

A total of 15%–20% of pregnancies end in spontaneous miscarriage, most before gestational week 12 (*3*). Approximately 50% of miscarriages are associated with chromosomal defects, which leaves many other miscarriages unexplained (*14*). Redline et al. examined first trimester miscarriages not associated with chromosomal abnormality, observing histologic changes of chronic intervillositis and increased perivillous fibrin (*15*). The mechanism of loss was thought to be related to a maternal inflammatory response to infectious antigen or an autoimmune phenomenon. Whether these findings can be extrapolated to second trimester losses is unknown. Second trimester miscarriages account for only 1%–2% of all pregnancy losses (*3*). Histopathologic features of these early second trimester miscarriages are not well studied, and descriptions are usually included in reports of fetal losses during 14–27 weeks, which include IUFD (>20 weeks) and miscarriage. Consequently, a direct causal relationship between influenza A and fetal demise has not been well established.

In our report, a retrospective investigation of the patient's products of conception with ultrastructural, immunohistochemical, and molecular methods that identified subtype H1N1 implies an exposure during her first trimester. Type-specific serologic analysis was not possible because she received influenza vaccine several weeks after presumed exposure. The maternal and fetal inflammatory responses (chronic intervillositis) after placental infection may have reduced villous surface area for oxygen and nutrient transport, resulting in growth restriction and ultimately second trimester loss.

Investigation of the effects of influenza in early pregnancy is needed to evaluate potential pathophysiological relationships between maternal exposure, infection, and fetal loss or adverse outcome. During the 2009–10 influenza pandemic, the Centers for Disease Control and Prevention (Atlanta, GA, USA) collected information from populations exposed to and infected with pandemic (H1N1) 2009 virus. Products of conception were not included among the samples cataloged. We recommend that future influenza outbreak evaluations of women exposed to influenza with first or early second trimester pregnancy loss include collections of placental and fetal tissue samples.

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References

1. Centers for Disease Control and Prevention. Novel influenza A (H1N1) virus infections in three pregnant women—United States, April–May 2009. MMWR Morb Mortal Wkly Rep. 2009;58:497–500. Erratum in MMWR Morb Mortal Wkly Rep. 2009;58:541.
2. Siston AM, Rasmussen SA, Honein MA, Fry AM, Seib K, Callaghan WM, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. JAMA. 2010; 303:1517–25.
3. Goldenberg RL, Culhane JF, Johnson DC. Maternal infection and adverse fetal and neonatal outcomes. Clin Perinatol. 2005;32:523–59. DOI: 10.1016/j.clp.2005.04.006
4. Redline RW. Placental pathology: a systematic approach with clinical correlations. Placenta. 2008;29(Suppl A):S86–91.
5. Harris JW. Influenza occurring in pregnant women: a statistical study of 1350 cases. J Am Med Assoc. 1919;72:970–2.
6. Hardy JM, Azarowicz EN, Mannini A, Donald N, Medearis J. The effect of Asian influenza on the outcome of pregnancy, Baltimore, 1957–1958. Am J Public Health Nations Health. 1961;51:1182–8. DOI: 10.2105/AJPH.51.8.1182
7. Stanwell-Smith R, Parker AM, Chakraverty P, Soltanpoor N, Simpson CN. Possible association of influenza A with fetal loss: investigation of a cluster of spontaneous abortions and stillbirths. Commun Dis Rep CDR Rev. 1994;4:R28–32.
8. Yawn DH, Pyeatte JC, Joseph JM, Eichler SL, Garcia-Bunuel R. Transplacental transfer of influenza virus. JAMA. 1971;216:1022–3. DOI: 10.1001/jama.216.6.1022
9. Mel'nikova VF, Tsinzerling VA, Aksenov OA, Tsinzerling AV. Chronic course of influenza with extrapulmonary involvement [in Russian]. Arkh Patol. 1994;56:33–8.
10. Gu J, Xie Z, Gao Z, Liu J, Korteweg C, Ye J, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study [comment]. Lancet. 2007;370:1137–45. DOI: 10.1016/S0140-6736-(07)61515-3
11. Acs N, Banhidy F, Puho E, Czeizel AE. Maternal influenza during pregnancy and risk of congenital abnormalities in offspring. Birth Defects Res Part A Clin Mol Teratol. 2005;73:989–96.
12. Selten JP, Frissen A, Lensvelt-Mulders G, Morgan VA, Selten J-P, Frissen A, et al. Schizophrenia and 1957 pandemic of influenza: meta-analysis. Schizophr Bull. 2010;36:219–28. DOI: 10.1093/schbul/sbp147
13. Uchide N, Ohyama K, Bessho T, Toyoda H. Induction of pro-inflammatory cytokine gene expression and apoptosis in human chorion cells of fetal membranes by influenza virus infection: possible implications for maintenance and interruption of pregnancy during infection. Med Sci Monit. 2005;11:RA7–16.
14. Goddijn M, Leschot NJ. Genetic aspects of miscarriage. Baillieres Best Pract Res Clin Obstet Gynaecol. 2000;14:855–65. DOI: 10.1053/beog.2000.0124
15. Redline RW, Zaragoza M, Hassold T. Prevalence of developmental and inflammatory lesions in nonmolar first-trimester spontaneous abortions. Hum Pathol. 1999;30:93–100. DOI: 10.1016/S0046-8177(99)90307-6

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Possible Interruption of Measles Virus Transmission, Uganda, 2006–2009

Frederick N. Baliraine, Josephine Bwogi, Henry Bukenya, Ronald Seguya, Theopista Kabaliisa, Annet Kisakye, William B. Mbabazi, and Sheilagh B. Smit

To determine what measles virus genotype(s) circulated in Uganda after strategic interventions aimed at controlling/eliminating measles, we examined samples obtained during 2006–2009 and found only genotype B3.1, which had not been previously detected. Kenya was the likely source, but other countries cannot be excluded.

In October 2002, Uganda implemented a 5-year (2002–2006) accelerated measles control strategy that began with a vigorous attempt to interrupt all chains of measles transmission by using a 5-day countrywide vaccination campaign. This brisk catch-up campaign was preceded by vaccine potency tests; meticulous planning to ensure political, religious, and tribal leaders' support; spirited social mobilization; training of health care workers and volunteers; and adequate provision of vaccination and cold chain materials at all vaccination posts, some of which were improvised structures (e.g., tents, schools, or under trees) for easy access. Community education was particularly vital to dispel commonly held myths that vaccinating children against measles or taking children having measles to the hospital (i.e., using foreign medicine) increases the risk for death. About 13.5 million (≈0.5 million above target) children ages 6–168 months were vaccinated, giving a national measles vaccine coverage rate of 104% (*1*). This was followed by keep-up campaigns in 15 high-risk districts for children ages 6–23 months in February 2005 and in April 2005 for all previously unvaccinated children ages 9–59 months. Uganda was virtually measles free in 2003–2005, but outbreaks resurfaced in 2006. Subsequently, nationwide follow-up supplemental measles vaccination campaigns were conducted for children ages 6–59 months during August–November 2006 (*1*) and for children ages 9–47 months in June 2009.

Virologic surveillance before initiating accelerated measles control activities enables genotypes to be cataloged in a country both before and after vaccination campaigns, which together with standard epidemiologic data can help detect imported viruses and evaluate control strategies (*2*). In Uganda, measles virus isolation began in 2000 (*3*). Our study sought to determine the measles virus genotype(s) circulating in Uganda after strategic interventions aimed at controlling/eliminating measles in the country were implemented.

The Study

As part of routine measles case investigations, urine samples and throat swab specimens for virus isolation were collected along with serum samples (0–12 days after rash onset) from patients across Uganda during 2006 through 2009. Infections were confirmed serologically or by virus isolation (*4*,*5*). Of the serum samples tested, 1,053 (15%) of 6,999 were positive for measles immunoglobulin (Ig) M; most were collected during 2006 (Figure 1). Twenty-two isolates (37%) were obtained from 59 samples from patients who had IgM against measles virus; 1 isolate was obtained from a patient who did not have IgM against measles virus; and another isolate was obtained from a patient who did not have serologic testing. Virus isolation was successful only in specimens collected within 5 days of rash onset (Table).

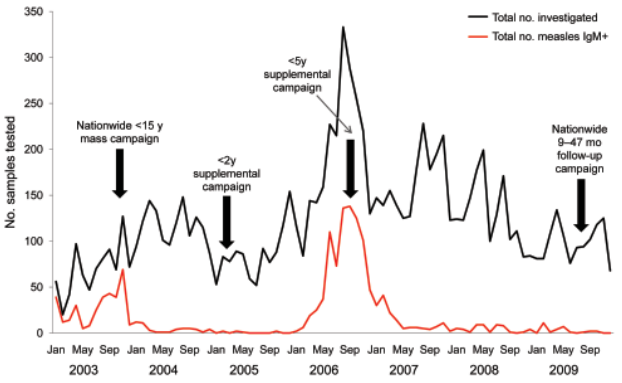

Figure 1. Laboratory-confirmed measles cases in Uganda, 2006–2009. Data from the accelerated measles control period 2003–2005 are included for comparison. The surge in measles cases during 2006 was caused by a resumption of measles outbreaks after a 3-year lag period, due to an accumulated number of susceptible persons (*1*).

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Table. Epidemiologic data associated with measles virus isolates analyzed, Uganda, 2006–2009*

Isolate name	District	Patient age, mo	Measles vaccine doses	Vaccination card seen	Date of onset	Interval, d†	Measles IgM	GenBank accession no.	Identity‡
MVi/Bushenyi.UGA/43.06	Bushenyi	19	1	No	2006 Oct	4	Pos	GU952229	A
MVi/Hoima.UGA/7.09/1	Hoima	36	X	No	2009 Feb	5	Pos	GU952246	B
MVi/Hoima.UGA/7.09/2	Hoima	36	X	No	2009 Feb	2	Neg		B
MVi/Kampala.UGA/26.06/1	Kampala	30	X	No	2006 Jun	1	Pos	GU952239	A
MVi/Kampala.UGA/26.06/2	Kampala	36	X	No	2006 Jun	4	Pos		A
MVi/Kampala.UGA/26.06/3	Kampala	18	0	No	2006 Jun	2	ND	GU952237	C
MVi/Kasese.UGA/7.07/1	Kasese	25	0	Yes	2007 Feb	2	Pos	GU952245	A
MVi/Kasese.UGA/7.07/2	Kasese	240	X	No	2007 Feb	3	Pos		A
MVi/Kitgum.UGA/28.06	Kitgum	60	2	No	2006 Jul	3	Pos	GU952235	A
MVi/Mukono.UGA/26.06	Mukono	96	0	No	2006 Jun	1	Pos	GU952238	D
MVi/Mukono.UGA/29.06	Mukono	276	X	No	2006 Jul	3	Pos	GU952233	E
MVi/Mukono.UGA/47.06	Mukono	48	1	Yes	2006 Nov	1	Pos	GU952243	F
MVi/Wakiso.UGA/26.06	Wakiso	36	2	No	2006 Jun	1	Pos	GU952240	A
MVi/Wakiso.UGA/27.06	Wakiso	96	0	No	2006 Jul	1	Pos	GU952236	C
MVi/Wakiso.UGA/29.06	Wakiso	33	0	Yes	2006 Jul	1	Pos	GU952234	A
MVi/Wakiso.UGA/31.06/1	Wakiso	19	1	No	2006 Jul	1	Pos	GU952232	G
MVi/Wakiso.UGA/31.06/2	Wakiso	25	0	No	2006 Aug	2	Pos	GU952231	A
MVi/Wakiso.UGA/32.06	Wakiso	11	X	No	2006 Aug	1	Pos	GU952230	A
MVi/Wakiso.UGA/41.06	Wakiso	27	0	No	2006 Oct	1	Pos	GU952241	A
MVi/Wakiso.UGA/45.06	Wakiso	120	2	No	2006 Nov	2	Pos	GU952242	H
MVi/Wakiso.UGA/49.06	Wakiso	216	1	No	2006 Dec	2	Pos	GU952244	A

*All were genotype B3.1. IgM, immunoglobulin M; pos, positive; neg, negative; X, unknown; ND, not done.
†Time between rash onset and sample collection.
‡Nucleotide sequences sharing the same letter are identical.

All isolates belonged to genotype B3.1 (Figure 2), which had not been previously detected in Uganda (*3*). Twelve (57%) of 21 sequences obtained were identical (Table) and also identical to isolates from the 2005 measles outbreak in Kenya (Figure 2). However, 9 (43%) of 21 showed neither 100% similarity with the other 12 Ugandan isolates (Table) nor with any other isolate available in GenBank.

Since the inception of Uganda's 2002–2006 accelerated measles control strategic plan, the number of measles cases in the country has declined dramatically (Figure 1). After the 2003 campaign, virtually no cases of measles occurred in Uganda for 3 years, until the outbreaks in 2006 (*1*). Our data show that 819 (78%) of 1,053 of the serologically confirmed measles cases for the 4-year surveillance period occurred during the 2006 outbreaks (Figure 1), confirming that the strategic interventions quickly subdued the 2006 transmission cycles. Moreover, the pattern of measles genotypes detected from 2000–2009 (2006–2009 reported in this study) suggests that transmission of the previously endemic genotype D10 in Uganda (*3*) has been interrupted and replaced by genotype B3.1. However, since molecular surveillance in Uganda only began 10 years ago (*3*), we cannot ascertain which genotypes were circulating in the country before 2000. By 2001, B3.1 was geographically restricted to the western and central African countries of Ghana, Nigeria, Cameroon, and Sudan (*6*,*7*); however, by the end of 2005, it had spread to Kenya, supposedly from Nigeria, where it caused a massive epidemic that was linked to subsequent infections in Europe and the Americas (*8*).

The fact that most isolates from Uganda were identical to those previously identified in neighboring Kenya indicates that Kenya was the most likely immediate source of the B3.1 viruses presently circulating in Uganda. However, the contribution of other African countries cannot be excluded, because molecular surveillance is still largely lacking in Africa (*5*).

Only 14 (23%) of 61 patients with laboratory-confirmed measles (whose specimens were screened for measles virus isolates) had been vaccinated, demonstrating a gap in vaccination coverage and possible vaccination failure in some cases and the need for timely catch-up vaccination campaigns even in the low-risk districts. In Uganda, routine childhood vaccination against measles began in 1983, but vaccine coverage was initially too low to interrupt indigenous transmission. The situation was not helped by the rampant poverty, limited health-care infrastructure, internal and external conflicts that have rocked the Great Lakes Region during the past 3 decades, high vaccination drop-out rates, and a high birth rate (second highest in the world, after Niger [*9*]). These conditions are typical in countries where vaccine-preventable infections remain a major problem (*10*). Nevertheless, education of Ugandan health workers, politicians, and development partners has been ongoing. This strategy has aroused strong interest and support for disease surveillance and

control activities from the political leadership, resulting in the creation of a special budget line for surveillance (*11*). Since 2003, routine vaccination has been strengthened by extending primary health care grants to all districts, and the implementation of the "Reaching Every District" strategy (*12*), among other interventions. Consequently, Uganda has recorded a tremendous rise in vaccination coverage, from 64% in 1997 to ≥85% by 2007 (*1*). This aggressive vaccination effort has been instrumental in interrupting indigenous measles strain transmission in the country.

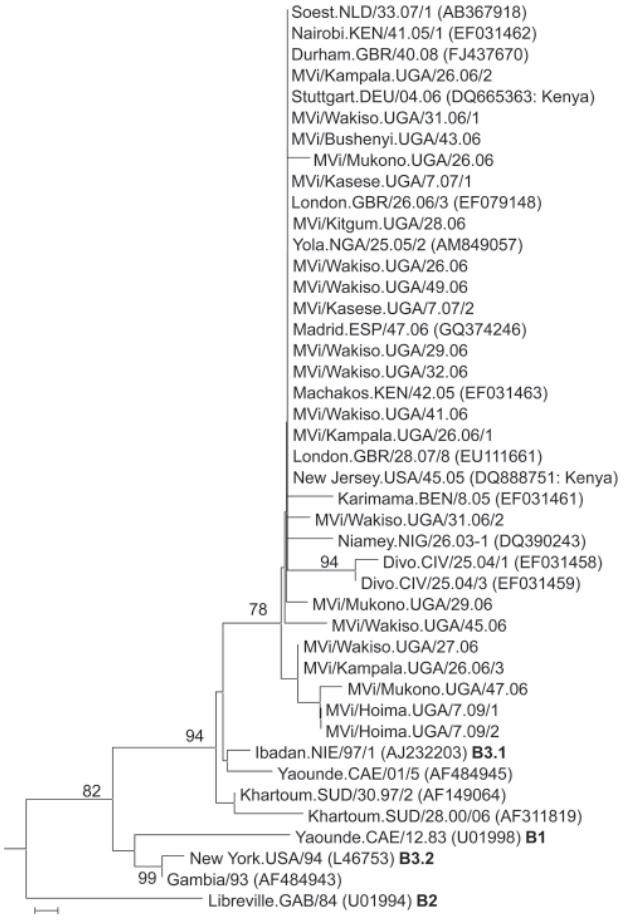

Figure 2. Phylogenetic analysis of the relationship between sequences of 21 Ugandan measles virus isolates obtained during 2006–2009 and 22 other recently described clade B nucleoprotein (N) gene sequences, including the World Health Organization reference strains for the B clade (*13*). **Boldface** indicates different genotypes. Analyses are based on sequences of the 450 nt encoding the COOH-terminal 150 nt of the N gene. The unrooted neighbor-joining consensus tree was generated by bootstrap analysis of 500 replicates by using MEGA4 software (www.megasoftware.net). Bootstrap percentages are shown when ≥75%. Only names of the isolates from Uganda (UGA) start with "MVi," and all comparison strains have their GenBank accession numbers indicated in parentheses. Genotypes of the World Health Organization reference sequences are indicated after the accession number. Comparison sequences were from viruses isolated in Benin (BEN), Cameroon (CAE), Côte d'Ivoire (CIV), Gabon (GAB), the Gambia, Germany (DEU), Great Britain (GBR), Kenya (KEN), Niger (NIG), Nigeria (NIE/NGA), Spain (ESP), Sudan (SUD), the Netherlands (NLD), and the United States (USA). Sequences from Uganda were most closely related to the B3.1 viruses of the September–December 2005 measles outbreak in Kenya (*8*). Scale bar indicates nucleotide substitutions per site.

All virus isolates from Uganda during 2000–2002, before accelerated measles control, belonged to genotype D10 (*3*). At that time, viruses isolated in countries bordering Uganda, such as Kenya, Sudan, and the Democratic Republic of the Congo, belonged to genotypes D4, B3, and B2 (*13*). However, without enhanced regional surveillance, should genotype D10 be isolated again, it would be difficult to determine whether it had truly been interrupted, without knowing if D10 was presently circulating in other African countries, which could serve as bases from which to reintroduce it into Uganda.

Conclusions

Our results show that, even under difficult circumstances (e.g., poverty), optimal resource allocation and mobilization of political will can interrupt vaccine-preventable diseases in Africa. These data provide molecular evidence that Uganda's 2002–2006 vaccination strategy was successful in interrupting indigenous measles transmission, but immunity gaps in the population allowed the establishment of an imported virus that was previously confined to western and central Africa. If national immunization programs across the region synchronized their vaccination strategies to eliminate sources of reintroduction, measles could be quickly eliminated from the entire continent. Vaccination success stories have already been noted in several African countries with routine coverage >80% (*14*,*15*). Therefore, continued education and cooperation are needed between countries, national policy makers, health care workers, and local communities throughout the continent to win the fight against measles.

Acknowledgments

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References

1. Mbabazi WB, Nanyunja M, Makumbi I, Braka F, Baliraine FN, Kisakye A, et al. Achieving measles control: lessons from the 2002–06 measles control strategy for Uganda. Health Policy Plan. 2009;24:261–9. DOI: 10.1093/heapol/czp008
2. Rota PA, Featherstone DA, Bellini WJ. Molecular epidemiology of measles virus. In: Griffin DE, Oldstone MBA, editors. Measles: pathogenesis and control. Berlin: Springer-Verlag; 2009. p. 129–50.
3. Muwonge A, Nanyunja M, Rota PA, Bwogi J, Lowe L, Liffick SL, et al. New measles genotype, Uganda. Emerg Infect Dis. 2005;11:1522–6.
4. World Health Organization. Manual for the laboratory diagnosis of measles and rubella virus infection. 2nd ed. Geneva (Switzerland); The Organization; 2007.
5. Smit SB, Hardie D, Tiemessen CT. Measles virus genotype B2 is not inactive: evidence of continued circulation in Africa. J Med Virol. 2005;77:550–7. DOI: 10.1002/jmv.20491
6. Kouomou DW, Nerrienet E, Mfoupouendoun J, Tene G, Whittle H, Wild TF. Measles virus strains circulating in central and west Africa: geographical distribution of two B3 genotypes. J Med Virol. 2002;68:433–40. DOI: 10.1002/jmv.10222
7. El Mubarak HS, van de Bildt MW, Mustafa OA, Vos HW, Mukhtar MM, Ibrahim SA, et al. Genetic characterization of wild-type measles viruses circulating in suburban Khartoum, 1997–2000. J Gen Virol. 2002;83:1437–43.
8. Rota J, Lowe L, Rota P, Bellini W, Redd S, Dayan G, et al. Identical genotype B3 sequences from measles patients in 4 countries, 2005. Emerg Infect Dis. 2006;12:1779–81.
9. Central Intelligence Agency. The world fact book: Uganda [cited 2010 Aug 18]. https://www.cia.gov/library/publications/the-world-factbook/geos/ug.html
10. Ferrari MJ, Grais RF, Bharti N, Conlan AJ, Bjornstad ON, Wolfson LJ, et al. The dynamics of measles in sub-Saharan Africa. Nature. 2008;451:679–84. DOI: 10.1038/nature06509
11. World Health Organization. Implementing intregrated disease surveillance and response. Wkly Epidemiol Rec. 2003;78:233–40.
12. World Health Organization. The RED strategy [cited 2010 May 13]. http://www.who.int/immunization_delivery/systems_policy/red/en
13. World Health Organization. New genotype of measles virus and update on global distribution of measles genotypes. Wkly Epidemiol Rec. 2005;80:348–51.
14. Biellik R, Madema S, Taole A, Kutsulukuta A, Allies E, Eggers R, et al. First 5 years of measles elimination in southern Africa: 1996–2000. Lancet. 2002;359:1564–8. DOI: 10.1016/S0140-6736-(02)08517-3
15. Otten MW Jr, Okwo-Bele JM, Kezaala R, Biellik R, Eggers R, Nshimirimana D. Impact of alternative approaches to accelerated measles control: experience in the African region, 1996–2002. J Infect Dis. 2003;187:S36–43. DOI: 10.1086/368272

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Babesiosis in Immunocompetent Patients, Europe

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We report 2 cases of babesiosis in immunocompetent patients in France. A severe influenza-like disease developed in both patients 2 weeks after they had been bitten by ticks. Diagnosis was obtained from blood smears, and *Babesia divergens* was identified by PCR in 1 case. Babesiosis in Europe occurs in healthy patients, not only in splenectomized patients.

Babesiosis, a tick-borne infectious disease that occurs worldwide, is caused by species of *Babesia*, an intraerythrocytic parasite (*1*). *Babesia* spp. parasites infect wild and domesticated animals and may cause a malaria-like syndrome. The first human case was described in 1957 in a splenectomized Yugoslavian farmer who died (*2*). More than 100 *Babesia* species infect animals, but human infection has been associated with only a few species, mainly *B. microti* and *B. divergens* (*1–3*). *B. microti* parasites are transmitted by *Ixodes scapularis* ticks and infect rodents. Since 1957, these parasites have caused hundreds of human babesiosis cases in the United States, the most affected country. Infections are found mainly in healthy persons and manifest as asymptomatic or mild to moderate illness; severe disease, even in immunocompromised or elderly patients, is seldom reported (*2,3*). *B. divergens* parasites are endemic to Europe; they are transmitted by *I. ricinus* ticks and infect bovines (*4*). In Europe, the disease is rare in humans; ≈40 cases have been reported (*2,3,5–7*). These cases are almost exclusively severe in immunocompromised patients, especially those whose spleens have been removed (*2,3,8*). *B. divergens* parasites are responsible for >70% of these cases (*2,8*), although the disease is not always confirmed by molecular-based methods.

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We report 2 cases of human babesiosis in Colmar, Alsace, a northeastern region of France in which Lyme disease is endemic. The disease was diagnosed in spring 2009 in healthy young persons without history of travel abroad who experienced a marked influenza-like syndrome and recovered. These cases should change the classic description of babesiosis in Europe, in which the disease was thought to affect immunocompromised patients exclusively. Our study indicates that this disease also occurs in Europe among immunocompetent patients.

Case Reports

Patient 1, a 37-year-old woman without known medical history, sought treatment on April 29, two weeks after a tick bite. She had a 38.5°C fever with chills, headaches, and arthromyalgia. Results of a physical examination were normal. Laboratory findings included leukopenia (3,300 leukocytes/μL, 45% polymorphonuclear cells, 37% lymphocytes), aspartate aminotransferase and alanine aminotransferase levels of 136 IU/L and 160 IU/L, respectively; γ-glutamyl transpeptidase 135 IU/L; alkaline phosphatase 131 IU/L; and elevated C-reactive protein level (48 mg/L). Serologic results for Lyme disease, tick-borne encephalitis virus, tularemia, *Anaplasma* spp., *Coxiella burnetii*, and *Rickettsia* spp., as well as blood cultures were negative.

A thin peripheral blood smear stained with May-Grünwald-Giemsa did not show any ehrlichial morulae in granulocytes, as suspected, but a retrospective examination of stored slides on May 22 (the same day that case 2 was characterized) showed pear forms and trophozoites of intraerythrocytic parasites (parasitemia level 0.29%), leading to the diagnosis of babesiosis (Figure 1). The patient had initially received doxycycline (200 mg/d) for a suspected bacterial tick-borne infection, and her symptoms rapidly resolved. The first blood sample was discarded, but on June 16 and June 24, additional serum and whole blood samples were collected in sodium citrate vacutainer tubes (Becton-Dickinson, Franklin Lakes, NJ, USA). The blood smears remained positive until July but were negative in August.

Patient 2, a 35-year-old man with an uneventful medical history, was hospitalized on May 21, two weeks after receiving 3 tick bites. He had a 39°C fever, severe headaches, and arthromyalgia. Results of a physical examination were normal. Laboratory findings showed leukopenia (1,860 leukocytes/μL, with 35% polymorphonuclear leukocytes, 49% lymphocytes), marked thrombopenia with 36,000 platelets/mm^3, elevated liver enzyme levels (aspartate aminotransferase 70 IU/L, alanine aminotransferase 77 IU/L, γ-glutamyl transferase 161 IU/L, and alkaline phosphatase 86 IU/L), and elevated C-reactive protein (124 mg/L). Tick-transmitted disease serologic results and blood cultures were negative.

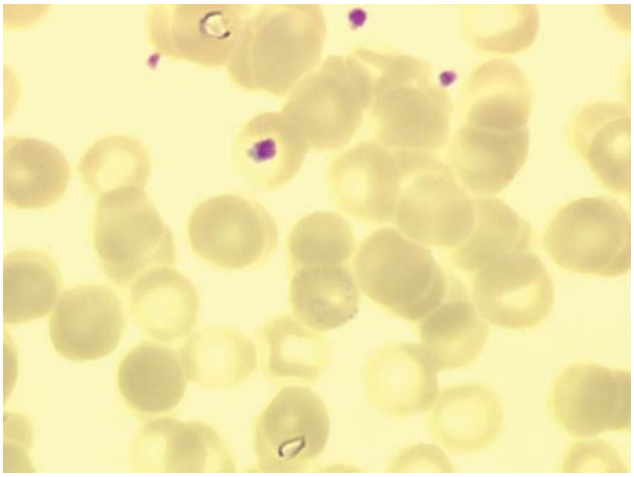

Figure 1. Two trophozoites, pear-shaped, of *Babesia divergens* in erythrocytes from case-patient 1 (original magnification ×1,000, May-Grünwald-Giemsa stain).

A thin peripheral blood smear stained with May-Grünwald-Giemsa showed intraerythrocytic *Babesia* spp. (parasitemia level 0.23%) (Figure 2). The patient received azithromycin 500 mg on day 1 then 250 mg/day plus atovaquone, and his illness rapidly resolved. Two samples of serum and whole blood were collected in sodium citrate vacutainer tubes on June 16 and July 21 and sent to the veterinary laboratory of Nantes for *B. divergens* serologic analysis (indirect immunofluorescent assay by using gerbil-derived strain *B. divergens* Rouen F5 antigen), erythrocyte cultures, and DNA extraction (Wizard genomic DNA Purification kit; Promega, Madison, WI, USA) for PCR *Babesia* spp. (*9*).

Serologic results and cultures remained negative for both patients. However, serologic analysis is neither sensitive nor specific (*7,10*), and cultures probably were inhibited because blood samples were collected after doxycycline or azithromycin proguanil treatments. The PCR for *Babesia* spp. is specific for an 18S rDNA 540-base long region of a variable part of the gene with Bab primers GF2 and GR2, was performed (*9,11*). Results were positive for patient 1. The sequencing of PCR products showed 100% homology with *B. divergens* human strains GenBank accession nos. FJ944822 and FJ944823 (*9*). PCR results were negative for patient 2. Samples from patient 2 were collected 1 month after treatment with atovaquone-proguanil, and the blood smear was negative. However, the clinical and biological data and the observation of trophozoites (especially 2 trophozoites in 1 erythrocyte) in the blood smear from patient 2 confirmed by a reference laboratory led us to strongly suspect babesiosis (Figure 2). In this case, the *Babesia* species remains unknown, and a non–*B. divergens* species cannot be ruled out, although it is rarer.

Conclusions

Our cases highlight that, in Europe, babesiosis can occur in healthy persons and manifest as moderate illness. The rarity of other reported cases in nonimmunocompromised patients in Europe may be related to the difficulty of diagnosing babesiosis. A stained thin blood smear is rarely performed in Europe after tick bite in healthy patients. The difficulty of detecting intra-erythrocytic forms of babesia coupled with frequent low levels of parasitemia, may result in accurate diagnoses, although acridine orange and fluorescent microscopy may assist in the detection of parasites (*1*). Other diagnostic tests, such as PCR and serologic analysis, are not routinely performed in France and require a reference laboratory (*8*).

Babesiosis, although difficult to diagnose, needs to be diagnosed for various reasons: 1) without treatment, babesiosis can lead to severe illness; 2) the disease can persist for a long period without symptoms, which could lead to posttransfusion cases (*12*); and 3) effective specific treatments are available (atovaquone plus azithromycin, or for severe cases, clindamycin and quinine) (*2*). These drugs are not usually prescribed in febrile tick-bite cases; doxycycline is the usual drug used to treat tick-borne bacterial diseases. Moreover, patients with moderate infection could benefit from an atovaquone plus azithromycin regimen, which is better tolerated (*13*).

Previous serosurveys from tick-exposed patients or healthy blood donors in France (*7*), Germany (*14*), and Switzerland (*15*) have demonstrated antibodies against *Babesia* spp. antigens ranging from 1.0% to 11.5%. These data suggest that *Babesia* spp. infections probably occur more frequently in Europe than previously believed and may affect healthy patients. Although most patients may be asymptomatic, our 2 cases demonstrate that babesiosis can result in a serious influenza-like syndrome in previously healthy

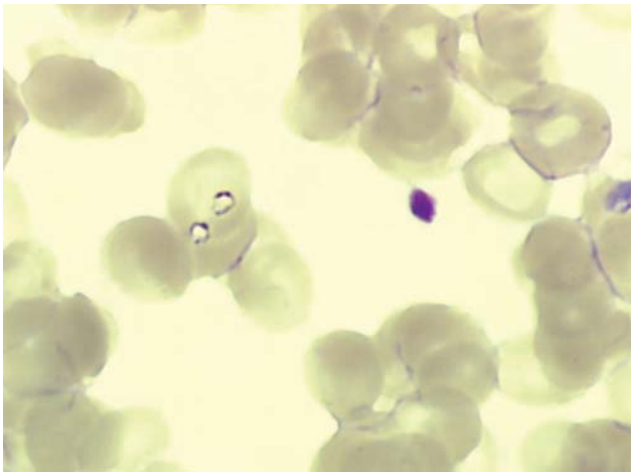

Figure 2. Two trophozoites of *Babesia* spp. in 1 erythrocyte from case-patient 2 (original magnification ×1,000, May-Grünwald-Giemsa stain).

patients. In Europe, babesiosis is probably underdiagnosed; thus, we suggest that when patients have influenza-like or malaria-like syndromes after confirmed or suspected tick bites, a blood smear be performed regardless of whether the patient is immunocompromised. Blood smear can identify not only *Babesia* spp. infection but also *Anaplasma* spp. infection, another emerging and underdiagnosed tick-borne illness. In cases of new European *Babesia* spp. infections, a deeper characterization of the strains by erythrocytes cultures and standardized PCR, as well as a systematic study of the patients' immune systems, should be undertaken to enable a better understanding of this disease.

Dr Martinot is a physician at the Department of Internal Medicine and Rheumatology, Hospital Pasteur, Colmar, France. His specialty is infectious diseases and primary research interests are tick-borne diseases, procalcitonin, and infections in immunocompromised patients (HIV).

References

1. Homer MJ, Persing DH. Human babesiosis. In: Goodman JL, Dennis DT, Sonenshine DE, editors. Tick-borne diseases of humans. Washington: ASM Press; 2005. p. 343–60.
2. Vannier E, Krause PJ. Update on babesiosis. Interdiscip Perspect Infect Dis. 2009;9845–68.
3. Gelfand JA, Vannier E. *Babesia* species. In: Mandell GL, Bennett JE, Dolin R, editors. Principles and practice of infectious diseases, 7th ed. Philadelphia: Elsevier; 2010. p. 3539–45.
4. L'Hostis M, Dumon H, Dorchies B, Boisdron F, Gorenflot A. Large scale survey of bovine babesiosis due to *Babesia divergens* in France. Vet Rec. 1995;136:36–8. DOI: 10.1136/vr.136.2.36
5. Hunfeld KP, Hildebrandt A, Gray JS. Babesiosis: recent insights into an ancient disease. Int J Parasitol. 2008;38:1219–37. DOI: 10.1016/j.ijpara.2008.03.001
6. Zintl A, Mulcahy G, Skerret HE, Taylor SM, Gray JS. *Babesia divergens*, a bovine blood parasite of veterinary and zoonotic importance. Clin Microbiol Rev. 2003;16:622–36. DOI: 10.1128/CMR.16.4.622-636.2003
7. Gorenflot A, Moubri K, Precigout E, Carcy B, Schetters TP. Human babesiosis. Ann Trop Med Parasitol. 1998;92:489–501.
8. Meliani P, Khatibi S, Randazzo S, Gorenflot A, Marchou B. Human babesiosis. Med Mal Infect. 2006;36:499–504. DOI: 10.1016/j.medmal.2006.07.002
9. Malandrin L, Jouglin M, Sun Y, Brisseau N, Chauvin A. Redescription of *Babesia capreoli* (Enigk and Friedhoff, 1962) from roe deer (*Capreolus capreolus*): isolation, cultivation, host specificity, molecular characterization and differentiation from *Babesia divergens*. Int J Parasitol. 2010;40:277–84. DOI: 10.1016/j.ijpara.2009.08.008
10. Rosenblatt JE. Laboratory diagnosis of infections due to blood and tissue parasites. Clin Infect Dis. 2009;49:1103–8. DOI: 10.1086/605574
11. Bonnet S, Jouglin M, L'hostis M, Chauvin A. *Babesia* sp. EU1 from roe deer and transmission within *Ixodes ricinus*. Emerg Infect Dis. 2007;13:1208–10.
12. Gubernot DM, Lucey CT, Lee KC, Conley GB, Holness LG, Wise RP. *Babesia* infection through blood transfusions: reports received by the US Food and Drug Administration. Clin Infect Dis. 2009;48:25–30. DOI: 10.1086/595010
13. Krause PJ, Lepore T, Sikand VK, Gadbaw J Jr, Burke G, Telford SR III, et al. Atovaquone and azithromycin for the treatment of babesiosis. N Engl J Med. 2000;343:1454–8. DOI: 10.1056/NEJM200011163432004
14. Hunfeld KP, Lambert A, Kampen H, Albert S, Epe C, Brade V, et al. Seroprevalence of *Babesia* infections in humans exposed to ticks in midwest Germany. J Clin Microbiol. 2002;40:2431–6. DOI: 10.1128/JCM.40.7.2431-2436.2002
15. Foppa IM, Krause PJ, Spielmann A, Goethert H, Gern L, Brand B, et al. Entomologic and serologic evidence of zoonotic transmission of *Babesia microti*, eastern Switzerland. Emerg Infect Dis. 2002;8:722–6.

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Echinostoma revolutum Infection in Children, Pursat Province, Cambodia

Woon-Mok Sohn, Jong-Yil Chai, Tai-Soon Yong, Keeseon S. Eom, Cheong-Ha Yoon, Muth Sinuon, Duong Socheat, and Soon-Hyung Lee

To determine the prevalence of helminthic infections in Pursat Province, Cambodia, we tested fecal specimens from 471 children, 10–14 years of age, in June 2007. The prevalence of infection with echinostome flukes ranged from 7.5% to 22.4% in 4 schools surveyed. Adult worms were identified as *Echinostoma revolutum.*

Echinostomes (family *Echinostomatidae*) are intestinal trematodes of birds and mammals, including humans. Echinostomiasis can result in severe epigastric or abdominal pain accompanied by diarrhea, easy fatigue, and malnutrition (*1*). Heavy worm loads may lead to death due to intestinal perforation or marked malnutrition and anemia, as has been reported for infection caused by an echinostome species, *Artyfechinostomum malayanum* (under the name *Artyfechinostomum mehrai*), in India (*1*).

A total of 20 species of echinostomes that belong to 8 genera (*Echinostoma*, *Echinochasmus*, *Acanthoparyphium*, *Artyfechinostomum*, *Episthmium*, *Himasthla*, *Hypoderaeum*, and *Isthmiophora*) infect humans worldwide (*1*). *Echinostoma revolutum*, the most widely distributed species, is found from Asia and Oceania to Europe and the Americas (*1*). The first reported human infection was in Taiwan in 1929 (*2*). The prevalence of *E. revolutum* flukes in Taiwan during 1929–1979 varied from 0.11% to 0.65% (*3*). Small *E. revolutum*–endemic foci or a few cases of human infection were discovered in the People's Republic of China, Indonesia, and Thailand until 1994 (*4,5*). However, no information is available about human *E. revolutum* infection after 1994, even in areas where the parasite was previously endemic.

In Cambodia, humans are commonly infected with intestinal nematodes and protozoa, including hookworms, *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Trichuris trichiura*, and *Giardia lamblia* (*6,7*). However, with the exception of the blood fluke *Schistosoma mekongi*, infection with trematodes or cestodes has seldom been reported (*8*). Echinostomatid eggs have been detected in schoolchildren in 2 provinces, Battambang and Kampongcham (*9,10*), but adult worms were not collected for identification. The Korea Association of Health Promotion, South Korea, and The National Institute of Malaria, Entomology, and Parasitology, Ministry of Health, Cambodia, have been conducting an international collaboration to control intestinal helminthiases in schoolchildren in Cambodia (2006–2011). In June 2007, we conducted a fecal survey in 4 primary schools in Pursat Province, Cambodia, and found that an average of 11.9% of schoolchildren had positive test results for echinostome eggs. Adult worms recovered after the children received treatment with praziquantel and underwent purgation with magnesium salts were identified as *E. revolutum*. We report echinostomiasis as an endemic trematode infection among schoolchildren in Pursat.

The Study

The surveyed areas were lakeside (the Tonle Sap Lake) villages in Pursat Province (Figure 1) where ≈12,000 persons, including 3,500 schoolchildren, live. For this study, 471 children (237 boys), 10–14 years of age, from 4 primary schools were selected. One fecal sample from each child was collected in June 2007. Samples were transported to the Malaria Station in Pursat within 2–3 days of collection and stored at 4°C until examination. The Kato-Katz thick

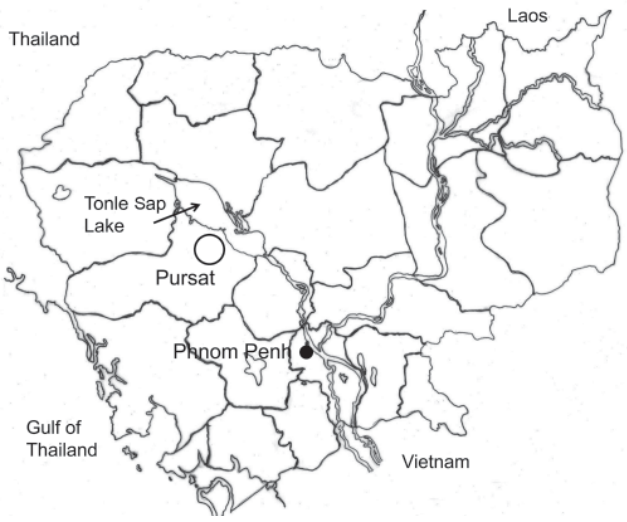

Figure 1. Surveyed area (circle) near Tonle Sap Lake, Pursat Province, Cambodia.

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Table 1. Prevalence of intestinal helminths among schoolchildren, Pursat Province, Cambodia, June 2007*

School	No. children examined	No. (%) positive results for helminth eggs; 95% CI egg positive rate				
		Echinostomes†	Hookworms	*Trichuris trichuira* eggs	Others‡	Total§
A	116	**26 (22.4); 14.8–30.0**	4 (3.4); 0.1–6.7	1 (0.9); 0.0–2.6	1 (0.9); 0.0–2.6	31 (26.7); 18.6–34.8
B	117	**12 (10.3); 4.8–15.8**	12 (10.3); 4.8–15.8	0	3 (2.6); 0.0–5.5	26 (22.2); 14.7–29.7
C	118	**9 (7.6); 2.8–12.4**	0	0	1 (0.8); 0.0–2.4	10 (8.5); 3.5–13.5
D	120	**9 (7.5); 2.8–12.2**	7 (5.8)	0	0	15 (12.5); 6.6–17.2
Total	471	56 (11.9); 9.0–14.8	23 (4.9); 3.0–6.8	1 (0.2); 0.0–0.6	5 (1.1); 0.2–2.0	82 (17.4); 14.0–20.8

*Determined by examination of feces using the Kato-Katz technique. **Boldface** indicates significant differences between schools A and B (p = 0.01); A and C (p = 0.004), and A and D (p = 0.004), as analyzed by *z* test.CI, confidence interval.
†Most of these are presumed to be eggs of *Echinostoma revolutum* worms.
‡Includes eggs of *Enterobius vermicularis* (schools A and B) and *Hymenolepis nana* (school C) worms.
§Total no. of schoolchildren positive for ≥1 helminth species.

smear technique was used to detect helminth eggs. Examination of feces and anthelmintic treatment were officially approved by the Ministry of Health, Cambodia, under the agreement of the Korea-Cambodia International Collaboration on Intestinal Parasite Control for Schoolchildren in Cambodia.

Four children who had positive test results for echinostomatid eggs and who had occasional, vague abdominal pain and discomfort were selected for anthelmintic treatment and adult worm recovery at the Malaria Station. After we obtained consent from their parents and the school guardian, the children's infections were treated with a single oral dose of 10 mg/kg praziquantel (Shinpoong Pharmceutical Co., Seoul, South Korea), and purged with 20 g magnesium sulfate. Whole diarrheic feces were collected 3–4 times and pooled individually. The diarrheic feces were processed as previously described (*11*). Worms were collected by using a wooden applicator and washed several times in water. They were fixed with 10% formalin under coverslip pressure, stained with acetocarmine, and identified by morphologic features.

A total of 17.4% of samples were positive for helminth eggs. Echinostomatid eggs were found most frequently, followed by hookworm and *Trichuris trichiura* eggs (Table 1). The percentages of echinostome eggs were significantly higher in school A than in schools B, C, and D (Table 1). However, prevalence did not differ significantly ($p<0.01$) between boys and girls (data not shown). A total of 20 echinostome adults (12, 3, 3, and 2 worms) were recovered from 4 children who showed 48–120 eggs per gram of feces (Table 2). The worms were leaflike, elongated (Figure 2), and an average of 8.8 mm long (8.0–9.5 mm) and 1.7 mm wide (1.2–2.1 mm) (n = 10). When first passed in the feces, they were pinkish red and coiled in a "c" or "e" shape. The eggs in uteri were an average of 105 μm long (97–117 μm) and 63 μm wide (61–65 μm) (n = 10). On the basis of these characteristics, the worms were identified as *E. revolutum* (Froelich, 1802) Looss, 1899.

The major sources of *E. revolutum* infection in humans are freshwater clams (*Corbicula producta*) in Taiwan and snails (*Physa occidentalis* or *Lymnaea* sp.) in Thailand (*1,5*). According to school personnel, the children were fond of eating undercooked snails or clams of unidentified species sold on the road to their homes after school. They stated that the mollusks are caught near Tonle Sap Lake. Reasons for the higher prevalence in school A than schools B, C and D are unclear.

Conclusions

Of the schoolchildren living near Tonle Sap Lake, Pursat Province, Cambodia, who participated in this study, 7.5%–22.4%, depending on school, were infected with *E. revolutum*. *E. revolutum* trematodes are endemic parasites in this area of Cambodia and a likely source of infection is freshwater snails or clams from the lake. The public health significance of echinostomiasis and educational and prevention efforts should be highlighted.

Echinostomiasis is not only an endemic infectious disease in Asian countries, including Cambodia, but also can be imported by overseas travelers from the United States or Europe. An outbreak of echinostomiasis was reported among US travelers returning from Kenya and Tanzania, although the source of infection was uncertain (*12*). This diagnosis should also be considered in patients with abdominal pain and diarrhea who have traveled to Southeast Asia and eaten snails or clams.

Despite the dangerous nature of echinostomes, the study of echinostomiasis has been neglected for many decades (*13,14*), possibly because physicians and laboratory personnel lack knowledge about this trematode parasite. In addition, no easy diagnostic technique is available to detect

Table 2. Recovery of *Echinostoma revolutum* worms from schoolchildren, Pursat Province, Cambodia, June 2007*

Child no.	Age, y	No. echinostome eggs/g†	No. *E. revolutum* specimens recovered‡
1	13	48	12
2	13	120	3
3	10	120	3
4	13	96	2

*All children were female. Fecal samples were collected individually 2–3 hours after administration of $MgSO_4$.
†Eggs/g of feces; amount in a typical sample was assumed to be 41.7 mg.
‡All recovered worms were adult worms that contained eggs.

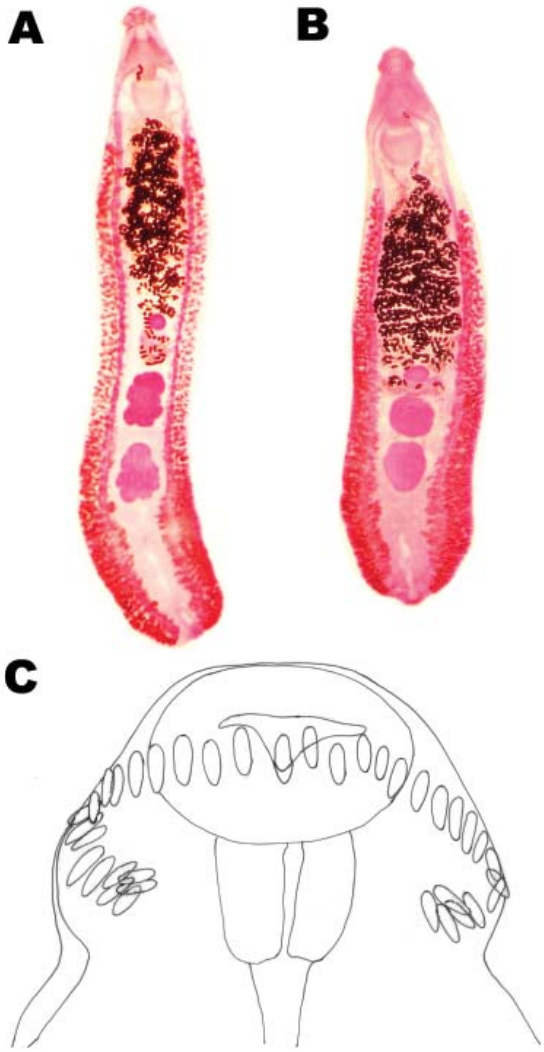

Figure 2. *Echinostoma revolutum* specimens recovered from schoolchildren in Pursat Province, Cambodia, which had 2 testes in the postequatorial region. A) An adult worm (8 mm long) showing lobulated testes. B) Another adult worm showing globular testes. C) Head collar of an adult specimen armed with 37 collar spines arranged in a single row, including 5 end-group spines on each side.

echinostome eggs, except for routine fecal examination. However, some microscopists seem to overlook or misinterpret the presence of echinostome eggs, particularly in Kato-Katz fecal smears. Even if echinostome eggs are detected, the specific diagnosis is not possible unless the adult worm is collected and identified. Thus, both the training of microscopists and emphasis on the clinical significance of echinostomiasis are urgently needed.

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References

1. Chai JY. Echinostomes in humans. In: Fried B, Toledo R, editors. The biology of echinostomes. New York: Springer; 2009. p. 147–83.
2. Anazawa K. On a human case of *Echinostoma revolutum* and its infection route [in Japanese]. Taiwan Igakkai Zasshi. 1929;288:221–41.
3. Lu SC. Echinostomiasis in Taiwan. Int J Zoonoses. 1982;9:33–8.
4. Radomyos P, Radomyos B, Tungtrongchitr A. Multi-infection with helminthes in adults from northeast Thailand as determined by post-treatment fecal examination of adult worms. Trop Med Parasitol. 1994;45:133–5.
5. Yu SH, Mott KE. Epidemiology and morbidity of food-borne intestinal trematode infections. Trop Dis Bull. 1994;91:R125–52.
6. Sinuon M, Anantaphruti MT, Socheat D. Intestinal helminthic infections in schoolchildren in Cambodia. Southeast Asian J Trop Med Public Health. 2003;34:254–8.
7. Copelovitch L, Ol OS, Taraquinio S, Chanpheaktra N. Childhood nephrotic syndrome in Cambodia: an association with gastrointestinal parasites. J Pediatr. 2010;156:76–81. DOI: 10.1016/j.jpeds.2009.06.049
8. Ohmae H, Sinuon M, Kirinoki M, Matsumoto J, Chigusa Y, Socheat D, et al. Schistosomiasis mekongi: from discovery to control. Parasitol Int. 2004;53:135–42. DOI: 10.1016/j.parint.2004.01.004
9. Lee KJ, Bae YT, Kim DH, Deung YK, Ryang YS, Kim HJ, et al. Status of intestinal parasites infection among primary school children in Kampongcham, Cambodia. Korean J Parasitol. 2002;40:153–5. DOI: 10.3347/kjp.2002.40.3.153
10. Park SK, Kim DH, Deung YK, Kim HJ, Yang EJ, Lim SJ, et al. Status of intestinal parasite infections among children in Bat Dambang, Cambodia. Korean J Parasitol. 2004;42:201–3. DOI: 10.3347/kjp.2004.42.4.201
11. Chai JY, Park JH, Han ET, Guk SM, Shin EH, Lin A, et al. Mixed infections with *Opisthorchis viverrini* and intestinal flukes in residents of Vientiane Municipality and Saravane Province in Laos. J Helminthol. 2005;79:283–9. DOI: 10.1079/JOH2005302
12. Poland GA, Navin TR, Sarosi GA. Outbreak of parasitic gastroenteritis among travelers returning from Africa. Arch Intern Med. 1985;145:2220–1. DOI: 10.1001/archinte.145.12.2220
13. Graczyk TK, Fried B. Echinostomiasis: a common but forgotten food-borne disease. Am J Trop Med Hyg. 1998;58:501–4.
14. Carney WP, Sudomo M. Purnomo. Echinostomiasis: a disease that disappeared. Trop Geogr Med. 1980;32:101–5.

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Streptococcus pneumoniae in Urinary Tracts of Children with Chronic Kidney Disease

Irene Burckhardt and Stefan Zimmermann

Streptococcus pneumoniae is not commonly considered an agent of urinary tract infections. We report 3 children with urinary tract abnormalities who had high numbers of *S. pneumoniae* in their urine (≥10^4 CFU/mL) and varying clinical symptoms.

The role of *Streptococcus pneumoniae* as an agent of septicemia, pneumonia, and meningitis is well known (*1*,*2*). However, published reports of urinary tract infection (UTI) associated with *S. pneumoniae* are scarce, and *S. pneumoniae* generally is not considered an agent of UTI in either adults or children (*3*,*4*). We report 3 children with urinary tract abnormalities and high levels of *S. pneumoniae* in their urine.

Case Reports

In June 2008, a 23-month-old boy (case-patient 1) received care for fever and clinical signs of a UTI at the emergency department of the University of Heidelberg Children's Hospital. He did not show signs of respiratory disease. His medical history was remarkable for bilateral cystic–dysplastic kidneys, a congenital urethral valve (surgery in October 2006), terminal kidney insufficiency and peritoneal dialysis since 2006, renal anemia, hyperparathyroidism, hyperphosphatemia, microcephalus, and failure to thrive. Blood was drawn for culture, and a urine sample was taken. After 9 hours of incubation, the blood culture was positive for *S. pneumoniae*, and the urine grew 10^5 CFU/mL of *S. pneumoniae*. Blood leukocyte levels were elevated (20 cells/nL [normal <13 cells/nL]), as were leukocyte levels in the urine (568 cells/μL [normal <4 cells/μL]). A throat swab was negative for *S. pneumoniae*. The boy's infection was treated successfully, and he was discharged 4 days after admission. Further analysis showed that both isolates were serotype 15B and were fully susceptible to all antibacterial drugs tested (Table 1).

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In September 2009, a 12-year-old boy (case-patient 2) sought care at the nephrology department of the University of Heidelberg Children's Hospital for his yearly control examination 7 years after kidney transplantation. He had no clinical signs of current infection. His medical history was remarkable for kidney insufficiency, congenital obstruction and reflux in the urethral valve, kidney transplantation in 2002, ileocecal pouch, chronic transplant nephropathy, metabolic acidosis, hypertension, and renal anemia. A urine sample showed 10^5 CFU/mL *S. pneumoniae* and 10^3 CFU/mL *Enterobacteriaceae*. Urine leukocyte levels were slightly elevated (16 cells/μL). Further analysis showed that *S. pneumoniae* was serotype 34 and fully susceptible to all antibacterial drugs tested (Table 1).

In November 2009, a 7-year-old girl (case-patient 3) was sent to the emergency department of the University of Heidelberg Children's Hospital by her pediatrician because of abnormal results in a control urine sample 4 weeks after percutaneous nephrolithoapraxia and concrement removal. She was known to have cystinuria and had already undergone extracorporeal shock wave treatment with concrement removal in 2004. Her temperature was slightly elevated (37.5°C), but she had no dysuria or pain. Urinalysis showed elevated leukocyte levels (158 cells/μL), and 10^4 CFU/mL *S. pneumoniae* could be grown. Because of the mild symptoms, no antimicrobial drug treatment was started. Further analysis showed that the isolate was a 19F serotype (Table 1).

For each patient, urine was routinely cultured as follows: 2 samples of 1 μL each were placed on a 5% sheep-blood agar plate and a MacConkey agar plate. chromID CPS medium (bioMérieux, Nürtingen, Germany) was injected with 10 μL of urine. All plates were incubated for 18–24 h at 36°C ± 1°C in ambient air (*5*). Susceptibility testing was performed by using the BD Phoenix Automated Microbiology System with SMIC/ID panels (Becton Dickinson, Heidelberg, Germany).

Discussion and Conclusions

The literature on urinary tract infections with *S. pneumoniae* is scarce. In 1980, Green and Selinger described a patient with a soft tissue abscess and a UTI caused by serotype 3 (*6*). In 2004, Dufke et al. described a patient with pyelonephritis and urosepsis caused by serotype 6A (*7*). In 1988, Nguyen and Penn determined the frequency of pneumococci in urine specimens from adults and found 38 (0.18%) of 22,744 samples positive for *S. pneumoniae* (*4*). Similarly, Miller et al. determined that the frequency of pneumococci in urine specimens from children was even lower: 43 (0.08%) of 53.499 samples (*3*). Of 28 patients, for whom clinical data were available, 5 had dysuria, and 2 had pyuria. Three asymptomatic children had medical histories of genitourinary abnormalities; 6 asymptomatic

Table 1. Characteristics of *Streptococcus pneumoniae* isolates in 3 children with chronic kidney disease, Germany, 2005–2010*

Characteristic	Case 1		Case 1		Case 2		Case 3	
Sample	Blood		Urine		Urine		Urine	
Serotype	15B		15B		34		19F	
Optochin	S		S		S		S	
Bile solubility	Positive		Positive		Positive		Positive	
Oxacillin, 1 μg	S		S		S		S	
Penicillin	≤0.03	S	≤0.03	S	≤0.03	S	0.06	S
Amoxicillin	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Cefotaxime	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S
Erythromycin	≤0.06	S	≤0.06	S	≤0.06	S	≥4.0	R
Clindamycin	0.06	S	0.06	S	0.06	S	≥2	R
Tetracycline	≤0.5	S	≤0.5	S	≤0.5	S	≥8	R
Levofloxacin	1	S	1	S	≤0.5	S	1.0	S
Meropenem	≤0.125	S	≤0.125	S	≤0.125	S	≤0.125	S
Vancomycin	≤0.5	S	≤0.5	S	≤0.5	S	≤0.5	S
Linezolid	≤1	S	≤1	S	≤1	S	≤1	S
Moxifloxacin	≤0.25	S	≤0.25	S	≤0.25	S	≤0.25	S
Co-trimoxazole	1/19	I	1/19	I	≤0.5/9.5	S	≤0.5/9.5	S

*Values are MICs (mg/mL). S, susceptible; R, resistant; I, intermediate.

children had medical histories of recurrent UTI with *Enterobacteriaceae*. The serotypes and antibiotic susceptibilities of the respective isolates were not reported.

A UTI is defined as bacteriuria ($\geq 10^5$ CFU/mL in adults, $\geq 10^4$ CFU/mL in children) of 1 uropathogen and typical clinical signs, i.e., dysuria and urgency. Depending on the age of the patient, clinical signs might be less typical, especially in children <2 years of age. Generally pyuria is present (*8*,*9*). By contrast, asymptomatic bacteriuria is defined as a uropathogen ($\geq 10^5$ CFU/mL in adults, $\geq 10^4$ CFU/mL in children) without pyuria (<10 leukocytes/μL) (*9*).

Applying these criteria to the 3 cases in this report yields the following results. Assuming that *S. pneumoniae* is a uropathogen, case 1 is a UTI accompanied by septicemia. No other focus of infection with *S. pneumoniae* was apparent or could be identified. We believe this is an ascending UTI in a boy with known oliguria from bilateral

Table 2. Results of urine samples of 12-year-old boy with chronic kidney disease, Germany, 2005–2010

Date sample arrived in laboratory	Type of urine sample	CFU/mL	Species	Leukocyte count, cells/μL
2005 Apr	Catheter	10^5	α-Hemolytic streptococci*	No data
		10^4	*Proteus mirabilis*	
2005 Jul	Midstream	10^5	α-Hemolytic streptococci	No data
		10^5	*Proteus mirabilis*	
2005 Sep	Midstream	10^5	*Streptococcus pyogenes*	No data
		10^3	α-Hemolytic streptococci	
2007 Mar	Midstream	10^4	*Streptococcus pneumoniae*	92
		10^4	*Proteus mirabilis*	
2008 Mar	Midstream	10^4	α-Hemolytic streptococci	54
		10^4	*Proteus mirabilis*	
2008 Jun	Midstream	10^5	*Proteus mirabilis*	33
		10^3	α-Hemolytic streptococci	
2008 Jul	Midstream	10^5	α-Hemolytic streptococci	150
2008 Nov	Midstream	10^4	α-Hemolytic streptococci	90
2009 Mar	Midstream	10^5	α-Hemolytic streptococci	46
2009 May	Midstream	10^5	α-Hemolytic streptococci	24
		10^4	*Proteus mirabilis*	
2009 Sep	Midstream	10^5	*Streptococcus pneumoniae*	16
		10^3	*Enterobacteriaceae*	
2010 Jan	Midstream	10^5	α-Hemolytic streptococci	94
		10^4	*Proteus mirabilis*	
2010 Jun	Midstream	10^5	α-Hemolytic streptococci	124
		10^4	*Proteus mirabilis*	

*Reference value is <4 cells/μL.

cystic–dysplastic kidneys. Because case-patient 3 showed only mild symptoms, diagnosis of UTI is not obligatory. Nevertheless, it is not a mere pneumococcosuria because of the high numbers of leukocytes in the urine (158 cells/μL).

Case 2 is more difficult to classify. The episode described might be pneumococcosuria because the leukocyte level is not high; nevertheless, it is above normal. Reassessment of all microbiological data of case-patient 2 indicated that since 2005 we have received 29 different urine samples. In March 2007, we had already identified *S. pneumoniae* (10^4 CFU/mL) and pyuria (92 CFU/μL). Additionally, 10^4 CFU/mL *Proteus mirabilis* had been present and considered the cause of the pyuria; 11 of 27 samples had contained α-hemolytic streptococci $\geq 10^3$ CFU/mL (Table 2). Unfortunately, no test to differentiate between pneumococci and the other α-hemolytic streptococci was originally performed on these bacteria. Therefore, we can only speculate whether at least some of these samples contained *S. pneumoniae*. Taking these facts into account, we believe that this might be a case of bacterial persistence according to the criteria described by Chang and Shortliffe (cultures ± same organism) (*8*).

Reassessment of all available laboratory data on case-patient 1 showed that we received 3 urine samples before the described episode and 8 afterward. In none of the samples did we find *S. pneumoniae* or α-hemolytic streptococci. From case-patient 3 we did not receive any other material than described.

Further analysis of the *S. pneumoniae* isolates indicated that all 3 children were affected by different serotypes. Therefore, we do not have an indication that a single serotype has a predilection for the urinary tract. In theory, 2 cases could have been avoided by vaccination, i.e., case 3 (serotype 19F) and case 1 (serotype 15B). However, case-patient 3 was 4 years of age when the routine vaccination program aimed at children <2 years of age started in Germany in 2006. Because no catch-up program existed, this child was never vaccinated against pneumococci. Case-patient 1 was regularly vaccinated with heptavalent pneumococcal conjugate vaccine in 2006–2007 but was just 23 months old at disease onset disease, i.e., 1 month too young to be eligible for the 23-valent pneumococcal polysaccharide vaccine. Case-patient 2 is not yet vaccinated against pneumococci. Thus, we suggest that *S. pneumoniae* be added to the potential UTI-causing pathogens in children with urinary tract abnormalities.

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Serotypes and bile solubility were determined at the National Reference Center for Streptococci, Aachen, Germany.

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References

1. O'Brien KL, Wolfson LJ, Watt JP, Henkle E, Deloria-Knoll M, McCall N, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. Lancet. 2009;374:893–902. DOI: 10.1016/S0140-6736(09)61204-6
2. van der Poll T, Opal SM. Pathogenesis, treatment, and prevention of pneumococcal pneumonia. Lancet. 2009;374:1543–56. DOI: 10.1016/S0140-6736(09)61114-4
3. Miller MA, Kaplan BS, Sorger S, Knowles KF. Pneumococcosuria in children. J Clin Microbiol. 1989;27:99–101.
4. Nguyen VQ, Penn RL. Pneumococcosuria in adults. J Clin Microbiol. 1988;26:1085–7.
5. Gatermann S, Fünfstück R, Handrick W, Leitritz L, Mauch H, Naber KG, et al. Harnwegsinfektionen. In: Mauch H, Podbielski A, Hermann M, editors. Qualitätsstandards in der mikrobiologisch-infektiologischen Diagnostik; Im Auftrag der Deutschen Gesellschaft für Hygiene und Mikrobiologie (DGHM). 2nd ed. Munich: Urban & Fischer Verlag; 2005. p. 1–70.
6. Green RF, Selinger DS. Urinary tract infection and soft tissue abscess caused by *Streptococcus pneumoniae*. South Med J. 1980;73:1550–1.
7. Dufke S, Kunze-Kronawitter H, Schubert S. Pyelonephritis and urosepsis caused by *Streptococcus pneumoniae*. J Clin Microbiol. 2004;42:4383–5. DOI: 10.1128/JCM.42.9.4383-4385.2004
8. Chang SL, Shortliffe LD. Pediatric urinary tract infections [vi]. Pediatr Clin North Am. 2006;53:379–400. DOI: 10.1016/j.pcl.2006.02.011
9. Prelog M, Fischer H, Fille M, Allerberger F, Zimmerhackl LB. Harnwegsinfektion im Kindesalter. Monatsschr Kinderheilkd. 2004;152:331–44. DOI: 10.1007/s00112-004-0907-2

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Foreign Travel and Decreased Ciprofloxacin Susceptibility in *Salmonella enterica* Infections

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To determine antimicrobial drug resistance patterns, we characterized nontyphoidal *Salmonella enterica* strains isolated in Liverpool, UK, January 2003 through December 2009. Decreased susceptibility to ciprofloxacin was found in 103 (20.9%) of 492 isolates. The lower susceptibility was associated with ciprofloxacin treatment failures and with particular serovars and phage types often acquired during foreign travel.

Nontyphoidal *Salmonella enterica* (NTS) isolates produce a common food-related infection that causes mild and self-limiting diarrhea and, occasionally, a potentially fatal invasive disease with bacteremia and endovascular infection (*1*). Antimicrobial drug therapy, often with the fluoroquinolone ciprofloxacin, is required for treating invasive NTS infections and severe diarrhea in immunocompromised patients. Full resistance to fluoroquinolones is relatively uncommon for NTS infections, but decreased susceptibility to ciprofloxacin (DCS), defined as MIC 0.1–1.0 μg/mL, has become common (*2*). Resistance to nalidixic acid is often used as a marker for DCS, although the validity of this assumption has been debated (*3*), and some have suggested that the ciprofloxacin disk susceptibility zone size breakpoints should be changed to detect such strains (*2*).

DCS is associated with ciprofloxacin treatment failures in patients with typhoid fever (*4*) and probably with invasive NTS infection, although the clinical significance of DCS in NTS has not been widely explored (*5*–*7*). Clinical clues to the presence of DCS in *S. enterica* infections could guide early empirical prescription of antimicrobial drug therapy. Links between DCS and foreign travel have been suggested in reports from Denmark (*8*) and Finland (*9*,*10*). In this study, we characterized the resistance patterns of NTS strains isolated in Liverpool and explored the value of nalidixic acid–resistance testing, the clinical significance of DCS, and possible links between DCS and foreign travel.

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The Study

We studied all isolates of NTS detected in samples submitted to the microbiology department at the Royal Liverpool University Hospital from patients in the Merseyside area from January 2003 through December 2009**.** Isolates were identified by standard biochemical and serologic methods. Disk susceptibility testing was performed for ampicillin, trimethoprim, ciprofloxacin, and ceftriaxone. Nalidixic acid testing was recommended but not consistently performed. The serovar, phage type, and susceptibility of each isolate were confirmed by the Laboratory for Gastrointestinal Pathogens, Health Protection Agency Centre for Infections, Colindale, London. Isolates were stored in glycerol broth at −70°C and later subcultured for determination of MICs to ciprofloxacin and nalidixic acid by using the agar dilution and the Etest strip methods. The control organisms *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used. Breakpoints were defined according to Clinical Laboratory Standards Institute guidelines (*11*). DCS was defined as MIC 0.1–1.0 μg/mL and resistance MIC >1 μg/mL.

Laboratory data, including serovar and antimicrobial drug susceptibility patterns, were merged with statutory notification of diseases data from the Cheshire and Merseyside Health Protection Unit of the Health Protection Agency. This second database contained the travel history (validated through an enhanced questionnaire) of the patients, detailing whether they had a relevant history of foreign travel and, if so, where they had traveled. The medical records of patients with bacteremia were retrieved and reviewed to establish whether ciprofloxacin had been used for therapy and the clinical outcome.

A total of 492 unique NTS patient isolates, including isolates from 116 inpatients, were identified during the study period. Diarrheal isolates accounted for 479 of the total; 11 were from blood, 1 was from a wrist aspirate, and 1 was from a pathology specimen of the large bowel. The number and proportion of isolates resistant to ampicillin were 39 (7.9%), to trimethoprim 22 (4.5%), to ciprofloxacin 2 (0.4%), and to ceftriaxone 6 (1.2%). Resistance to nalidixic acid was determined at isolation for 281 (57.1%) of 492 isolates and was found for 53 (18.9%). When all 492 patient isolates were tested, 103 (20.9%) were resistant to nalidixic acid, including both isolates that were cip-

rofloxacin resistant. Of 397 (81%) isolates retrievable for MIC testing, all 304 with a MIC of ciprofloxacin <0.1 μg/mL were also susceptible to nalidixic acid, 91 had DCS (0.1–1.0 μg/mL), all but 1 were resistant to nalidixic acid, and 2 were resistant to nalidixic acid and ciprofloxacin with a ciprofloxacin MIC >4.0 μg/mL. Nalidixic acid resistance correlated well with DCS.

Forty-eight named serovars of *S. enterica* were found among the 492 characterized isolates. The serovars most commonly isolated from patients with nalidixic acid resistance were Enteritidis phage type 1 (PT1), Virchow, Newport, and Enteritidis PT21; the first 3 of these were also commonly associated with a history of foreign travel (Table). The higher levels of DCS in serovars Enteritidis and Virchow and lower levels in serovar Typhimurium are consistent with data from Europe, as is the association with particular serovar Enteritidis phage types (*12*,*13*).

A relevant foreign travel history was reported for 110 (22.4%) of 492 patients; destinations were identifiable for 105, including 36 countries from across Asia, Europe, South America, and Africa. Countries most commonly implicated were Spain (22; 20.0%), Egypt (11; 10.0%), Turkey (10; 9.1%), India (8; 7.3%), and Thailand (5; 4.6%). Among the 110 isolates from patients who had traveled, 35 (31.8%) were resistant to nalidixic acid compared with 68 (17.8%) from the 382 patients with no history of foreign travel (odds ratio [OR] 2.15, 95% confidence interval [CI] 1.30–3.57; p<0.001). Travel to Egypt (OR 5.3, 95% CI 1.59–17.99; p = 0.007), Spain (OR 3.08, CI 1.27–7.48; p = 0.018), and Thailand (OR 17.51, CI 2.8–109.33; p = 0.002) was associated with DCS; these countries were also identified in other studies (*8–10*). High levels of DCS or resistance to ciprofloxacin have been observed among isolates of NTS from Spain and Thailand (*14*,*15*). This study does not include rates of travel to various destinations, so the higher numbers of *S. enterica* isolates with DCS in travelers to some destinations may simply reflect travel patterns.

In a multivariate analysis, after different serovars and phage types and history of foreign travel were adjusted for, DCS was independently associated with serovars Enteritidis PT1 (OR 14.42, CI 6.41–32.43; p<0.001), Enteritidis PT21 (OR 5.81, CI 2.1–16.08; p = 0.001), Newport (OR 9.38, CI 2.8–31.38; p<0.001), and Virchow (OR 62.33, CI 7.37–526.82; p<0.001) but not with a history of foreign travel (OR 1.54, CI 0.82–2.85; p = 0.178). This finding suggests that the association with particular serovars and phage types is greater than any association with foreign travel and that travel is a factor only because travel can facilitate importation of these serovars.

The clinical features of the 11 patients with bacteremia are summarized in the online Appendix Table (www.cdc.gov/EID/content/17/1/123-appT.htm); 10 either were immunosuppressed or had gall bladder disease. Five blood culture isolates were resistant to nalidixic acid with DCS. Ciprofloxacin was the initial drug choice for 3 of the patients infected with a DCS isolate, but in each instance, the drug was changed to an alternative (ceftriaxone for 2 patients, ampicillin for 1) because of an unsatisfactory clinical response. The other 2 patients initially received a cephalosporin, and outcome was acceptable. Although extended-spectrum cephalosporins are the principal alternative antimicrobial drugs for treatment of bacteremic infections, resistance is also emerging (*2*,*13*). Six of the isolates in this study were resistant to ceftriaxone, including 1 of the isolates from a patient with bacteremia, which was susceptible to ciprofloxacin.

Conclusions

Our data show that one fifth of NTS isolates in Liverpool demonstrated nalidixic acid resistance and that this was a good marker for DCS. The data also suggest that DCS may compromise ciprofloxacin therapy for invasive disease caused by NTS. Infection with particular serovars and phage type, frequently associated with foreign travel, were significant risk factors for infection with an *S. enterica* isolate with DCS, and this information can help guide initial empirical antimicrobial drug choices. Early detection of DCS is essential, but nalidixic acid–resistance testing

Table. Relationship between nontyphoidal *Salmonella enterica* serovar, phage type, nalidixic acid resistance, and history of foreign travel, Liverpool, UK, 2003–2009*

Serovar	Total no. (%) patient isolates	No. (%) patients with foreign travel history	No. (%) patients with nalidixic acid–resistant isolate	No. (%) patients with foreign travel history and nalidixic acid–resistant isolate
All	492 (100.0)	110 (22.4)	103 (20.9)	35 (31.8)
Enteritidis PT1	53 (10.8)	16 (30.2)	36 (67.9)	9 (56.3)
Enteritidis PT4	93 (18.9)	12 (12.9)	4 (4.3)	2 (16.7)
Enteritidis PT8	43 (8.7)	8 (18.6)	1 (2.3)	1 (12.5)
Enteritidis PT21	22 (4.5)	5 (22.7)	10 (45.4)	1 (20.0)
Enteritidis (other PT)	104 (21.1)	13 (12.5)	16 (15.4)	3 (23.1)
Typhimurium	44 (8.9)	11 (25.0)	3 (6.8)	1 (9.1)
Newport	14 (2.8)	3 (21.4)	8 (57.1)	3 (100.0)
Virchow	12 (2.4)	9 (75.0)	11 (91.7)	8 (88.9)
Other	107 (21.7)	33 (30.8)	14 (13.1)	7 (21.2)

*PT, phage type.

was not always performed at the time of isolation. Revision of ciprofloxacin disk susceptibility breakpoints would allow such isolates to be detected more easily.

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References

1. Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis. 2001;32:263–9. DOI: 10.1086/318457
2. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and non-typhoidal salmonellae. Curr Opin Infect Dis. 2008;21:531–8. DOI: 10.1097/QCO.0b013e32830f453a
3. Hakanen AJ, Lindgren M, Huovinen P, Jalava J, Siitonen A, Kotilainen P. New quinolone resistance phenomenon in *Salmonella enterica*: nalidixic acidsusceptible isolates with reduced fluoroquinolone susceptibility. J Clin Microbiol. 2005;43:5775–8. DOI: 10.1128/JCM.43.11.5775-5778.2005
4. Crump JA, Kretsinger K, Gay K, Hoekstra RM, Vugia DJ, Hurd S, et al. Clinical response and outcome of infection with *Salmonella enterica* serotype Typhi with decreased susceptibility to fluoroquinolones: a United States FoodNet multicentre retrospective cohort study. Antimicrob Agents Chemother. 2008;52:1278–84. DOI: 10.1128/AAC.01509-07
5. Mølbak K, Baggesen DL, Aarestrup FM, Ebbesen JM, Engberg J, Frydendahl K, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium DT 104. N Engl J Med. 1999;341:1420–5. DOI: 10.1056/NEJM199911043411902
6. Helms M, Simonsen J, Mølbak K. Quinolone resistance is associated with increased risk of invasive illness or death during infection with *Salmonella* serotype Typhimurium. J Infect Dis. 2004;190:1652–4. DOI: 10.1086/424570
7. Varma JK, Molbak K, Barrett TJ, Beebe JL, Jones TF, Rabatsky-Ehr T, et al. Antimicrobial-resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. J Infect Dis. 2005;191:554–61. DOI: 10.1086/427263
8. Mølbak K, Gerner-Smidt P, Wegener HC. Increasing quinolone resistance in *Salmonella enterica* serotype Enteritidis. Emerg Infect Dis. 2002;8:514–5.
9. Hakanen A, Kotilainen P, Huovinen P, Helenius H, Siitonen A. Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from Southeast Asia. Emerg Infect Dis. 2001;7:996–1003. DOI: 10.3201/eid0706.010613
10. Hakanen AJ, Kotilainen P, Pitkänen S, Huikko S, Siitonen A, Huovinen P. Reduction in fluoroquinolone susceptibility among non-typhoidal strains of *Salmonella enterica* isolated from Finnish patients. J Antimicrob Chemother. 2006;57:569–72. DOI: 10.1093/jac/dkl002
11. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement. M100-S20. Wayne (PA): The Institute; 2010.
12. Murray A, Coia JE, Mather H, Brown DJ. Ciprofloxacin resistance in non-typhoidal *Salmonella* serotypes in Scotland, 1993–2003. J Antimicrob Chemother. 2005;56:110–4. DOI: 10.1093/jac/dki164
13. Meakins S, Fisher IST, Berghold C, Gerner-Smidt P, Tschäpe H, Comican M, et al. Antimicrobial drug resistance in human nontyphoidal *Salmonella* isolates in Europe 2000–2004: a report from the Enter-net International Surveillance Network. Microb Drug Resist. 2008;14:31–5. DOI: 10.1089/mdr.2008.0777
14. Marimón JM, Gomáriz M, Zigorraga C, Cilla G, Pérez-Trallero E. Increasing prevalence of quinolone resistance in human nontyphoid *Salmonella enterica* isolates obtained in Spain from 1981 to 2003. Antimicrob Agents Chemother. 2004;48:3789–93. DOI: 10.1128/AAC.48.10.3789-3793.2004
15. Lee HY, Su LH, Tsai MH, Kim SW, Chang HH, Jung SI, et al. High rate of reduced susceptibility to ciprofloxacin and ceftriaxone among nontyphoidal *Salmonella* clinical isolates in Asia. Antimicrob Agents Chemother. 2009;53:2696–9. DOI: 10.1128/AAC.01297-08

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How Safe Is Our Food?

J. Glenn Morris, Jr.

How safe is our food? Put another way, how much illness in the United States is caused by foodborne pathogens? It sounds like a simple question. Getting a reasonable answer, however, is far from simple. The basic problem lies in the fact that only a small fraction of foodborne disease cases get reported through official (or unofficial) reporting systems. Calculating the "real" rate of foodborne illness requires development of models that use reported cases as a starting point to estimate underlying disease rates. Given the plethora of pathogens that can be transmitted through foodborne routes, this is a complex, and somewhat daunting, process. It is, however, necessary for assessing the safety of foods and developing strategies for disease prevention. The articles by Scallan et al. (*1*,*2*) in this issue represent the latest efforts to develop such estimates of the magnitude of foodborne illness in the United States.

In 1999, Mead et al. (*3*) published initial estimates of foodborne disease in the United States. This landmark undertaking was the first to provide a comprehensive compilation of data from a variety of sources, including the Centers for Disease Control and Prevention (CDC) and the medical literature. It resulted in the often-cited estimates that foodborne pathogens cause 76 million episodes of illness, 325,000 hospitalizations, and 5,000 deaths each year in the United States. (Hereafter, episodes of illness are referred to as illnesses.) During the past decade, these numbers have strongly driven ongoing efforts to implement or reform regulatory systems to protect the public from foodborne illness. However, some aspects of the methods have been criticized, particularly the high degree of uncertainty of particular parameters and thus of the results themselves (*4*–*6*). These concerns have led to requests for CDC to repeat and update the work of Mead et al., using better methods and parameter estimates that more closely reflect current realities.

Now, ≈11 years later, Scallan et al. have produced "Sons of Mead," which include substantial improvements to the methods used by Mead et al. and to the quality and timeliness of data (*1*,*2*). Scallan et al. should be commended, especially for 2 specific improvements: their advanced treatment of statistical uncertainty and variability and their transparent inclusion of voluminous appendixes of data, models, and assumptions. These authors followed the same basic approach as Mead et al. but chose to report their estimates in 2 articles. In the first article, they based their estimates of illnesses caused by 24 major pathogens (e.g., *Salmonella* spp., *Escherichia coli* O157:H7) primarily on data from the Foodborne Diseases Active Surveillance Network (FoodNet) and other pathogen-specific surveillance systems. In the second article, they estimated illnesses caused by unknown (or unspecified) pathogens by subtracting illnesses caused by known pathogens from the annual estimated number of cases of acute gastroenteritis in the US population and adjusting the result by the percentage assumed to be acquired domestically through food. If these 2 estimates are combined, as they were by Mead et al., the new totals are 47.8 million foodborne illnesses, 127,839 hospitalizations, and 3,037 deaths per year in the United States.

When one compares the 1999 and 2010 estimates (76 million vs. 47.8 million illnesses), the immediate response is to ask: Does this mean that food in this country is safer than it was 11 years ago? Unfortunately, the Scallan et al. articles do not enable us to answer this question. The methods, underlying assumptions, and parameter estimates used to generate these new numbers differ sufficiently from those used ≈11 years ago to preclude comparisons. In fact, if one looks simply at rates of overall gastrointestinal illness in the United States, based on FoodNet Population Surveys (*2*), one might infer that overall rates of acute gastrointestinal illness have increased during this period, from 0.49 episodes per person per year in 2000–2001, to 0.54 in 2002–2003, and to 0.73 in 2006–2007 (see [*7*] for a discussion of some methodologic issues with regard to the 2006–2007 survey). For the Scallan et al. articles, these 3 numbers were averaged to arrive at a rate of 0.6 episodes of acute gastroenteritis per person per year over the past decade. In contrast, Mead et al. used an estimate of 0.79 episodes of gastroenteritis per person per year, based on FoodNet data but also on older community surveys; they also used a somewhat different definition of acute gastrointestinal illness. This difference in estimated annual rates

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of acute gastroenteritis, when combined with a lower assumed proportion of gastroenteritis that is foodborne, explains much of the dramatic drop in total annual episodes of foodborne disease. Had Scallan et al. elected to use the 2006–2007 FoodNet estimate of 0.73 cases per person per year rather than use the average of 0.6 cases, their numbers would have been substantially higher and closer to the Mead et al. estimates.

Thus, if we can't use the Scallan estimates for comparison, is there any way to say whether food in the United States is safer now than it was 11 years ago? The best answer to this question comes from the FoodNet system (*8*), an active laboratory-based sentinel surveillance system that was established to monitor the public health impact of the 1995 US Department of Agriculture (USDA) Pathogen Reduction: Hazard Analysis and Critical Control Point (HAACP) System regulations (the first major revision of USDA food safety regulations since 1906). FoodNet provides annual data from designated sentinel surveillance sites on numbers of laboratory-diagnosed cases of 10 predominantly foodborne bacterial and parasitic pathogens; it reports actual case totals, not estimates. Despite year-to-year variability (including significant decreases in incidence of *Shigella* spp. and *E. coli* O157:H7 for 2009) (*8*), the overall trends show an initial drop in incidence of infection with the major bacterial foodborne pathogens after implementation of the 1995 USDA regulations, followed by a leveling off of incidence in subsequent years. One exception is infections caused by *Vibrio* spp., which are increasing, partly because climate change is affecting coastal environments (*9*). Bottom line: with the exception of *Vibrio* spp., things don't seem to be getting worse; however, after the initial decline since the USDA regulatory changes in 1995, one does not see evidence of sustained improvement.

How do numbers from the United States compare with those from Europe and the rest of the world? Again, differences in methods used by Scallan et al. make it difficult, if not impossible, to directly compare these numbers with those being published by other countries, including Canada, Australia, and members of the European Union (*10*–*12*). Although these new estimates cannot be compared directly with previous estimates or with estimates from other countries, these articles nonetheless constitute a necessary starting point for generation of more robust and regularly updated numbers. Looking across time, use of a consistent method, with regular updating of data (ideally annually), would provide a basis for assessing the effect of changes in regulation and other interventions at a national level. Similarly, if the methods are further modified in keeping with current international discussions on standardization of foodborne disease estimates (*13*), direct comparison of US numbers with those from other countries may become possible.

Estimates of the relative burden of disease caused by specific pathogens are crucial for improving our understanding of foodborne illness risks, but they are insufficient on their own. To target interventions (which are almost always food specific), illnesses must be quantified in terms of food–pathogen combinations. Doing so, in turn, requires development of what have been termed food attribution data (*14*,*15*). That is, how much salmonellosis is caused by eating contaminated chicken versus eggs, beef, or pork? How often is beef, compared with produce, the source of infection with *E. coli* O157:H7? Likewise, summary statistics such as number of cases, hospitalizations, and deaths ignore at-risk subpopulations and chronic sequelae such as end-stage renal disease, congenital toxoplasmosis, and irritable bowel syndrome. As such, the World Health Organization and many industrialized countries are increasingly reporting integrated measures of disease, such as disability-adjusted life years, which more fully capture disease symptoms and severities (*13*). Furthermore, to reduce specific foodborne hazards, we need information about the many factors along the complex farm-to-table pathway that can lead to the introduction or amplification of pathogens that contaminate food. This information would also help determine feasibility and efficacy of potential interventions.

As outlined in a recent Institute of Medicine report (*16*), implementation of a modern, risk-based food safety system in the United States will ultimately require much better data and a strong analytic capacity at the federal level that cuts across current agency lines. Although we still have a long way to go to bring our food safety system into the current century, the articles by Scallan et al. are critical steps in the right direction.

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References

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011;17:7–15.
2. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis. 2011;17:16–22.
3. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C, et al. Food-related illness and death in the United States. Emerg Infect Dis. 1999;5:607–24. DOI: 10.3201/eid0505.990502

4. Powell M, Ebel E, Schlosser W. Considering uncertainty in comparing the burden of illness due to foodborne microbial pathogens. Int J Food Microbiol. 2001;69:209–15. DOI: 10.1016/S0168-1605(01)00495-0
5. Phillips CV, LaPole LM. 2003. Quantifying errors without random sampling. BMC Medical Research Methodology 2003;3:9 [cited 2010 Nov 15]. http://www.biomedcentral.com/1471-2288/3/9
6. Frenzen PD. Deaths due to unknown foodborne agents. Emerg Infect Dis. 2004;10:1536–43.
7. Cantwell LB, Henao OL, Hoekstra RM, Scallan E. The effect of different recall periods on estimates of acute gastroenteritis in the United States, FoodNet Population Survey 2006–2007. Foodborne Pathog Dis. 2010;7:1225–8. DOI: 10.1089/fpd.2010.0567
8. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. MMWR Morb Mortal Wkly Rep. 2010;59:418–22.
9. Lipp EK, Huq A, Colwell RR. Effects of global climate on infectious disease: the cholera model. Clin Microbiol Rev. 2002;15:757–70. DOI: 10.1128/CMR.15.4.757-770.2002
10. Adak GK, Long SM, O'Brien SJ. Trends in indigenous foodborne disease and deaths, England and Wales: 1992–2000. Gut. 2002;51:832–41. DOI: 10.1136/gut.51.6.832
11. OzFoodNet Net Working Group. Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: annual report of the OzFoodNet Network, 2008. Commun Dis Intell. 2009;33:389–413.
12. Flint JA, Van Duynhoven YT, Angulo FJ, DeLong SM, Braun P, Kirk M, et al. Estimating the burden of acute gastroenteritis, foodborne disease, and pathogens commonly transmitted by food: an international review. Clin Infect Dis. 2005;41:698–704. DOI: 10.1086/432064
13. Kuchenmuller T, Hird S, Stein C, Kramarz P, Nanda A, Havelaar AH. Estimating the global burden of foodborne diseases—a collaborative effort. Euro Surveill. 2009;14:pii:19195.
14. Batz MB, Doyle MP, Morris JG, Painter J, Singh R, Tauxe RV, et al.; Food Attribution Working Group. Linking illness to food: summary of a workshop on food attribution. Emerg Infect Dis. 2005;11:993–9.
15. Pires SM, Evers EE, van Pelt W, Ayers T, Scallan E, Angulo FJ, et al.; Med-Vet-Net Workpackage 28 Working Group. Attributing the human disease burden of foodborne infections to specific sources. Foodborne Pathog Dis. 2009;6:417–24. DOI: 10.1089/fpd.2008.0208
16. Institute of Medicine. Enhancing food safety: the role of the Food and Drug Administration. Washington: National Academy Press; 2010. p. 1–576.

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Emergence of New Delhi Metallo-β-Lactamase, Austria

To the Editor: Extended-spectrum β-lactamase–producing *Enterobacteriaceae* strains have emerged as a major public health problem throughout the world, particularly in India and Pakistan. The widespread use of carbapenems, the only agents reliably active against these bacteria, resulted in the emergence of a new resistance mechanism. New Delhi metallo-β-lactamase (NDM-1) was first detected in a *Klebsiella pneumonia*e isolate in 2008 from a Swedish patient of Indian origin; it has since been reported in increasing numbers of infections in patients from India, Pakistan, and the United Kingdom (*1*–*3*).

NDM-1 shares very little identity with other metallo-β-lactamase enzymes; *Enterobacteriaceae* isolates with NDM-1 show high resistance to nearly all commonly used antibacterial agents (*4*). Most NDM-1 patients in Europe and the United States had received medical care in India or Pakistan before isolation of the strain. However, the emergence of NDM-1 poses the risk of plasmid-mediated transfer of the carbapenemase enzyme $bla_{\text{NDM-1}}$ between different bacterial strains, which could lead to serious public health issues (*3,5*). We report the emergence of NDM-1–positive *K. pneumoniae* in Austria in 2009–2010.

Primers for PCR detection of NDM-1 were designed according to GenBank (National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD, USA) database entry AB571289.1 (www.ncbi.nlm.nih.gov/nuccore/300422615). The forward primer NDM-1gf 5′-ACC GCC TGG ACC GAT GAC CA-3′ (positions 80–99), and reverse primer NDM-1gr 5′-GCC AAA GTT GGG CGC GGT TG-3′ (positions 343–324) were used.

PCR conditions were the following: initial denaturation at 94°C for 5 min; 35 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 30 s; and final incubation for 10 min at 72°C. Taq DNA polymerase and dNTPs from QIAGEN (Hilden, Germany) were used. The 264-bp fragment was sequenced and compared with the GenBank entry for NDM-1.

Carbapenemase-producing *K. pneumoniae* has been detected in 26 isolates obtained during September 2007 through August 2010 from 6 patients at the University Hospital, Graz, Austria. Eight isolates from 2 patients were found to carry the plasmid NDM-1. The first case involving NDM-1 occurred in November 2009, and the second occurred in August 2010. Automated repetitive element PCR, conducted with the DiversiLab system (bioMérieux, Marcy l'Etoile, France) (*6*) showed a genetic relatedness of isolates from the 2 patients of ≤81.1% (5 band differences), which indicated independent clones. Isolated NDM-1 strains exhibited resistance to nearly all antibacterial agents, including aztreonam, ciprofloxacin, and gentamicin, and were susceptible to only colistin, tigecycline, and amikacin (Table).

Patient 1, a 30-year-old Austrian man, was admitted to University Hospital (Graz, Austria) in November 2009. His medical history showed he had experienced multiple open fractures of his upper and lower left leg as well as rectal laceration because of a motorcycle accident in Pakistan. His treatment had taken place primarily in surgery departments in Pakistan and India. During his hospitalization in Austria, multiple resistant gram-negative bacteria were isolated, including highly resistant NDM-1–producing *K. pneumoniae*. The NDM-1 strain was isolated twice, from a sacral decubitus ulcer and from stool. After 5 months of recurrent hospitalizations with various infectious complications, multiple anti-infective regimens, and surgical interventions required to treat fractures resulting from the patient's motorcycle accident, the patient was released without further medical problems.

In August 2010, patient 2, a 14-year-old boy from Kosovo, was transferred from a hospital in that country to the Department of Pediatrics, University Hospital (Graz, Austria) with multiple intra-abdominal abscesses and peritonitis. He had undergone an appendectomy in Pristina, Kosovo, in April 2010, after which abdominal sepsis developed. His travel history was completely unremarkable. On the day of admission, multiple-drug resistant *K. pneumoniae* was isolated from 5 sites (2 swab samples from the abdominal wound, 1 sample from the throat, 1 sample of secretion from an abdominal fistula, and 1 sample from stool). As of November 2010, the patient still required medical care and remained hospitalized.

Most plasmids with the carbapenemase enzyme $bla_{\text{NDM-1}}$ were shown to be readily transferable and prone to rearrangement, which indicates a potential to spread among bacterial populations (*3*). So far, NDM-1 carbapenemase has been detected in *K. pneumoniae*, *Escherichia coli*,

Table. Antimicrobial drug susceptibilities of New Delhi metallo-β-lactamase strains isolated from 2 patients, Graz, Austria, 2010*

	MIC, mg/L	
Drug	Patient 1 isolate	Patient 2 isolate
Colistin	0.125	0.125
Tigecycline	2	0.125
Amikacin	8	2

*Only substances for which isolates had susceptibility are listed. MICs were determined by the Etest method (AB BIODISK, Solna, Sweden). Susceptibility was determined according to relevant testing conditions and the new susceptibility interpretation standards proposed by the Clinical and Laboratory Standards Institute (www.clsi.org).

Citrobacter freundii, *Enterobacter cloacae,* and *Morganella morganii* and has shown resistance to nearly all classes of antibacterial agents, except polymyxins and tigecycline (*2*,*3*). Kumarasamy et al. recently reported the identification of 37 isolates with NDM-1 in the United Kingdom. The isolates came from 29 patients, of whom at least 17 had traveled to India or Pakistan in the year preceding identification of NDM-1; 14 patients had been admitted to a hospital in those countries (*2*).

NDM-1 has also been isolated from 3 patients in the United States, all of whom had recently received medical care in India (*7*). In contrast, 1 of the 2 patients with *K. pneumoniae*–carrying NDM-1 reported here was transferred to our hospital from Kosovo in southeastern Europe and had an unremarkable travel history. Immediate action is needed to control the spread of NDM-1 and avoid a worldwide public health problem.

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References

1. Yong D, Toleman MA, Giske CG, Walsh TR. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–54. DOI: 10.1128/AAC.00774-09
2. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010. Epub 2010 Aug 10.
3. Muir A, Weinbren MJ. New Delhi metallo-beta-lactamase: a cautionary tale. J Hosp Infect. 2010;75:239–40. DOI: 10.1016/j.jhin.2010.02.005
4. Krishna BV. New Delhi metallo-beta-lactamases: a wake-up call for microbiologists. Indian J Med Microbiol. 2010;28:265–6. DOI: 10.4103/0255-0857.66477
5. Carattoli A, Miriagou V, Bertini A, Loli A, Colinon C, Villa L, et al. Replicon typing of plasmids encoding resistance to newer beta-lactams. Emerg Infect Dis. 2006;12:1145–8.
6. Grisold AJ, Zarfel G, Strenger V, Feierl G, Leitner E, Masoud L, et al. Use of automated repetitive-sequence–based PCR for rapid laboratory confirmation of nosocomial outbreaks. J Infect. 2010;60:44–51. DOI: 10.1016/j.jinf.2009.10.045
7. Centers for Disease Control and Prevention. Detection of *Enterobacteriaceae* isolates carrying metallo-beta-lactamase—United States, 2010. MMWR Morb Mortal Wkly Rep. 2010;59:750.

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Carbapenemases in Enterobacteria, Hong Kong, China, 2009

To the Editor: Carbapenems are often the recommended treatment for serious infections caused by extended-spectrum β-lactamase–producing enterobacteria. However, enzyme-mediated carbapenem resistance is increasingly reported worldwide. Carbapenemases are represented by 3 molecular classes of β-lactamase: A, B, and D (*1*). The best known class A carbapenemase is *Klebsiella pneumoniae* carbapenemase (KPC); KPC-producing enterobacteria are responsible for many hospital outbreaks. Class B carbapenemases are metallo-β-lactamases (MBL), which have the widest substrate spectrum. Class D OXA-type carbapenemases are found mainly in nonfermenting bacteria, except for OXA-48, which has been found only in enterobacteria.

In Hong Kong Special Administrative Region, People's Republic of China, the Public Health Laboratory Centre routinely provides microbiological diagnostic services for government outpatient clinics and confirms the identity of bacterial isolates referred by other clinical laboratories. In 2009, among 18 enterobacteria isolates determined to be not susceptible to carbapenem, only 4 isolates—*Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, and *K. pneumoniae*—were confirmed to produce carbapenemase. The *E. coli* isolate was from a government outpatient clinic; the others were from a regional hospital laboratory (Table).

For all 4 isolates, the modified Hodge test (*2*) demonstrated enzyme activity against ertapenem and meropenem. Previously described PCR and sequencing methods (*1*) identified the MBL IMP-4 in the *C. freundii* and *K. pneumoniae* isolates; the *C. freundii* isolate also possessed extended-spec-

trum β-lactamase CTX-M-9. The *E. coli* isolate harbored the recently described MBL called New Delhi metallo-β-lactamase (NDM-1) (GenBank accession no. FN396876) from India (*3*). The *E. cloacae* isolate possessed a class A carbapenemase IMI-like (Nmc-type) gene, and DNA sequencing confirmed its 97.2% nt and 97.6% aa identity to IMI-1. This IMI allele was subsequently designated IMI-3 (GenBank accession no. GU015024). For all 4 enterobacteria isolates, PCR was negative for OXA-48.

MIC determination by Etest and VITEK 2 (bioMérieux, Marcy l'Etoile, France) showed that all 4 isolates were resistant to ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefoxitin, cefuroxime, cefotaxime, and ceftazidime, according to Clinical and Laboratory Standards Institute breakpoints (*2*). Because IMI-1 was inhibited by clavulanate and tazobactam, the corresponding resistance in the IMI-3 positive *E. cloacae* isolate might result from other mechanisms, possibly AmpC β-lactamase, although PCR results for common AmpC alleles were negative (*4*).

All 4 isolates showed resistance to all 3 carbapenems according to the Clinical and Laboratory Standards Institute MIC criteria updated in June 2010 (Table), except for the NDM-1 positive *E. coli* isolate, which had an intermediate MIC for meropenem of 2 μg/mL. The IMP-4 positive *C. freundii* and *K. pneumoniae* isolates also seemed to be more multidrug resistant; they were resistant to nalidixic acid, ciprofloxacin, nitrofurantoin, and co-trimoxazole and susceptible to only amikacin and gentamicin. Conversely, the 2 organisms harboring IMI-3 and NDM-1 were susceptible to all these agents except for the NDM-1–positive *E. coli*, which was resistant to amikacin and gentamicin.

IMP-4 in *Acinetobacter* spp. was first described in 2001 in a teaching hospital in Hong Kong (*5*). Since then, IMP-4 has been detected in several enterobacteria from mainland China and Australia. IMP-4 has spread throughout Hong Kong, crossing geographic and genus barriers; other new carbapenemases are also emerging. The association of IMP-4 with integrons and conjugative plasmids has been documented and possibly contributed to its propensity to spread. IMI-1 in *E. cloacae* was originally described in the United States in 1996. In 2005, IMI-2 (99% aa identity to IMI-1) in *Enterobacter asburiae* isolated from rivers in the United States was reported. (*6*), and in 2006, a blood culture *E. cloacae* was found to possess IMI-2 in Hangzhou, China (*7*).

We report IMI-3 (aa identity 97.6% to IMI-2) in a urine isolate of *E. cloacae*, possibly a colonizer rather than the causative agent of the urinary tract infection because the urine specimen did not contain any leukocytes. The 2 IMP-4–positive enterobacteria isolates were also only transiently present; repeated cultures did not yield any carbapenem-resistant organisms despite the patients not having received any targeted therapy. Nonetheless, the presence of these transferable resistance determinants among patients with prolonged hospitalization is cause for concern. The NDM-1–positive *E. coli* isolate came from an outpatient of Indian ethnicity, who had hypertension, diabetes, and a urinary tract infection that responded to ciprofloxacin. This isolate was thought to have originated from the Indian subcontinent, where the patient had spent 3 weeks in March 2009; he had not been hospitalized in India. A similar case of travel-related NDM-1–positive *E. coli* isolated from urine has also been recently reported in Australia (8).

NDM-1 has the potential to be a worldwide public health problem (*9*). Our findings highlight the threat of carbapenemase-mediated resistance. Scrupulous surveillance must be maintained, and clinical microbiology laboratories should have adequate knowledge and capacity to identify these resistance determinants. To control the dissemination of these resistance determinants, coordinated infection control responses are needed at local, national, and international levels (*10*).

This work was supported by the Government of Hong Kong, China.

Table. Antimicrobial susceptibility results and ESBL detected for 4 carbapenemase-harboring enterobacteria isolates, Hong Kong, 2009*

Organism	Patient age, y/ sex	Patient location	Specimen	MIC, μg/mL (CLSI breakpoint for resistance)†									ESBL/ carbapenemase detected
				IMP (≥4)	MEM (≥4)	ERT (≥1)	NA (≥32)	CIP (≥4)	NIT (≥128)	AK (≥64)	GN (≥16)	SXT (≥80)	
Citrobacter freundii	69/M	Hospital	Sputum	8	≥16	≥8	≥32	≥4	128	≤2	8	≥320	IMP-4, CTX-M-9
Klebsiella pneumoniae	60/M	Hospital	Bedsore	≥16	≥16	≥8	≥32	≥4	≥512	16	≤1	≥320	IMP-4
Enterobacter cloacae	68/F	Hospital	Urine	≥16	≥16	≥8	4	≤0.25	64	≤2	≤1	≤20	IMI-3
Escherichia coli	64/M	Outpatient clinic	Urine	4	2	4	≤2	≤0.25	≤16	≥256	≥16	≤20	NDM-1

*ESBL, extended-spectrum β-lactamase; IMP, imipenem; MEM, meropenem; ERT, ertapenem; NA, nalidixic acid; CIP, ciprofloxacin; NIT, nitrofurantoin; AK, amikacin; GN, gentamicin; SXT, co-trimoxazole; NDM-1, New Delhi metallo-β-lactamase.
†CLSI, Clinical and Laboratory Standards Institute, updated June 2010.

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References

1. Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. Clin Microbiol Rev. 2007;20:440–58. DOI: 10.1128/CMR.00001-07
2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 20th informational supplement (update 2010 Jun). Document M100–S20-U. Wayne (PA): The Institute; 2010.
3. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, et al. Characterization of a new metallo-β-lactamase gene, bla_{NDM-1}, and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob Agents Chemother. 2009;53:5046–54. DOI: 10.1128/AAC.00774-09
4. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev. 2009;22:161–81. DOI: 10.1128/CMR.00036-08
5. Chu YW, Afzal-Shah M, Houang ET, Palepou MI, Lyon DJ, Woodford N, et al. IMP-4, a novel metallo-beta-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. Antimicrob Agents Chemother. 2001;45:710–4. DOI: 10.1128/AAC.45.3.710-714.2001
6. Aubron C, Poirel L, Ash RJ, Nordmann P. Carbapenemase-producing *Enterobacteriaceae*, US rivers. Emerg Infect Dis. 2005;11:260–4.
7. Yu YS, Du XX, Zhou ZH, Chen YG, Li LJ. First Isolation of bla_{IMI-2} in an *Enterobacter cloacae* clinical isolate from China. Antimicrob Agents Chemother. 2006;50:1610–1. DOI: 10.1128/AAC.50.4.1610-1611.2006
8. Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P. Emergence of metallo-beta-lactamase NDM-1-producing multidrug resistant *Escherichia coli* in Australia. Antimicrob Agents Chemother. 2010;54:4914–6. DOI: 10.1128/AAC.00878-10
9. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010;10:597–602. DOI: 10.1016/S1473-3099(10)70143-2
10. Bilavsky E, Schwaber MJ, Carmeli Y. How to stem the tide of carbapenemase-producing *Enterobacteriaceae*?: proactive versus reactive strategies. Curr Opin Infect Dis. 2010;23:327–31. DOI: 10.1097/QCO.0b013e32833b3571

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Change in Age Pattern of Persons with Dengue, Northeastern Brazil

To the Editor: Approximately 40% of the world's population is at risk for dengue (*1*). Epidemiologic characteristics of dengue differ by region, and disease incidence varies by patient age. In Southeast Asia, incidence of dengue fever (DF) and dengue hemorrhagic fever (DHF) is highest among children (*2*,*3*); in the Western Hemisphere, incidence of these diseases is higher among adults.

In Brazil, which has ≈80% of dengue cases in the Western Hemisphere, adults are at risk for dengue virus (DENV) infection (*3*,*4*). However, in 2007, a total of 53% of persons in Brazil hospitalized with DHF were children <15 years of age; this proportion was highest (65.4%) in children in northeastern Brazil (*5*).

In Ceará, a state in northeastern Brazil, DENV-1 epidemics have occurred since 1987. DHF cases have been reported since 1994 when DENV-2 was identified. In 2003, a severe DENV-3 epidemic occurred, and DHF incidence was high among adults (*6*). However, since 2007, incidence of DENV infection has been highest among children (*7*). To better understand factors that could affect this change in risk by age group, we studied the temporal progression of age distribution of persons with dengue during 1998–2008 in Ceará.

We used data for Ceará from the National System of Notifiable Diseases (DF and DHF cases), the Hospital Admission Data System (dengue hospitalizations) (*8*), and the Central Public Health Laboratory (virus isolation). For each age group (<10, 10–19, 20–59, and ≥60 years), we calculated incidence of DF and hospitalization rate for DHF. We also calculated proportions of dengue serotypes per year (2001–2008). Medians for continuous variables were compared by using the Kruskal-Wallis test. Analyses were performed by using Epi Info version 6.0 software (Centers for Disease Control and Prevention, Atlanta, GA, USA).

From 1998 (10.8 cases/100,000 persons) through 2007 (236.7 cases/100,000 persons), DF incidence was lowest among persons <10 years of age. However, the incidence was highest (599.4 cases/100,000 persons) for this age group in 2008. In 2007, incidence among persons <10 years of age (236.7 cases/100,000 persons) was similar to that among persons 10–19, 20–59, and ≥60 years of age (305.6, 331.5, and 249.9 cases/100,000 persons, respectively).

Since 2007, the incidence of DHF among children was already higher (4,884 cases/100,000 persons) than among the other age groups (3,261, 3,387 and 2,789 cases/100,000 persons, respectively). In 2008, incidence of DHF among children was 8,992 cases/100,000 persons, which was 2× that among persons 10–19 and 20–59

years of age and >3× that among persons ≥60 years of age. Median age of persons with DHF decreased from 38 years in 2001 to 18 years in 2008 (p<0.0001). Children <10 years age, who in 2001 accounted for 5% of all cases, accounted for 33% of cases in 2008.

The hospitalization rate for dengue among children in Ceará followed a pattern similar to that for DHF and increased for children ≤10 years of age. In 2008, this pattern was greater for this age group (1.449/1,000 hospitalizations) than in any other age group. DENV-2 (52.3%) and DENV-1 (47.7%) were co-circulating in 2002. DENV-3 was isolated in 2003 and represented >40% of isolations. At this time, DENV-2 and DENV-1 represented 7.4% and 48.5% of isolations, respectively. DENV-3 then predominated in Ceará until 2006 when DENV-2 reemerged (1.4%). DENV-2 became the predominant serotype in 2007 (84%) and 2008 (76.1%) (Table).

The increase in DHF incidence among children in Ceará during 2007–2008 was greater than the overall increase in Brazil (*4*,*5*). Because the predominant serotype in Ceará in 2007–2008 was DENV-2, two hypotheses may explain this phenomenon.

First, a more virulent DENV-2 may have been introduced. Genetic sequencing of DENV-2 circulating in another state in Brazil during the 2008 epidemic, compared with the 1990 and 1998 epidemics, showed that all isolates had the same genotype (American/Asiatic); only a 2% had a phylogenetic change (*9*). Such a small difference cannot explain this change in the age group affected by dengue.

Second, the time when 3 serotypes circulated in Ceará may not have favored development of antibodies against DENV-3 in children <10 years of age, although they were susceptible to DENV-2. DENV-1 and DENV-2 were circulating in Ceará before 2002 and caused DF epidemics and a few DHF cases. However, these diseases occurred predominantly in adults. Conversely, children had little likelihood of being infected with DENV-2 because the incidence of dengue before 2002 was low and the 2000 birth cohort had little contact with DENV-2, which was no longer circulating. Thus, most persons susceptible to DENV-2 were children.

DENV-3 was circulating during 2003–2006 and affected persons of all ages. Thus, when DENV-2 reemerged in 2006, many adults in Ceará already had antibodies against it. However, children had no antibodies against DENV-2, although some had antibodies against DENV-3. This immunologic difference may have caused the higher incidence of dengue among children in Ceará during 2007–2008, particularly in view of severity of the 2 epidemics, increased risk for DHF (*10*), and number of hospitalizations for dengue during this period.

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Table. Confirmed cases of DF and DHF, Ceará, Brazil, 1998–2008*

Characteristic	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
No. DF cases	3,581	9,757	13,645	34,390	16,465	23,796	3,094	22,817	25,569	25,026	44,508
No. DHF cases	4	3	4	82	72	292	14	199	173	280	408
Serotype of DENV isolated, %											
1	–	–	–	47.7	48.5	1.9	0	2.5	0	0	0
2	–	–	–	52.3	7.4	1.9	0	0.0	1.4	84.0	76.1
3	–	–	–	0	44.1	96.2	100.0	97.5	98.6	16.0	23.9
Incidence of DF and DHF by age, y†											
<10	10.8	37.8	65.4	174.7	78.5	128.0	14.3	126.5	116.0	236.7	599.4
10–19	26.7	95.4	129.3	321.4	160.6	250.4	34.3	198.2	247.9	305.6	574.4
20–59	68.3	198.7	263.6	659.3	304.3	416.9	53.6	365.2	412.6	331.5	521.9
≥60	143.1	210.0	194.8	423.4	223.3	313.0	39.1	441.5	422.2	249.9	301.0
Incidence of DHF by age, y†											
<10	0.000	0.000	0.000	0.236	0.114	1.423	0.068	1.011	0.890	4.884	8.992
10–19	0.062	0.000	0.059	0.796	0.449	3.601	0.065	2.512	1.771	3.261	4.968
20–59	0.064	0.094	0.058	1.361	1.474	4.655	0.318	3.074	3.053	3.387	4.358
≥60	0.174	0.000	0.152	1.890	1.289	5.134	0.000	2.650	0.832	2.789	2.868
Hospitalizations for dengue (classic and hemorrhagic) by age, y‡											
<10	0.008	0.014	0.015	0.050	0.145	0.352	0.073	0.395	0.266	0.667	1.449
10–19	0.006	0.010	0.020	0.131	0.255	0.632	0.156	0.646	0.497	0.750	1.128
20–59	0.005	0.011	0.024	0.256	0.440	0.847	0.255	0.804	0.572	0.710	0.786
≥60	0.009	0.009	0.021	0.401	0.658	1.159	0.389	1.012	0.664	0.954	0.922

*Source: Hospitalization Information System, Notifiable Disease Information System, and Central Public Health Laboratory of Ceará. DF, dengue fever; DHF, dengue hemorrhagic fever, DENV, dengue virus; –, no information.
†Per 100,000 persons.
‡Per 1,000 persons.

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References

1. World Organization Health. Dengue and dengue haemorragic fever fact sheet 217. 2009 [cited 2010 Aug 10]. http://www.who.int/mediacentre/factsheets/fs117/en/index.html.
2. Halstead SB. Dengue in the Americas and Southeast Asia: do they differ? Rev Panam Salud Publica. 2006;20:407–15. DOI: 10.1590/S1020-49892006001100007
3. Siqueira JB Jr, Martelli CM, Coelho GE, Simplício AC, Hatch DL. Dengue and dengue hemorrhagic fever, Brazil, 1981–2002. Emerg Infect Dis. 2005;11:48–53.
4. Teixeira MG, Costa Mda C, Barreto F, Barreto ML. Dengue: twenty-five years since reemergence in Brazil. Cad Saude Publica. 2009;25(Suppl 1):S7–18. DOI: 10.1590/S0102-311X2009001300002
5. Teixeira MG, Costa MC, Coelho GE, Barreto ML. Recent shift in age pattern of dengue hemorrhagic fever, Brazil. Emerg Infect Dis. 2008;14:1663. DOI: 10.3201/eid1410.071164
6. Cavalcanti LP, Coelho IC, Vilar DC, Holanda SG, Escóssia KN, Souza-Santos R. Clinical and epidemiological characterization of dengue hemorrhagic fever cases in northeastern Brazil. Rev Soc Bras Med Trop. 2010;43:355–8.
7. Vilar DC. Aspectos clínicos e epidemiológicos do dengue hemorrágico no Ceará, no período de 1994 a 2006. Dissertação (Mestrado). Ceará (Brazil): Escola Nacional de Saúde Pública; 2008 [cited 2010 Aug 10]. http://bvssp.icict.fiocruz.br/lildbi/docsonline/4/3/1734-Vilardclfm.pdf
8. Brasil, Ministério da Saúde. Datasus [cited 2010 Aug 10]. http://www2.datasus.gov.br/DATASUS/index.php?area=0206
9. Oliveira MF, Araújo JM, Ferreira OC Jr, Ferreira DF, Lima DB, Santos FB, et al. Two lineages of dengue virus type 2, Brazil. Emerg Infect Dis. 2010;16:576–8.
10. Halstead SB. The Alexander D. Langmuir lecture. The pathogenesis of dengue. Molecular epidemiology in infections disease. Am J Epidemiol. 1981;114:632–48.

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Apophysomyces variabilis Infections in Humans

To the Editor: The fungus *Apophysomyces elegans* (order Mucorales) is a thermotolerant species that causes severe infections among humans. In contrast to other fungi that cause zygomycosis, which have a worldwide distribution and are rarely found in immunocompetent hosts, *A. elegans* has been reported mainly in areas with warm climates as an emerging pathogen that causes mostly cutaneous infections after injury to the skin (*1*). This fungus was discovered in 1979 (*2*) and until recently was considered the only species in the genus.

A polyphasic study of clinical and environmental strains of *A. elegans,* including analysis of several genes, showed that the genus contained 4 well-characterized species (*3*). Of 16 isolates tested in this study, only 2 from soil in India were *A. elegans*. Most of the isolates were *A. variabilis*. The incidence of *A. variabilis* in humans is unknown and difficult to ascertain because most cases had isolates that were not properly preserved. These fungi usually cause necrotizing fasciitis, but rhino-orbito-cerebral or renal infections have also been reported (*1*). Whether these infections are produced by different *Apophysomyces* spp., have different responses to antifungal drugs, or have differences in virulence is unknown.

To assess incidence of *Apophysomyces* spp. in a tertiary hospital (Government Medical College Hospital, Chandigarh, India), which usually receives patients with zygomycosis, a retrospective study was conducted during November 2001–April 2009. Nine patients were identified as having primary cutaneous zygomycosis. For 4 patients, fungal isolates were morphologically identified as *A. elegans.* A description of clinical findings, their management, and outcomes for these 9 patients has been reported (*4*). The 4 isolates were sent to the Universitat Rovira i Virgili (Reus, Spain) for molecular analysis.

The internal transcribed spacer region of these isolates was sequenced and compared with those of type strains of *Apophysomyces* spp. Fungi were identified by morphologic (Figure, panel A) and molecular analysis as *A. variabilis* (99.6%–99.7% sequence identity with sequence of type strain CBS 658.93 [FN556436]). GenBank accession nos. of the 4 isolates are FN813491, FN813490, FN556442, and FN813492.

Another patient was also infected with *A. variabilis* fungi. The patient was a 45-year-old woman with diabetes from Derabassi (Punjab), India, who was hospitalized because of swelling in her right breast and blackening of overlying skin. A diagnosis of right breast gangrene was made. Therefore, local debridement of the swelling was conducted, and tissue samples were tested by microbiologic culture and histopathologic analysis.

A KOH wet mount showed broad aseptate hyphae with right-angled branching. The fungal isolate was tentatively identified as *A. elegans*. Histopathologic analysis confirmed a diagnosis of zygomycosis. The patient was treated under local anesthesia by debridement of infected tissue and some of the healthy surrounding tissue (Figure, panel B). However, an antifungal regimen could not be given because she had disturbed renal function. Her condition deteriorated, septicemia was observed, and she died from sudden cardiac arrest on the sixth day after admission. The fungal isolate was also identified as *A. variabilis* (98.9% identity, GenBank accession no. FN556443).

Although most cases of infection with *A. variabilis* fungi have been reported in India (*5*), infections with this fungus may have a wider distribution. A recent study demonstrated that this species represented 0.5% of fungi

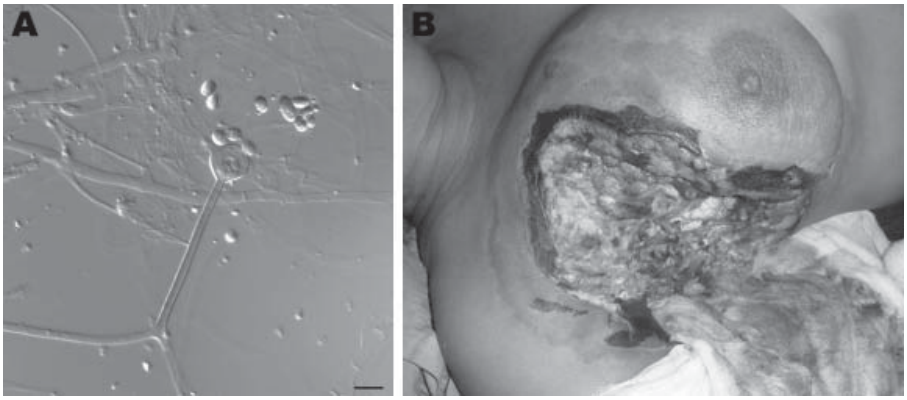

Figure. A) Sporangiophore (center) and sporangiospores of *Apophysomyces variabilis* fungi. Scale bar = 10 μm. B) Clinical manifestations in a woman infected with *A. variabilis* fungi in the upper part of the chest and the breast. A color version of this figure is available online (www.cdc.gov/EID/content/17/1/134-F.htm).

of the order Mucorales isolated from clinical samples in the United States (*6*). Furthermore, a high mortality rate and the fact that most of these infections involve otherwise healthy patients make this a serious infection.

The number of infections with *Apophysomyces* spp. is underestimated because these fungi do not usually sporulate on standard fungal culture media used in clinical laboratories. These fungi require special nutrient-deficient growth medium (Czapek agar), a high temperature in comparison to other human pathogens (37°C–42°C), and prolonged incubation (7–10 days) (*7*).

A difference in mortality rate was observed when we compared patients in our study (80%) with those reported by Chakrabarti et al. (*5*) (28.5%) in India, even though treatment was generally similar, i.e., local débridement and amphotericin B. Other differences in our study were that the infection in 4 patients was preceded by intramuscular injection, and these 2 patients had diabetes mellitus.

In conclusion, *A. variabilis* is an emerging pathogenic fungus that can cause rapid and fatal infections in humans. As more isolates of *Apophysomyces* fungi become available, molecular typing studies must be conducted to better understand the epidemiology and distribution of different *Apophysomyces* spp.

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References

1. Meis JF, Chakrabarti A. Changing epidemiology of an emerging infection: zygomycosis. Clin Microbiol Infect. 2009;15(Suppl 5):10–4. DOI: 10.1111/j.1469-0691.2009.02973.x
2. Misra PC, Srivastava KJ, Lata K. *Apophysomyces*, a new genus of the Mucorales. Mycotaxon. 1979;8:377–82.
3. Alvarez E, Stchigel AM, Cano J, Sutton DA, Fothergill AW, Chander J, et al. Molecular phylogenetic diversity of the emerging mucoralean fungus *Apophysomyces*: proposal of three new species. Rev Iberoam Micol. 2010;27:80–9. DOI: 10.1016/j.riam.2010.01.006
4. Chander J, Kaur J, Attri A, Mohan H. Primary cutaneous zygomycosis from a tertiary care centre in north-west India. Indian J Med Res. 2010;131:765–70.
5. Chakrabarti A, Ghosh A, Prasad GS, David JK, Gupta S, Das A, et al. *Apophysomyces elegans*: an emerging zygomycete in India. J Clin Microbiol. 2003;41:783–8. DOI: 10.1128/JCM.41.2.783-788.2003
6. Alvarez E, Sutton DA, Cano J, Fothergill AW, Stchigel A, Rinaldi MG, et al. Spectrum of zygomycete species identified in clinically significant specimens in the United States. J Clin Microbiol. 2009;47:1650–6. DOI: 10.1128/JCM.00036-09
7. Padhye AA, Ajello L. Simple method of inducing sporulation by *Apophysomyces elegans* and *Saksenaea vasiformis.* J Clin Microbiol. 1988;26:1861–3.

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Letters

Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 Figure or Table and should not be divided into sections. All letters should contain material not previously published and include a word count.

Fatal *Vibrio vulnificus* Infection Associated with Eating Raw Oysters, New Caledonia

To the Editor: The bacterium *Vibrio vulnificus* is a marine flora saprophyte that can cause necrotic skin infection and septicemia in humans who eat shellfish. Symptoms of septicemia (mortality rate >50%) have been described mostly in Florida and Japan among persons who ate raw filter-feeding shellfish when seawater temperatures are >20°C (*1*).

V. vulnificus–related septicemia introduced through the digestive system appears within 7 days after ingestion (*2*). Clinical signs and symptoms include fever, collapse, and metastatic necrotic skin lesions. We report 3 patients from New Caledonia who died after *V. vulnificus* infection, which they probably acquired by eating contaminated oysters. These patients were hospitalized during February–May 2008 at Noumea Hospital (Noumea, New Caledonia).

Patient 1 was a 51-year-old man with fever, muscle pains, bleeding gums, and a history of alcohol abuse; within 48 hours after symptom onset, he died of septic shock, with diffuse ecchymoses and purpura. Patient 2 was a 67-year-old woman with no known concurrent conditions who was admitted to the hospital with chills, diarrhea, and vomiting; septic shock developed, with painful erythematous plaques on the lower limbs becoming foamy, confluent, and necrotic. Patient 3 was a 74-year-old woman with untreated lupus who was hospitalized with lower-limb edema, hypotension, hypothermia, and erythematous skin lesions. All 3 patients received cephalosporins but died of multiple organ failure within 12 hours after hospital admission.

Peripheral blood aerobic–anaerobic samples were taken from all patients, stored in BacT/Alert FA vials (bioMérieux, Marcy-l'Etoile, France), and incubated in the BacT/Alert 3D system (bioMérieux). Curved mobile gram-negative bacilli were isolated from blood samples cultured on conventional media without additional salt within 24 h after incubation at 37°C in a 5% CO_2-enriched atmosphere. *V. vulnificus* was identified through the Vitek2 system (bioMérieux) and confirmed by using the Api 20E system (bioMérieux).

Strains were sent to the Centre National de Reference des Vibrions et du Choléra, (Institut Pasteur, Paris, France), which by PCR confirmed the gene encoding virulence-associated hemolysin, a species-specific marker (*3*). Molecular typing by pulsed-field gel electrophoresis was performed to assess possible clonality of the strains.

Several studies have shown the genomic diversity among environmental and clinical *V. vulnificus* isolates. The use of genotyping methods has identified >100 *V. vulnificus* strains in a single oyster (*4*) and notable heterogeneity among clinical isolates from multiple patients, even if a unique pathogenic strain causes the infection in each patient. Thus, *V. vulnificus* infections within a large population at risk may result from rare events controlled more by the host than by the bacterial strain (*5*).

Pulsed-field gel electrophoresis genotype analysis enabled us to divide the strains into 2 groups. One group included the isolate from patient 1, and the other group included isolates from patients 2 and 3, which despite having slightly different *Not*I and *Sfi*I patterns reflecting genetic rearrangement, clearly belonged to a single clone. Isolation of strains with such a high degree of homogeneity is not common, raising the question of the existence of *V. vulnificus* clones that are particularly virulent or adapted to humans. Currently, however, reliable markers for determining *V. vulnificus* virulence do not exist. Thus, no genotyping system is likely to be useful for rapidly identifying strains that affect public health (*6*). *V. vulnificus*–related analysis requires the assumption that all strains are virulent.

Epidemiologic information collected from patients' families indicated recent consumption of raw oysters. Two of the 3 cases occurred within a short time frame and were associated with eating local oysters harvested on the west coast of New Caledonia.

The literature mentions few cases of *V. vulnificus* infection in the South Pacific. Cases described were isolated, rarely fatal, and involved infection through the skin (*7*–*10*). The *V. vulnificus* infections we report may be related to the emergence of a new clone or to changes in the climate or environmental conditions. New Caledonia experienced unusual weather conditions during the first half of 2008 (heavy rains and exceptionally high temperatures). These specific conditions may have favored higher sea surface temperatures, lower salinity, increased turbidity, and subsequent multiplication of *V. vulnificus* in seawater.

A range of projects were implemented to train practitioners to recognize potential *V. vulnificus* infections. Local health authorities issued criteria for defining suspected cases of *V. vulnificus* infection and recommendations for early medical care of patients with clinical symptoms. Methods of detecting the bacterium in human and animal health laboratories were improved, particularly by the systematic use of selective media in the event of suspected clinical *V. vulnificus* infection and standardized reporting of *V. vulnificus* isolation. Preventive measures, such as improving microbial surveillance and warning consumers about risks associated with eating raw seafood, are essential to help reduce the risk for *V. vulnificus*–induced illness.

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References

1. Koenig KL, Mueller J, Rose T. *Vibrio vulnificus*: hazard on half shell. West J Med. 1991;155:400–3.
2. Hlady WG, Klontz KC. The epidemiology of *Vibrio* infections in Florida, 1981–1993. J Infect Dis. 1996;173:1176–83.
3. Brauns LA, Hudson MC, Oliver JD. Use of the polymerase chain reaction in detection of culturable and nonculturable *Vibrio vulnificus* cells. Appl Environ Microbiol. 1991;57:2651–5.
4. Buchrieser C, Gangar VV, Murphree RL, Tamplin ML, Kaspar CW. Multiple *Vibrio vulnificus* strains in oysters as demonstrated by clamped homogeneous electric field gel electrophoresis. Appl Environ Microbiol. 1995;61:1163–8.
5. Tamplin ML, Jackson JK, Buchrieser C, Murphree RL, Portier KM, Gangar V, et al. Pulsed-field gel electrophoresis and ribotype profiles of clinical and environmental *Vibrio vulnificus* isolates. Appl Environ Microbiol. 1996;62:3572–80.
6. Sanjuán E, Fouz B, Oliver JD, Amaro C. Evaluation of genotypic and phenotypic methods to distinguish clinical from environmental *Vibrio vulnificus* strains. Appl Environ Microbiol. 2009;75:1604–13. DOI: 10.1128/AEM.01594-08
7. Ralph A, Currie BJ. *Vibrio vulnificus* and *V. parahaemolyticus* necrotising fasciitis in fishermen visiting an estuarine tropical northern Australian location. J Infect. 2007;54:e111–4. DOI: 10.1016/j.jinf.2006.06.015
8. Wise KA, Newton PJ. A fatal case of *Vibrio vulnificus* septicemia. Pathology. 1992;24:121–2. DOI: 10.3109/00313029209063637
9. Preda TC, Preda VA, Mekisic AP. Septic shock from penetrating leg injury with *Vibrio vulnificus* infection. Med J Aust. 2009;190:716.
10. Upton A, Taylor S. *Vibrio vulnificus* necrotising fasciitis and septicaemia. N Z Med J. 2002;115:108–9.

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Vibrio vulnificus

[vĭb’re-o vŭl-nĭf’ĭ-kəs]

From the Latin *vibrio* (to move) and *vulnificus* (causing wounds). *Vibrio vulnificus* is a virulent, gram-negative, comma-shaped, motile bacterium that belongs to the family Vibrionaceae. In 1976, researchers at the Centers for Disease Control identified it as a *Vibrio* sp. and possible emerging pathogen. Because of its association with blistering skin infections, the bacterium was named *Vibrio vulnificus* in 1979.

Source: Farmer JJ III. Vibrio (“*Beneckea*”) *vulnificus*, the bacterium associated with sepsis, septicaemia and the sea. Lancet. 1979;2:903; Hollis DG, Weaver RE, Baker CN, Thornsberry C. Halophilic *Vibrio* species isolated from blood cultures. J Clin Microbiol. 1976;3:425–31; Todar K. Todar’s online textbook of bacteriology. *Vibrio vulnificus*. [cited 2010 Nov 24]. http://textbookofbacteriology.net/v.vulnificus.html; Dorland’s illustrated medical dictionary. 31st ed. Philadelphia: Saunders Elsevier; 2007.

Empyema caused by MRSA ST398 with Atypical Resistance Profile, Spain

To the Editor: We report a case of empyema caused by methicillin-resistant *Staphylococcus aureus* (MRSA) sequence type ST398 in a 79-year-old man in Spain who had severe chronic obstructive pulmonary disease. In 2009, the patient was hospitalized in the intensive care unit because of decompensation of his chronic obstructive pulmonary disease, profound iliofemoral venous thrombosis, right pneumothorax, and lung carcinoma. Thoracic drainage, support measures, and intravenous levofloxacin were initiated, but no clinical improvement was seen. Purulent exudates from the thoracic drainage tube and of a tracheal aspirate were cultured. MRSA was isolated from both samples and from a nasal swab. Antimicrobial drug therapy was changed from levofloxacin to intravenous linezolid, but the patient's clinical situation rapidly worsened, and he died of multiorgan failure.

The 3 MRSA isolates were typed (multilocus sequence typing-, *spa*-, staphylococcal cassette chromosome [SCC] *mec*-, and *agr*-typing, in addition to pulsed-field gel electrophoresis [PFGE]), likewise, the antimicrobial drug–resistance phenotypes and genotypes, and virulence genes were determined (*1*,*2*). All 3 MRSA isolates were typed as sequence type (ST) 398, *spa*-type t011, SCC*mec*V, and *agr*I. The 3 isolates had the same resistance phenotype, including to β-lactams, tetracycline, clindamycin (but not erythromycin), ciprofloxacin, and levofloxacin. We confirmed the presence of *mec*A, *tet*M, *tet*K, and *vga*(A) genes by PCR and sequencing; however, PCRs for *lnu*(A), *lnu*(B), *lnu*(C), *lnu*(D), *cfr*, *vga*(C), *lsa*(B), and *tetL* genes were negative. Primers used for detection of *vga*(A) gene were F-5′-GAAACTCTTATTC GAACYATTCTAGC-3′ and R-5′-GGTTCAATACTCAATCGACT-GAG-3′. Specific amino acid changes in the quinolone-determining region of GyrA and ParC proteins (S84L and S80F, respectively) were detected by PCR and sequencing (*1*). All 3 MRSA isolates were negative by PCR for the Panton-Valentine leukocidin, toxic shock syndrome toxin 1, and exfoliative toxins A and B.

The patient lived with his wife and 2 sons near a pig farm. Both sons worked on the farm; the patient, but not his wife, helped sporadically on the farm. Nasal samples from the 3 family members indicated MRSA carriage in 1 son but not in the other son or the patient's wife. The characteristics of the nasal MRSA strain recovered from the son were identical to those previously detected in MRSA strains from the patient (Table). In addition, nasal swabs from 18 pigs on the farm were randomly taken, and MRSA isolates were detected in 9 (50%) pigs; 1 MRSA isolate per animal was further characterized. Eight isolates were typed as ST398/t011/SCC*mec*V/*agr*I, and the remaining one as ST398/t1451/SCC*mec*V/*agr*I. All animal isolates had the same resistance phenotype and genotype as the MRSA isolates from the patient and son. None harbored the studied virulence factors (Table). All isolates had an unusual clindamycin-resistance/erythromycin-susceptibility phenotype and harbored the *vga*(A) gene.

We analyzed all MRSA isolates of human and animal origins by *Apa*I-PFGE (*2*) and compared patterns as previously recommended (*3*). Only 1 pulsotype (A) and 3 closely related subtypes were identified (A1, A2, and A3). One MRSA isolate obtained from pleural fluid of the patient, 2 isolates from nasal swabs (patient and son), and most isolates from animals showed the same PFGE pulsotype and subtype (A1). Alternatively, 1 MRSA isolate from bronchial aspirate of the patient and 2 isolates from animals showed closely related patterns (subtypes A2 and A3).

Other studies have suggested clonal spread and transmission of MRSA ST398 between pigs and persons who work with them (*4*,*5*). This microorganism has been generally associated with skin and soft tissue infections in humans (*6*). Nevertheless, severe infections by ST398 also have been sporadically described, and the report of 7 pneumonia cases associated with mechanical ventilation in central Europe is relevant (*7*). In general, ST398 isolates have fewer virulence factors than do other clones of MRSA (*2*); nonetheless, human infections from Panton-Valentine leukocidin–positive ST398 isolates have been reported (*8*). The immunocompromised status of patients in intensive care units could favor dissemination of ST398 in this environment.

MRSA ST398 implicated in the described empyema was resistant to the first-line antimicrobial agent used for treatment (levofloxacin, MIC 4 mg/L) that was associated with amino acid changes in GyrA and ParC proteins, which could have accelerated the deteriorating evolution of the patient's respiratory infection. The atypical clindamycin-resistance/erythromycin-susceptibility phenotype detected in our human and animal MRSA strains is infrequently detected in clinical MRSA isolates from humans. Nevertheless, this phenotype might be emerging among livestock MRSA isolates, as we and others (*9*,*10*) have observed. The *vga*(A) gene detected in these isolates could be responsible for this resistant phenotype, as has been recently reported by others (*10*).

In conclusion, we report potential pig-to-human transmission of MRSA ST398. MRSA ST398 can be associated with severe respiratory pathology in immunocompromised patients, and these microorganisms could also be resistant to other first-line antimicrobial agents, such as fluoroquino-

Table. Characteristics of methicillin-resistant *Staphylococcus aureus* strains recovered from humans and animals, Spain, 2009*

Strain	Origin	SCC*mec* type	MLST/*spa* type	*agr*	PFGE	Antimicrobial resistance phenotype	Resistance genes detected	Amino acid change	
								GrlA	GyrA
C2355	Patient, pleural fluid	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2354	Patient, bronchial aspirate	V	ST398/t011	I	A2	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2634	Patient, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2664	Son, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2669	Pig 1, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2670	Pig 2, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2694	Pig 3, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2695	Pig 4, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2697	Pig 5, nasal swab	V	ST398/t011	I	A3	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2698	Pig 6, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2700	Pig 7, nasal swab	V	ST398/t011	I	A3	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2704	Pig 8, nasal swab	V	ST398/t011	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L
C2706	Pig 9, nasal swab	V	ST398/t1451	I	A1	OXA-CLI-TET-CIP-LEV	*mecA*, *tetK*, *tetM*, *vga*(A)	S80F	S84L

*All strains were susceptible to fusidic acid, fosfomycin, gentamicin, tobramycin, mupirocin, trimethoprim-sulfamethoxazole, vancomycin, teicoplanin, quinupristin/dalfopristin, and tigecycline. SCC, staphylococcal cassette chromosome; MLST, multilocus sequence typing; PFGE, pulsed-field gel electrophoresis; ST, sequence type; OXA, oxacillin; CLI, clindamycin; TET, tetracycline; CIP, ciprofloxacin; LEV, levofloxacin.

lones, used to treat these infections. Moreover, the unusual clindamycin-resistance/erythromycin-susceptibility phenotype might be a key marker (in addition to tetracycline resistance) for the possible presence of livestock-associated MRSA.

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References

1. Aspiroz C, Lozano C, Vindel A, Lasarte JJ, Zarazaga M, Torres C. Skin lesion caused by ST398 and ST1 MRSA, Spain. Emerg Infect Dis. 2010;16:157–9. DOI: 10.3201/eid1601.090694
2. Kadlec K, Ehricht R, Monecke S, Steinacker U, Kaspar H, Mankertz J, et al. Diversity of antimicrobial resistance pheno- and genotypes of methicillin-resistant *Staphylococcus aureus* ST398 from diseased swine. J Antimicrob Chemother. 2009;64:1156–64. DOI: 10.1093/jac/dkp350
3. 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.
4. Huijsdens XW, van Dijke BJ, Spalburg E, van Santen-Verheuvel MG, Heck ME, Pluister GN, et al. Community-acquired MRSA and pig-farming. Ann Clin Microbiol Antimicrob. 2006;5:26. DOI: 10.1186/1476-0711-5-26
5. Morgan M. Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? J Antimicrob Chemother. 2008;62:1181–7. DOI: 10.1093/jac/dkn405
6. van Loo I, Huisdens X, Tiemersma E, de Neeling A, van de Sande-Bruinsma N, Bearijlan D, et al. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. Emerg Infect Dis. 2007;13:1834–9.
7. Witte W, Strommenger B, Stanek C, Cuny C. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg Infect Dis. 2007;13:255–8. DOI: 10.3201/eid1302.060924
8. Welinder-Olsson C, Florén-Johansson K, Larsson L, Oberg S, Karlsson L, Ahrén C. Infection with Panton-Valentine leukocidin-positive methicillin-resistant *Staphylococcus aureus* t034. Emerg Infect Dis. 2008;14:1271–2. DOI: 10.3201/eid1408.071427

9. 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. DOI: 10.1371/journal.pone.0004258
10. Kadlec K, Pomba CF, Couto N, Schwarz S. Small plasmids carrying vga(A) or vga(C) genes mediate resistance to lincosamides, pleuromutilins and streptogramin A antibiotics in methicillin-resistant *Staphylococcus aureus* ST398 from swine. J Antimicrob Chemother. 2010;65:2692–3. DOI: 10.1093/jac/dkq365

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Intensive Care Unit Admission for Pandemic (H1N1) 2009, Reunion Island, 2009

To the Editor: We report results of the prospective surveillance system established in the largest intensive care unit (ICU) of Reunion Island (25 beds). This system covers 500,000 residents (62% of the total population) and monitors the daily status of patients >17 years of age who had a positive reverse transcription–PCR (RT-PCR) for pandemic (H1N1) 2009 virus. Reunion Island is a French overseas territory in the Southern Hemisphere, with health care facilities similar to those of mainland France. Patients were followed up until discharge from the ICU or death. Data were collected during July 15–September 30, 2009.

Of 148 patients with confirmed pandemic (H1N1) 2009 infection admitted to the hospital, 13 (9%) patients (8 female) were admitted to the ICU. These corresponded to 7% of all 187 patients admitted to the ICU during the same period. Median age was 39.4 (±19) years (range 17–69 years). Ten patients were admitted for respiratory failure related to viral pneumonitis, 1 for pulmonary edema with severe chronic coronary insufficiency, 1 for congenital adrenal insufficiency with reversible multiple organ failure, and 1 for status epilepticus. Eleven (85%) patients had underlying concurrent medical conditions: 3 were overweight (body mass indexes 38, 32, and 29.3 kg/m^2); 1 was pregnant and had asthma.

Four (31%) patients died. One was a 28-year-old woman with cerebral motor infirmity and severe chronic restrictive respiratory failure. An 18-year-old woman with aplasia after receipt of an allograft for Hodgkin lymphoma died of cerebral hemorrhage while receiving extracorporeal membrane oxygenation. A 52-year-old man admitted for pulmonary edema with severe coronary insufficiency died of multiple organ failure. A 33-year-old man with no known concurrent medical conditions died of acute respiratory distress syndrome. Time from ICU admission to death ranged from 15 to 85 days (mean 36.5 ± 32 days). Mean age of patients who died was 32.5 ± 14.3 years.

Chest radiographic findings were abnormal for all patients except 1, who was admitted for fever and convulsions (Huntington chorea). Bilateral pulmonary embolism was confirmed in an obese patient who survived.

Mean time between onset of clinical signs and ICU admission was 6.9 ± 3.2 days. Mean time between admission to ICU with diagnosis confirmed by RT-PCR and initiation of antiviral treatment was 1.8 ± 1.7 days and between illness onset and initiation of antiviral treatment, 8.8 ± 3 days (range 4–16 days). Mean length of ICU stay was 26.3 ± 29.3 days. Patients remained in the ICU for a total of 201 bed-days (402 per million residents). The maximum daily occupancy of the ICU was 10 beds per million residents.

Five patients received steroids for severe hypotension or asthma-like clinical illness. Severe hypotension developed in 5 patients, and they received vasopressors. No patient received intravenous immunoglobulins. Ten (77%) patients required mechanical ventilation for a median of 11.5 ± 12.2 days. One patient required high-frequency ventilation, 3 required extracorporeal membrane oxygenation, and 1 required hemodialysis. Multiple organ failure developed in 3. All patients were empirically given antibacterial drugs. Secondary infections were either documented or strongly suspected for 5 patients.

All patients received oral oseltamivir beginning 4–16 days after illness onset and continuing for 2–17 days (mean 7.2 ± 4.3). Zanamivir was administered 1 time by inhalation through the ventilator. Viral loads in respiratory specimens ranged from 4×10^3 to 6.9×10^7 copies/mL (mean 1.4×10^5). Two patients excreted virus in their bronchoalveolar lavage specimens for a prolonged time (14 days).

The most prominent biological findings were elevated serum levels of procalcitonin, C-reactive protein, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and creatine kinase. Eight patients had lymphopenia (<1,200 cells/mm^3).

Our findings are consistent with findings of other studies of severe or fatal viral pneumonia in younger patients than are usually affected in a normal influenza season (*1–4*), particularly in patients with concurrent medical conditions. In our study, the 3 overweight patients survived. Obesity is associated with increased severity of illness, but not always with death, in critically ill patients (*5*). We confirm that previously healthy young persons can die of pandemic (H1N1) 2009, although at a much lower rate than those infected in the initial outbreaks

in Mexico (*6*) and the United States (*7*). In the United States, several pregnant women died, and the hospitalization rate for pregnant women was 4× higher than for the general population (*8*). Despite a fairly high birth rate on Reunion Island (19 births/1,000 population), our small series does not support these findings.

During the epidemic (July 20–September 20, 2009), acute respiratory infections, including presumed cases of pandemic (H1N1) 2009, accounted for 20.6% of the total case load of physicians on the island. The attack rate was ≈12.9% among the 810,000 inhabitants, and 8 deaths among persons with confirmed infection were reported. Therefore, the minimal overall death rate was ≈7.5 per million population and the case-fatality rate, 1 per 10,000 population.

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References

1. Chowell G, Bertozzi SM, Colchero MA, Lopez-Gatell H, Alpuche-Aranda C, Hernandez M, et al. Severe respiratory disease concurrent with the circulation of H1N1 influenza. N Engl J Med. 2009;361:674–9. DOI: 10.1056/NEJMoa0904023
2. ANZIC Influenza Investigators, Webb SA, Pettilä V, Seppelt I, Bellomo R, Bailey M, et al. Critical care services and 2009 H1N1 influenza in Australia and New Zealand. N Engl J Med. 2009;361:1925–34. DOI: 10.1056/NEJMoa0908481
3. Kumar A, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lacroix J, et al. Critically ill patients with 2009 influenza A(H1N1) infection in Canada. JAMA. 2009;302:1872–9. DOI: 10.1001/jama.2009.1496
4. Rello J, Rodríguez A, Ibañez P, Socias L, Cebrian J, Marques A, et al. Intensive care adult patients with severe respiratory failure caused by influenza A (H1N1)v in Spain. Crit Care. 2009;13:R148. DOI: 10.1186/cc8044
5. Sakr Y, Madl C, Filipescu D, Moreno R, Groeneveld J, Artigas A, et al. Obesity is associated with increased morbidity but not mortality in critically ill patients. Intensive Care Med. 2008;34:1999–2009. DOI: 10.1007/s00134-008-1243-0
6. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quiñones-Falconi F, Bautista E, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med. 2009;361:680–9. DOI: 10.1056/NEJMoa0904252
7. Centers for Disease Control and Prevention. Intensive care patients with severe novel influenza A(H1N1) virus infection—Michigan, June 2009. MMWR Morb Mortal Wkly Rep. 2009;58:10.
8. Jamieson DJ, Honein MA, Rasmussen SA, Williams JL, Swerdlow DL, Biggerstaff MS, et al. H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet. 2009;374:451–8. DOI: 10.1016/S0140-6736(09)61304-0

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Crimean-Congo Hemorrhagic Fever Virus, Northeastern Greece

To the Editor: Crimean-Congo hemorrhagic fever virus (CCHFV) causes a disease in humans that is characterized by fever and hemorrhagic manifestations, with death rates up to 30%. Humans are infected through tick bites or contact with the viremic blood of patients or livestock. CCHFV belongs to the genus Nairovirus (family *Bunyaviridae*), which contains 7 serogroups: CCHFV, Dugbe virus, Hughes virus, Sakhalin virus, Dera Ghazi Khan virus, Qalyub virus, and Thiafora virus.

A CCHFV strain, AP92, was isolated from *Rhipicephalus bursa* ticks collected in 1975 from goats in Vergina, a village in northern Greece (*1*). Seroprevalence among Vergina residents was 6.1% (*2*). During 1981–1988, the seroprevalence among 3,388 persons in Greece was 1.1% (range 0%–9.6%) (*3*). The first Crimean-Congo hemorrhagic fever case in Greece was reported in 2008, when a woman died in Komotini in northeastern Greece (*4*). The causative strain (Rodopi) differs from strain AP92 (*5*).

To determine the prevalence of CCHFV antibodies in the human population of northeastern Greece, serum samples were collected prospectively during November 2008–April 2009 from 1,178 residents of Drama, Kavala, Xanthi, Rodopi, and Evros prefectures. A predefined number of participants were enrolled in the study on the basis of prefecture population. Participants were selected randomly among persons who were referred to health care settings for blood testing, regardless of reason for testing, and regardless of CCHFV risk factors. Oral consent was given by all participants. A questionnaire was completed concerning age, sex, occupation, place of residence, history of tick bite, symp-

toms after the bite, contact with animals, and any other situation related with increased risk for tick bite. All age groups were included (range 0–97 years, mean ± SE age 53.2 ± 0.63).

Serum samples were tested for CCHFV immunoglobulin (Ig) G by ELISA (Vektor-Best, Koltsovo, Novosibirsk, Russia). The data were analyzed by using Stata Special Edition 9 (StataCorp LP, College Station, TX, USA). Multivariate logistic regression modeling was adopted to identify potential risk factors for acquisition of CCHFV infection. Odds ratios (ORs) with 95% confidence intervals (CIs) were obtained. p values <0.05 were considered significant.

In total, 37 (3.14%) of 1,178 persons were positive for CCHFV by IgG. The mean ± SE age of seropositive and seronegative persons was 68.7 ± 2.54 years (range 0–87 years) and 55.6 ± 0.79 years (range 0–97 years). The female:male ratio was 1.6 among tested persons and 0.6 among seropositive persons. Seroprevalence differed among prefectures: Rodopi, where the fatal Crimean-Congo hemorrhagic fever case was observed, and Evros had the highest values (4.95% and 4.49%), Drama and Xanthi the lowest (1.34% and 1.09%), and no IgG-positive person was found in Kavala. The distribution of regions where IgG-positive persons were found is presented in the Figure. Seropositive persons lived in rural areas at an altitude of 10m to 270 m; however, this factor was not significant (p = 0.358).

Crude analysis showed that age, sex, prefecture, occupation, contact with goats and sheep, slaughtering, and history of tick bite were significantly associated with seropositivity. Multivariate analysis showed that the following variables were significant risk factors for acquisition of CCHFV infection: age (OR 1.05, 95% CI 1.02–1.08; p = 0.002), residence in Rodopi prefecture (with Drama prefecture as reference category) (OR 6.55, 95% CI 1.36–31.60; p = 0.019), contact with goats (OR 3.40, 95% CI 1.22–9.43; p = 0.019), history of slaughtering (OR 2.53, 95% CI 1.01–6.45; p = 0.048), and history of tick bite (OR 2.51, 95% CI 1.03–6.15; p = 0.044).

When we compared our results with those of Antoniadis et al. (*3*), marked differences were seen: seroprevalence in Rodopi, Evros, Xanthi, and Drama was 0.5%, 0%, 1.2%, and 0%, respectively, compared with 4.95%, 4.49%, 1.09%, and 1.34% in the present study, which suggests that during the past 20 years CCHFV was introduced in some areas in Greece or increased its circulation in others. Climatic and environmental changes and infested livestock movements (legal or illegal) in a habitat suitable for ticks might play a role in the current situation (*6*).

Further studies are necessary to estimate the seroprevalence in the whole country and detect disease-endemic foci of the disease. In addition, surveys for CCHFV in Ixodid ticks are necessary to enable the construction of risk maps and risk assessment analysis.

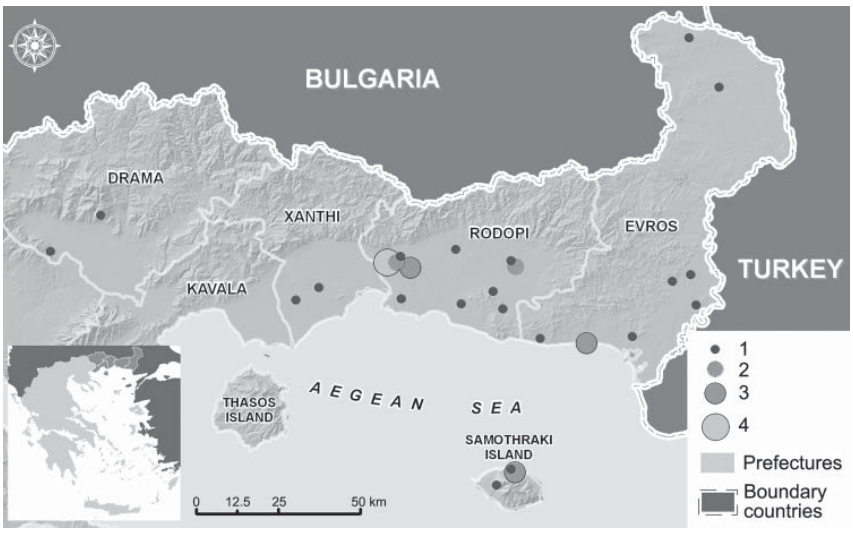

Figure. Five prefectures in northeastern Greece (inset), showing locations of persons who were immunoglobulin G–positive for Crimean-Congo hemorrhagic fever virus (solid circles), 2008–2009. Size of circle indicates number of persons with positive test results in each location.

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References

1. Papadopoulos O, Koptopoulos G. Crimean-Congo hemorrhagic fever (CCHF) in Greece: Isolation of the virus from *Rhipicephalus bursa* ticks and a preliminary serological survey. In: Vesenjak-Hirjan J, Porterfield JS, editors. Arboviruses in the Mediterranean countries. Stuttgart (Germany): Gustav Fisher Verlag; 1980. p. 117–21.

2. Antoniadis A, Casals J. Serological evidence of human infection with Congo-Crimean hemorrhagic fever virus in Greece. Am J Trop Med Hyg. 1982;31:1066–7.
3. Antoniadis A, Alexiou-Daniel S, Malisiovas N, Doutsos I, Polyzoni T, Leduc JW, et al. Seroepidemiological survey for antibodies to arboviruses in Greece. Arch Virol 1990;Suppl 1:277–85.
4. Papa A, Maltezou HC, Tsiodras S, Dalla VG, Papadimitriou T, Pierroutsakos I, et al. A case of Crimean-Congo haemorrhagic fever in Greece, June 2008. Euro Surveill. 2008;13(33):18952.
5. Papa A, Dalla V, Papadimitriou E, Kartalis GN, Antoniadis A. Emergence of Crimean-Congo haemorrhagic fever in Greece. Clin Microbiol Infect. 2010;16:843–7.
6. Maltezou HC, Papa A, Tsiodras S, Dalla V, Maltezos E, Antoniadis A. Crimean-Congo hemorrhagic fever in Greece: a public health perspective. Int J Infect Dis. 2009;13:713–6. DOI: 10.1016/j.ijid.2008.11.011

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Class D OXA-48 Carbapenemase in Multidrug-Resistant Enterobacteria, Senegal

To the Editor: Class D OXA β-lactamases are characterized as penicillinases that can hydrolyze oxacillin and cloxacillin and are poorly inhibited by clavulanic acid and EDTA. OXA-48 is one of the few members of this family to possess notable carbapenem-hydrolyzing activity (*1*). First described in 2004 in Turkey, OXA-48 has recently started to spread in Europe and the Middle East (*2*). We report the recent emergence of the plasmid-encoded $bla_{\text{OXA-48}}$ gene in multidrug-resistant *Enterobacteriaceae* recovered from patients in Dakar, Senegal, in hospitals and in the community.

From November 2008 through October 2009, 11 *Enterobacteriaceae* isolates (8 *Klebsiella pneumoniae*, 1 *Escherichia coli*, 1 *Enterobacter cloacae*, and 1 *Enterobacter sakazakii*) with reduced susceptibility to imipenem were identified at the Institut Pasteur (Dakar, Senegal). Antibacterial drug susceptibility was determined by the disk diffusion method and interpreted according to the European Committee on Antimicrobial Susceptibility Testing guidelines (www.eucast.org). Nine isolates were resistant to expanded-spectrum cephalosporins and also to other antibacterial drug classes.

The isolates were recovered from 6 patients with urinary tract infections, 4 patients with surgical infections, and 1 patient with omphalitis. Nine infections were hospital acquired (Le Dantec and Principal Hospitals). Because the patients died before antibacterial drug susceptibility testing could be completed, all 5 patients with surgical infections or omphalitis received only empirical therapy with amoxicillin/clavulanate. One patient with a nosocomial urinary tract infection caused by a co-trimoxazole–susceptible strain was successfully treated with this antibacterial agent. The antibacterial drug regimens of the remaining 4 patients were not known, and they were lost to follow-up. We determined the MICs of imipenem, meropenem, and ertapenem by using the Etest method (AB Biodisk, Solna, Sweden), which showed that 9 isolates were susceptible to imipenem and meropenem but either intermediately susceptible or resistant to ertapenem (Table). The 2 imipenem-nonsusceptible isolates were susceptible or intermediately susceptible to meropenem, and both were resistant to ertapenem.

We used previously described PCRs (*1*,*3*–*7*) to screen for carbapenem-hydrolyzing β-lactamase genes (bla_{VIM}, bla_{IMP}, bla_{KPC}, and $bla_{\text{OXA-48}}$), as well as plasmid-encoded $bla_{\text{CTX-M}}$, bla_{AmpC}, $bla_{\text{OXA-1}}$, and bla_{TEM} β-lactamase genes; the *aac(6')-Ib* aminoglycoside resistance gene; the quinolone resistance genes *qnrA,B,S*; the tetracycline resistance genes *tetA,B,D*; and class 1 integron. The $bla_{\text{OXA-48}}$, $bla_{\text{CTX-M}}$, bla_{AmpC}, and *aac(6')-Ib* genes and the variable region of class 1 integron were then characterized by direct DNA sequencing of the PCR products. $bla_{\text{OXA-48}}$ was present in all 11 isolates. bla_{VIM}, bla_{IMP}, and bla_{KPC} were not detected. The *qnr* genes were present in 7 isolates resistant to ciprofloxacin. The *aac(6')-Ib-cr* variant was present in 7 isolates resistant to gentamicin, tobramycin, and ciprofloxacin.

The 9 isolates resistant to expanded-spectrum cephalosporins all harbored the $bla_{\text{CTX-M-15}}$ gene. The *E. coli* isolate also harbored the plasmid-encoded bla_{AmpC} gene ACT-1; $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, bla_{TEM}, and *aac(6')lb-cr* were associated in 6 isolates. Long-range PCRs showed that these latter 4 genes were located in the same "multidrug resistance region," as described in Senegal (*6*). Positive conjugation experiments with sodium azide–resistant *E. coli* J53 showed through PCR results, plasmid DNA extraction, and antibiogram patterns of the obtained transconjugants that $bla_{\text{OXA-48}}$ was located on a 70-kb self-conjugative plasmid.

The genetic environment of $bla_{\text{OXA-48}}$ was then investigated by PCR with primers specific for insertion sequence IS*1999* and for the 5′ part of $bla_{\text{OXA-48}}$ (*1*). $bla_{\text{OXA-48}}$ was found to be part of a *Tn1999* composite transposon composed of 2 copies of the insertion sequence IS*1999*, as reported (*2*). Further sequencing of the IS*1999* located upstream of $bla_{\text{OXA-48}}$ showed that it was consistently truncated by the insertion sequence IS*1R*, as initially described in Turkey and more recently in Lebanon and Egypt (*2*,*8*).

*Xba*I pulsed-field gel electrophoresis was then used to study the genetic relatedness of the 8 *K. pneumoniae*

isolates. Three isolates had similar restriction profiles and had been recovered from 3 patients concurrently hospitalized at Le Dantec Hospital, suggesting nosocomial transmission. A class 1 integron harboring the *dfrA1* trimethoprim-resistance gene was detected in the 3 clonal isolates.

Together, these findings show the recent emergence of bla_{OXA-48} in Senegal in community and hospital settings. They may also suggest the spread of the same major carrying plasmid between the Middle East and Africa. Although 9 of the 11 isolates were found to be susceptible to imipenem on the basis of their MICs, their MICs were nonetheless higher than those of bla_{OXA-48}–negative isolates. This raises 2 issues. First, these strains might go undetected during routine antibacterial drug susceptibility testing, a problem that could be overcome by using ertapenem, a compound more susceptible to carbapenemases. Second, the clinical efficacy of imipenem on such strains is uncertain. The frequency of acquired carbapenemases, which emerged early after imipenem introduction in Senegal (2008), is probably strongly underestimated, partly owing to the limited availability of reliable clinical laboratories (*9*). Because multidrug resistance is prevalent among *Enterobacteriaceae* isolated in Dakar hospitals (B. Garin, unpub. data) and in rural communities (*6*), the emergence of bla_{OXA-48} is a clear cause for concern.

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References

1. Poirel L, Heritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2004;48:15–22. DOI: 10.1128/AAC.48.1.15-22.2004
2. Carrër A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, et al. Emerging spread of OXA-48-encoding plasmid from Turkey and beyond. Antimicrob Agents Chemother. 2010;54:1369–73. DOI: 10.1128/AAC.01312-09
3. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev. 2007;20:440–58. DOI: 10.1128/CMR.00001-07
4. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, et al. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother. 2000;44:891–7. DOI: 10.1128/AAC.44.4.891-897.2000
5. Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol. 2002;40:2153–62. DOI: 10.1128/JCM.40.6.2153-2162.2002
6. Ruppé E, Woerther PL, Diop A, Sene AM, Da Costa A, Arlet G, et al. Carriage of CTX-M-15-producing *Escherichia coli* isolates among children living in a remote village in Senegal. Antimicrob Agents Chemother. 2009;53:3135–7. DOI: 10.1128/AAC.00139-09
7. Guessennd N, Bremont S, Gbonon V, Kacou-Ndouba A, Ekaza E, Lambert T, et al. Qnr-type quinolone resistance in extended-spectrum beta-lactamase producing enterobacteria in Abidjan, Ivory Coast [in French]. Pathol Biol (Paris). 2008;56:439–46. DOI: 10.1016/j.patbio.2008.07.025
8. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48-positive carbapenem-resistant *Klebsiella pneumoniae* isolates in Istanbul, Turkey. Antimicrob Agents Chemother. 2008;52:2950–4. DOI: 10.1128/AAC.01672-07
9. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. Clin Infect Dis. 2006;42:377–82. DOI: 10.1086/499363

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Table. Resistance genes and carbapenem MICs of 11 *Enterbacteriaceae* isolates, Senegal, 2008–2009*

Isolate	Species	Origin†	Resistance genes								MIC (μg/mL)		
			bla_{OXA-48}	$bla_{CTX-M-15}$	bla_{OXA-1}	bla_{TEM}	*aac-6'-Ib*	*qnr*	*tet*	*dfr*	IPM	MEM	ERT
17176	*Klebsiella pneumoniae*	1	+	+	–	–	–	–	*A,D*	–	1	0.38	1.5
22184	*K. pneumoniae*‡	2	+	+	+	+	*cr*	*S*	*A*	*A1*	1	0.38	1.5
20254	*K. pneumoniae*‡	3	+	+	+	+	*cr*	*S*	*A*	*A1*	1	0.38	1.5
10243	*K .pneumoniae*‡	3	+	+	+	+	*cr*	*S*	*A*	*A1*	1	0.38	1.5
19220	*K. pneumoniae*	2	+	+	+	+	*cr*	*B*	*A,D*	–	0.5	0.19	0.75
18212	*K. pneumoniae*	2	+	–	–	–	–	–	–	–	3	2	12
18220	*K. pneumoniae*	3	+	+	–	+	–	*S*	*D*	–	1	0.38	1.5
06003	*K. pneumoniae*	4	+	–	–	–	–	–	–	–	2	0.25	0.75
HPD	*Enterobacter cloacae*	3	+	+	+	+	*cr*	*B,S*	*B*	–	4	3	8
20247	*Enterobacter sakazakii*	5	+	+	+	+	*cr*	–	–	–	0.5	1	3
24246	*Escherichia. coli*	4	+	+	+	–	*cr*	*S*	–	–	2	0.5	2
J53	*E. coli*		–	–	–	–	–	–	–	–	0.12	0.03	0.03
TC	*E. coli*		+	–	–	–	–	–	–	–	0.5	0.19	0.75

*IPM, imipenem; MEM, meropenem; ERT, ertapenem; TC, transconjugants.
†Origin: 1, postsurgical visceral infection; 2, postsurgical orthopedic infection; 3, nosocomial urinary tract infection; 4, community-acquired urinary tract infection; 5, omphalocele infection.
‡Clonally related isolates.

Identification of *Legionella feeleii* Cellulitis

To the Editor: In general, reports of extrapulmonary *Legionella* spp. infections are scarce. For example, *L. micdadei* infection was found with the following manifestations: a mass on the left side of the neck and low-grade fever in a healthy 9-year-old girl (*1*); multiple liver and lung abscesses in a 7-year-old girl with acute lymphoblastic leukemia who had undergone allogeneic cord blood transplantation (*2*); and a cerebral abscess in a patient with legionellosis (*3*).

L. feeleii was first described in 1984 as the causative agent of a Pontiac fever outbreak (*4*). *L. feeleii* was responsible, according to a recent review, for only 10 reported cases of infections, all of which were pneumonia, only 1 complicated by endocarditis (*5*). Unlike lung abscesses, cutaneous lesions caused by *Legionella* spp. are uncommon. Recurrent soft tissue abscesses of the jaw, wrist, and arm caused by *L. cincinnatiensis* were described in a 73-year-old woman with nephrotic syndrome and idiopathic immunoglobulin gammopathy (*6*). *L. micdadei* has been found in a cutaneous abscess of the leg of a 62-year-old immunosuppressed woman, and it was responsible for necrotizing cellulitis that resulted in amputation of the left arm of a recipient of a cadaveric renal transplant (*7*). *L. pneumophila* with mixed flora was identified in a perirectal abscess (*8*) and in skin samples from a patient with lymphoma and cellulitis associated with pneumonia (*9*). The infrequency of reporting *Legionella* spp. cutaneous infections may be explained in part by the fact that *Legionella* spp. agar is not routinely a part of media inoculated for cases of cutaneous abscess. Here we report the identification of *L. feeleii* in a cutaneous infection through the use of a shell vial culture protocol.

In late October 2009, a 66-year-old woman was admitted to Hôpital Nord, Marseille, France, for a papular lesion complicated by cellulitis and an abscess, centered on her right leg (Figure). The patient's history noted that she had been bitten by an insect or spider (suspected to be a spider) on October 9. The next day, the patient had a fever of 39°C, and a papular lesion appeared around the bite. Four days later, the fever had persisted, and she was given amoxicillin-clavulanate and local wound care. Two days later, the lesion became necrotic, and levofloxacin was added to the medication regimen. At day 10 after the bite, cellulitis with a central abscess appeared on her leg. The patient was transferred to Hôpital Nord. At admission, she had leukocytosis (16.9×10^9 cells/L) with neutropenia (0.51×10^9 cells/L), slight anemia (104 g/dL), and inflammatory syndrome (C-reactive protein 72 mg/L, erythrocyte sedimentation rate 150 mm after 1 h); she was also still febrile (38°C). She had a history of chronic lymphocytic leukemia. A cutaneous biopsy sample showed inflamed and necrotic tissue, which suggested squamous cell evocating carcinoma.

Gram staining of tissue samples did not show any bacteria, and conventional cultures incubated under aerobic and anaerobic conditions did not lead to growth after 3 weeks of incubation. *Je ne sais pas* (or "I don't know [what I'm growing]" [*10*]) shell vial culture protocol was done on a skin biopsy sample of the lesion. Culturing was performed by the centrifugation shell vial technique with 3.7 mL human embryonic lung fibroblast cell monolayers (Sterilin, Felthan, UK) inoculated with the skin biopsy sample previously triturated in cell culture medium. Small extra- and intracellular bacilli were observed directly inside the shell vial by using Gimenez and Gram staining. DNA extraction, partial 16S rRNA gene amplification and sequencing, and *mip* and *rpoB* gene amplification and sequencing were done on shell vial supernatant. Partial sequence of 16S rRNA identified a *Legionella* sp. Subsequently, *L. feeleii* was identified with 100% and 96.5% sequence similarity for *mip* and *rpoB* genes, respectively. The biopsy sample was unfrozen and then injected onto *Legionella* spp.–buffered charcoal yeast extract agar as the superna-

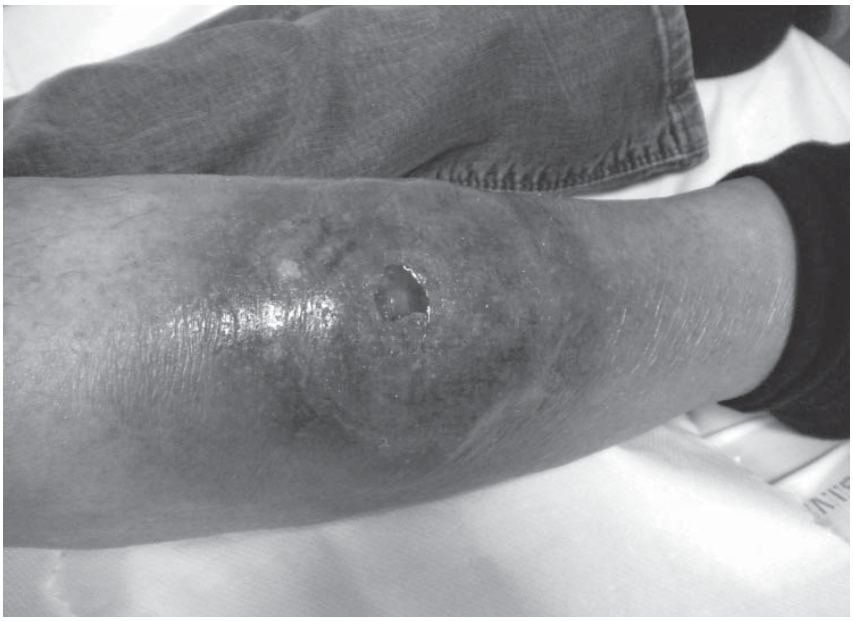

Figure. Cellulitis with a central abscess present at time of patient's admission to hospital, Marseille, France, 2010. A color version of this figure is available online (www.cdc.gov/EID/content/17/1/145-F.htm).

tant of the shell vial. No growth was obtained from the biopsy sample, but *L. feelii* was identified similarly from colonies growing on buffered charcoal yeast extract agar plates injected with shell vial supernatant. The patient's necrotic tissues were surgically excised; a vacuum-assisted closure system was used. Reexamination of the tissue biopsy samples ruled out the diagnosis of carcinoma.

Finally, despite the fact that the shell vial technique requires specialized equipment and trained personnel, this method was performed in a reference center to improve the accuracy of a microbiologic diagnosis and, consequently, the care of the patient in uncommon situations (*10*). This improvement in diagnosis and care was also noted in an unusual *L. pneumophila* infection described by our team (*2*).

In our laboratory, we have been performing the *je ne sais pas* protocol almost routinely since 1996 (*10*). Cell cultures provide supplemental tools to elucidate the cause of microbial diseases when results of PCR and classical agar procedures are negative. Furthermore, this procedure provides a means for the isolation of a wide range of intracellular bacteria, even when little biopsy material is available.

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References

1. Qin X, Abe PM, Weissman SJ, Manning SC. Extrapulmonary *Legionella micdadei* infection in a previously healthy child. Pediatr Infect Dis J. 2002;21:1174–6. DOI: 10.1097/00006454-200212000-00022
2. La Scola B, Michel G, Raoult D. Isolation of *Legionella pneumophila* by centrifugation of shell vial cell cultures from multiple liver and lung abscesses. J Clin Microbiol. 1999;37:785–7.
3. Andersen BB, Sogaard I. Legionnaires' disease and brain abscess. Neurology. 1987;37:333–4.
4. Herwaldt LA, Gorman GW, McGrath T, Toma S, Brake B, Hightower AW. A new *Legionella* species, *Legionella feeleii* species nova, causes Pontiac fever in an automobile plant. Ann Intern Med. 1984;100:333–8.
5. Lee J, Caplivski D, Wu M, Huprikar S. Pneumonia due to *Legionella feeleii*: case report and review of the literature. Transpl Infect Dis. 2009;11:337–40. DOI: 10.1111/j.1399-3062.2009.00390.x
6. Gubler JGH, Schorr M, Gaia V, Zbinden R, Altwegg M. Recurrent soft tissue abscesses caused by *Legionella cincinnatiensis.* J Clin Microbiol. 2001;39:4568–70. DOI: 10.1128/JCM.39.12.4568-4570.2001
7. Kilborn JA, Manz LA, O'Brien M, Douglass MC, Horst HM, Kupin W, et al. Necrotizing cellulitis caused by *Legionella micdadei.* Am J Med. 1992;92:104–6. DOI: 10.1016/0002-9343(92)90024-6
8. Arnow PM, Boyko EJ, Friedman EL. Perirectal abscess caused by *Legionella pneumophila* and mixed anaerobic bacteria. Ann Intern Med. 1983;98:184–5.
9. Waldor MK, Wilson B, Swartz M. Cellulitis caused by *Legionella pneumophila.* Clin Infect Dis. 1993;16:51–3.
10. Gouriet F, Fenollar F, Patrice JY, Drancourt M, Raoult D. Use of shell-vial cell culture assay for isolation of bacteria from clinical specimens: 13 years of experience. J Clin Microbiol. 2005;43:4993–5002. DOI: 10.1128/JCM.43.10.4993-5002.2005

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Sparganosis, Henan Province, Central China

To the Editor: Sparganosis is a parasitic zoonosis caused by invasion of the spargana, the plerocercoid larvae of various diphyllobothroid tapeworms belonging to the genus *Spirometra* (*1*). Although human sparganosis is cosmopolitan, it is most frequently found in eastern and southeastern Asia (*2*). During 1927–2009 in the People's Republic of China, >1,000 cases in humans in 27 provinces were reported; most cases were in southern China, where human infections were mainly acquired by eating raw or insufficiently cooked meat of frogs and snakes or by placing frog or snake flesh on open wounds for treatment of skin ulcers or on eyes to treat inflammation (*3*,*4*).

Sparganosis is rarely seen in central and northern China. Before 2006, only 3 imported cases from southern China had been reported in Henan Province in central China (*5*). However, since 2006 in Henan Province, 20 autochthonous cases caused by ingestion of live tadpoles have emerged. To assess the risk for human infection with sparganosis in this province and to strengthen public safety awareness, we investigated spargana infection in the animal hosts of *Spirometra* tapeworms.

During July 2007–July 2010, wild frogs and frog tadpoles were collected from the cities of Shangqiu, Zhoukou, and Luohe in Henan Province. Necropsies identified plerocercoids in 11.93% (163/1,366) of tadpoles and in 26.58% (172/647) of frogs. By frog species, plerocercoids were found in 31.09% (111/357) of *Rana limmocharis* and 26.29% (61/232) of *R. nigromaculata* frogs, each significantly ($p<0.05$) more numerous in these species than in *R. temporaria* frogs (0/58). In addition, 177 wild frogs sold at markets in Luohe were also exam-

ined; plerocercoids were detected in 30.39% (31/102) of *R. limmocharis* and 28% (21/75) of *R. nigromaculata* frogs. Thus, in Henan Province, *R. limmocharis* and *R. nigromaculata* frogs are the main intermediate hosts of *Spirometra* tapeworms.

No pathologic changes associated with the tapeworms were found in dissected tadpoles and frogs. We found 250 plerocercoids in tadpoles and 1,387 in frogs. Tadpoles contained 1–14 (mean 1.53) and frogs 1–87 (mean 6.85) tapeworms. In frogs, most plerocercoids were located in the muscles of hind legs and back; some were in the muscles of the abdominal wall and forelegs. Plerocercoids dissected from tadpole and frog tissues were wrinkled, whitish, and ribbon-shaped and continuously moved while in normal saline. Plerocercoids from tadpoles were 1–8 mm long and 0.2–0.5 mm wide; those from frogs were 1–13 cm long and 1–2.5 mm wide.

Cyclops were collected from ponds and ditches by using a 425-μm mesh (no. 40) sieve and species were identified by microscopic appearance as *Mesocyclops leuckarti* (*6*,*7*). Procercoids were microscopically found in the hemocele of 3.53% (3/85) of cyclops; 3–5 worms per cyclop were found.

Fecal examination of dogs and cats found *Spirometra mansoni* tapeworm eggs in 19.35% (6/31) of dogs and 33.33% (1/3) cats. In addition, a 3-month-old specific pathogen–free cat was orally inoculated with 33 plerocercoids from tadpoles, fecal samples were microscopically examined by sedimentation during 10–25 days postinfection (dpi), and the cat was euthanized and examined for adult worms. *S. mansoni* tapeworm eggs were found in the feces during 12–25 dpi, and 17 adult worms, 26–45 cm long, were recovered from the small intestines at 25 dpi; however, no plerocercoids were seen in the tissues. The adult worms were morphologically identified as *S. mansoni* according to the following features: scolex with 2 longitudinal grooves, mature and gravid proglottids with conspicuous uterus at the center of segments, spiral-shaped uterus (unlike the rosette-shaped uterus of *Diphyllobothrium latum*), and cirrus and vaginal pore with separate openings (*8*,*9*).

Although in recent years in Henan Province wild frogs have been sold clandestinely at markets, persons in this province do not routinely eat raw frog meat or use raw meat as poultices. However, some villagers in Henan do believe that eating live tadpoles has a medicinal role for skin diseases and, thus, they contract sparganosis. Accordingly, the route of plerocercoid infection for humans in Henan Province differs from that in southern China. Because this disease is rare in central and northern China, sparganosis is often neglected and misdiagnosed. Our survey showed that in Henan Province, 11.93% of tadpoles are infected with plerocercoids and 3.53% of cyclops are infected with procercoids. Therefore, eating live tadpoles poses a high risk for plerocercoid infection and must be discouraged. In addition, drinking raw water containing cyclops also poses a slight risk for sparganosis.

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References

1. Fukushima T, Yamane Y. How does the sparganosis occur? Parasitology Today. 1999;15:124. DOI: 10.1016/S0169-4758-(99)01405-2
2. Wiwanitkit V. A review of human sparganosis in Thailand. Int J Infect Dis. 2005;9:312–6. DOI: 10.1016/j.ijid.2004.08.003
3. Qiu MH, Qiu MD. Human plerocercoidosis and sparganosis. II. A historical review on pathology, clinics, epidemiology and control [in Chinese]. Chinese Journal of Parasitology and Parasitic Diseases. 2009;27:251–60.
4. Li MW, Lin HY, Xie WT, Gao MJ, Huang ZW, Wu JP, et al. Enzootic sparganosis in Guangdong, People's Republic of China. Emerg Infect Dis. 2009;15:1317–8. DOI: 10.3201/eid1508.090099
5. Wang ZQ. The rare helminthes in Henan Province [in Chinese]. Henan Journal of Preventive Medicine. 1996;7:44–7.
6. Roberts LS, Janovy J Jr, Schmidt GD. Foundations of parasitology. 8th ed. New York: McGraw-Hill; 2009.
7. Li CP. Medical arthropodology [in Chinese]. Beijing: People's Health; 2009.
8. Chen XT. Medical parasitology [in Chinese]. 2nd ed. Beijing: People's Health; 1965.
9. Wardle RA, McLeod JA. The zoology of tapeworms. Minneapolis (MN): University of Minnesota Press; 1952.

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Ceftriaxone-Resistant *Neisseria gonorrhoeae*, Japan

To the Editor: Spread of multidrug-resistant *Neisseria gonorrhoeae* is a major public health concern. Effective antimicrobial therapy is a key element in gonorrhea control. However, *N. gonorrhoeae* has developed resistance to multiple classes of antimicrobial drugs, including β-lactams, tetracyclines, and fluoroquinolones (*1*–*3*). Even an extended-spectrum oral cephalosporin-resistant, cefixime-resistant *N. gonorrhoeae* has emerged, and cefixime has now been withdrawn from use in Japan. Best practice treatment is limited to injectable extended-spectrum cephalosporins, such as ceftriaxone and spectinomycin. The emergence of ceftriaxone-resistant *N. gonorrhoeae* threatens effective disease control.

We identified a novel ceftriaxone-resistant *N. gonorrhoeae* isolated from a 31-year-old female commercial sex worker; MIC of ceftriaxone for this isolate was high (2 μg/mL). The woman visited a clinic in Kyoto for a routine examination for sexually transmitted infections in January 2009. Although she had no obvious symptoms or signs, a throat sample collected on her first visit yielded a positive result for *N. gonorrhoeae* by the strand displacement amplification test (ProbeTec ET, Becton Dickinson, Franklin Lakes, NJ, USA), but a vaginal sample taken at the same time was negative. After 2 weeks, another throat sample was positive for *N. gonorrhoeae* when cultured on Thayer-Martin medium, and the patient subsequently received 1 g ceftriaxone intravenously. Her pharyngeal sample was also *N. gonorrhoeae* positive by strand displacement amplification test on the third visit 2 weeks later, and further ceftriaxone treatment was prescribed. However, a culture for test of cure was not conducted because reinfection was considered. A negative result was finally obtained in April 2009.

The culture showed positive reactions in oxidase and catalase tests. Gram staining showed gram-negative diplococci. The ID-test HN-20 Rapid system (Nissui, Tokyo, Japan) classified the bacterium as *N. gonorrhoeae*. Susceptibility was determined by the agar dilution method (*4*). For this strain, named H041, MIC of ceftriaxone was high (2 μg/mL), and the strain was highly resistant to penicillin G (4 μg/mL), cefixime (8 μg/mL), and levofloxacin (32 μg/mL). However, it demonstrated susceptibility to spectinomycin (16 μg/mL) and reduced susceptibility to azithromycin (0.5 μg/mL).

To characterize the ceftriaxone-resistant *N. gonorrhoeae* H041, multilocus sequence typing characterized the strain as ST7363 (*5*), which is the predominant sequence type (ST) among cefixime-resistant clones (*6*). *N. gonorrhoea* multiantigen sequence typing (NG-MAST) was also performed (*7*). The NG-MAST strategy uses 2 genes, *por* and *tbpB*, for porin and a transferrin-binding protein, respectively. NG-MAST indicated that the strain H041 was ST4220 and contained the *por2594* allele and the *tbpB10* allele. NG-MAST 4220 is a novel ST. However, the *tbpB10* allele is the most frequently observed allele (76.5%) among multilocus sequence typing-ST7363 *N. gonorrhoeae* strains (n = 81) (M. Ohnishi, unpub. data).

Molecular typing suggested that the novel ceftriaxone-resistant *N. gonorrhoeae*, H041, is closely related to the ST7363 cefixime-resistant *N. gonorrhoeae*. Therefore, we compared *Spe*I-digested genomic DNA banding patterns of strain H041 with those of other *N. gonorrhoeae* strains by using pulsed-field gel electrophoresis as described (*8*). Four ST7363 strains, including *N. gonorrhoeae* H041, and 4 ST1901 strains (another major ST among cefixime-resistant *N. gonorrhoeae* strains) (*6*) were analyzed. The banding pattern of *Spe*I digested H041 genomic DNA was similar to that of other ST7363 strains and indistinguishable from that of cefixime-resistant but ceftriaxone-susceptible NG0207 (Figure).

We describe the emergence of ceftriaxone-resistant *N. gonorrhoeae*, isolated from a pharyngeal specimen from a female commercial sex worker. At 2 μg/mL, the MIC was 4-fold higher than that of the previously reported ceftriaxone nonsusceptible strain (*9*). Our susceptibility testing suggests that only azithromycin and spectinomycin are effective drugs for treating this strain. In this case, eradication was successful, although *N. gonorrhoeae* colonization of the pharynx may just be tempory because

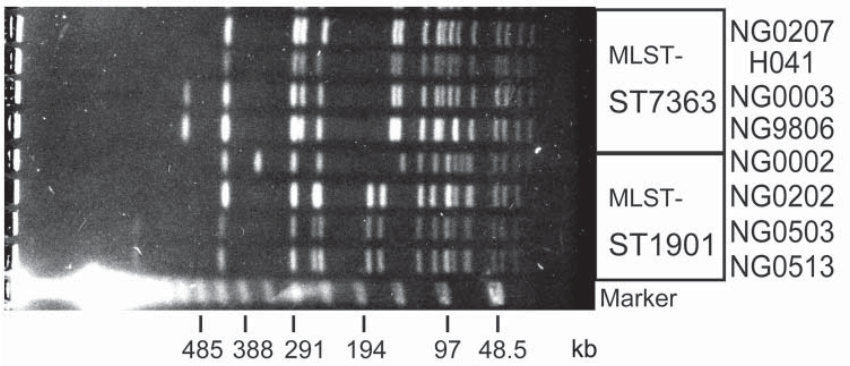

Figure. Pulsed-field gel electrophoresis patterns of ceftriaxone-resistant *Neisseria gonorrhoeae* strain H041 and other multilocus sequence typing (MLST) ST7363 and ST1901 strains. *Spe*I-digested genomic DNA from ceftriaxone-resistant *N. gonorrhoeae* H041, 3 of the MLST ST7363 strains and 4 of the MLST ST1901 strains were analyzed by pulsed-field gel electrophoresis. A lambda ladder standard (Bio-Rad, Hercules, CA, USA) was used as a molecular size marker.

the pharynx is not an ideal site for *N. gonorrhoeae* growth. From the routine examinations of commercial sex workers during January–March 2009, 40 *N. gonorrhoeae* were isolated in the clinic, but no other ceftriaxone-resistant strains were isolated. There is no evidence of dissemination of this strain in Kyoto.

Three independent molecular subtyping methods indicated that the ceftriaxone-resistant H041 strain was *N. gonorrhoeae*, and it might originate from an ST7363 cefixime-resistant *N. gonorrhoeae* clone. There are several possible mechanisms for the acquisition of resistance, including formation of a new mosaic type *penA* allele as *penA-X* cefixime resistance and acquisition of an extended-spectrum β-lactamase gene. The H041 strain did not produce β-lactamase in a nitrocephin test. Further molecular analysis is needed to elucidate the precise mechanism of the ceftriaxone resistance of the H041 strain.

The emergence of ceftriaxone-resistant *N. gonorrhoeae* raises concerns for controlling gonorrhea because ceftriaxone is widely recommended and the first-line treatment for gonorrhea around the world. *N. gonorrhoeae* has a potential to gain an extraordinarily high MIC to ceftriaxone. Surveillance for ceftriaxone-resistant *N. gonorrhoeae* should be strengthened.

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References

1. Tapsall JW, Ndowa F, Lewis DA, Unemo M. Meeting the public health challenge of multidrug- and extensively drug-resistant *Neisseria gonorrhoeae*. Expert Rev Anti Infect Ther. 2009;7:821–34. DOI: 10.1586/eri.09.63
2. Workowski KA, Berman SM, Douglas JM Jr. Emerging antimicrobial resistance in *Neisseria gonorrhoeae*: urgent need to strengthen prevention strategies. Ann Intern Med. 2008;148:606–13.
3. Tapsall J. Antibiotic resistance in Neisseria gonorrhoeae is diminishing available treatment options for gonorrhea: some possible remedies. Expert Rev Anti Infect Ther. 2006;4:619–28. DOI: 10.1586/14787210.4.4.619
4. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 19th informational supplement. CLSI document M100–S19. Wayne (PA): The Institute; 2009.
5. Jolley KA. Multi-locus sequence typing. In: Pollard AJ Maiden MCJ, editors. Meningococcal disease: methods and protocols. Totowa (NJ): Humana Press; 2001. p. 173–86.
6. Ohnishi M, Watanabe Y, Ono E, Takahashi C, Oya H, Kuroki T, et al. Spreading of a chromosomal cefixime resistant penA gene among different *Neisseria gonorrhoeae* lineages. Antimicrob Agents Chemother. 2010;54:1060–7. DOI: 10.1128/AAC.01010-09
7. Martin IM, Ison CA, Aanensen DM, Fenton KA, Spratt BG. Rapid sequence-based identification of gonococcal transmission clusters in a large metropolitan area. J Infect Dis. 2004;189:1497–505. DOI: 10.1086/383047
8. Ito M, Yasuda M, Yokoi S, Ito S, Takahashi Y, Ishihara S, et al. Remarkable increase in central Japan in 2001–2002 of *Neisseria gonorrhoeae* isolates with decreased susceptibility to penicillin, tetracycline, oral cepharosporins, and fluoroquinolones. Antimicrob Agents Chemother. 2004;48:3185–7. DOI: 10.1128/AAC.48.8.3185-3187.2004
9. Tanaka M, Nakayama H, Huruya K, Konomi I, Irie S, Kanayama A, et al. Analysis of mutations within multiple genes associated with resistance in a clinical isolate of Neisseria gonorrhoeae with reduced ceftriaxone susceptibility that shows a multidrug-resistant phenotype. Int J Antimicrob Agents. 2006;27:20–6. DOI: 10.1016/j.ijantimicag.2005.08.021

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Role of National Travel Health Network and Centre Website during Pandemic (H1N1) 2009

To the Editor: The National Travel Health Network and Centre (NaTHNaC) was created in 2002 by the Department of Health in England to provide authoritative guidance in travel medicine. The open-access NaTHNaC website (www.nathnac.org) is a key mode of communication, with both health professionals' and travelers' areas. Website country information pages (CIP) provide specific guidance for travel to each country of the world, and an outbreak surveillance database (OSD) detailing global outbreaks of disease is updated daily.

In late April 2009, influenza A virus (H1N1) of swine origin was identified in 2 children from California, USA (*1*). These cases were traced to travel to Mexico, and a widespread outbreak of influenza A (H1N1) in Mexico subsequently was recognized. On June 11, 2009, the World Health

Organization declared a global influenza pandemic (*2*). We reviewed use of the NaTHNaC website during the early recognition of pandemic (H1N1) 2009. During this phase, before widespread community transmission in the United Kingdom, assessing the international situation was necessary because travel abroad represented the highest risk for infection (*3*).

NaTHNaC, the national authority for travel health advice, posted multiple information resources on pandemic (H1N1) 2009. A daily table of internationally reported cases and deaths was compiled from official sources. A more detailed report of confirmed and suspected cases was circulated to key NaTHNaC stakeholders, including the Health Protection Agency (HPA) and the Foreign and Commonwealth Office (FCO). The OSD listed progression of the pandemic by date, country, and region. Reports of the pandemic and advice on preventive measures for travelers, termed Clinical Updates, were written daily, posted, and circulated to stakeholders.

NaTHNaC website statistics were obtained from Google Analytics. Use for the first 8 weeks of the pandemic period (April 24–June 18, 2009) was extracted, analyzed by using STATA version 9.1 (StataCorp LP, College Station, TX, USA), and compared with use for the 8 weeks preceding the start of the pandemic influenza (prepandemic period, February 27–April 23, 2009).

During the pandemic period, the daily number of visits to the website increased 28.1% over the prepandemic period (Table; online Technical Appendix Figure 1, www.cdc.gov/EID/content/17/1/149-Techapp.pdf). More new visitors accessed the website (63.6% vs. 61.7%), particularly through the Health Professionals portal (50.7% vs. 46.1%; p<0.001).

The number of website visitors from Mexico and the number of visits to the Mexico CIP also increased; Mexico was the most frequently searched country on the OSD (Table). Visits to the Mexico CIP (633 visits) and the Mexico OSD (129 visits) pages peaked on April 27, the Monday after pandemic (H1N1) 2009 was recognized. The pandemic (H1N1) 2009 home page that hosted clinical updates, news items, and an information sheet about subtype H1N1 became the seventh most viewed page (11,009 views). Visits for advice on seasonal influenza also increased markedly.

During the pandemic period, the website was accessed more often through referring websites (46.3%) than it was during the prepandemic period (39.9%; p<0.001). The most frequent referral website was the FCO (Table), accounting for 56.4% of all referrals during the pandemic period, with a peak on April 27 (online Technical Appendix, Figure 2). A large increase also occurred in referrals from the HPA.

Our analysis documents increased use of a national resource during the emergence of pandemic (H1N1) 2009. Information accessed included specific country information for Mexico and the United States, the countries first reporting cases, and information about and guidance for the prevention of pandemic (H1N1) 2009. The 28% increase in access to the website most likely reflected widespread interest in the pandemic, new links to the NaTHNaC website from UK authorities (e.g., FCO and HPA), and daily communication with stakeholders within the United Kingdom. In addition, NaTHNaC collaborated with these stakeholders and public health agencies to report progression of the outbreak and to help set policy on travel to influenza-affected countries.

The internet is a major resource for travel health information for health professionals and travelers. In 2008, ≈83% of internet users and 61% of all US adults used the Internet to acquire health information; 9% searched for travel health information (*4*). Public health agencies also use the Internet to assess global disease threats. Many use informal Internet sources, such as news articles and media outlets, to monitor potential threats in a more timely fashion than through the often delayed public health reporting mechanisms (*5*–*7*).

During a rapidly evolving global health situation, such as pandemic influenza, timely, accurate information is needed. The World Health Organization provided daily, and often twice daily, information (*8*); the US Centers for Disease Control and Prevention and the European Centre for Disease Prevention and Control used new and existing reporting systems (*9*,*10*). The

Table. Number of visits to or searches on the National Travel Health Network and Centre website during the 8 weeks before and after recognition of pandemic (H1N1) 2009

Visits or searches	No. before*	No. after†	% Change
Daily website visits	1,664 ± 655	2,132 ± 885	+28.1
Website access from Mexico	55	210	+281.8
Visits to Mexico Country Information Page	2,040	4,090	+100.5
Mexico searches on the Outbreak Surveillance Database	50	459	+818.0
Visits to US country information page	654	2003	+206.3
Visits to seasonal influenza information sheet	34	1,572	+4,523.5
Visits to Outbreak Surveillance Database home page	2,050	5,110	+149.3
Referral traffic from Foreign and Commonwealth Office	21,604	31,200	+44.4
Referral traffic from Health Protection Agency	1,399	7,247	+418.0

*Data from before the beginning of the pandemic (February 27–April 23, 2009).
†Data from the first 8 weeks of the pandemic (April 24–June 18, 2009).

experience of NaTHNaC indicates that acquisition and coordination of information with health authorities, rapid and direct communication of findings and recommendations to stakeholders, and posting of this information for access by travelers and health professionals can increase communication about global health events.

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References

1. Centers for Disease Control and Prevention. Swine influenza A (H1N1) infection in two children—southern California, March–April, 2009. MMWR Morb Mortal Wkly Rep. 2009;58:400–2.
2. World Health Organization. 11 June 2009: DG statement following the meeting of the Emergency Committee [cited 2010 Sep 3]. http://www.who.int/csr/disease/swineflu/4th_meeting_ihr/en/index.html
3. Health Protection Agency. Update on confirmed swine flu cases in England. 30 April 2009 [cited 2010 Sep 3]. http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1241048759904
4. Fox S, Jones S. The social life of health information. Americans' pursuit of health takes place within a widening network of both online and offline resources [cited 2010 Sep 3]. http://www.pewinternet.org/~/media/Files/Reports/2009/PIP_Health_2009
5. Marano C, Freedman DO. Global health surveillance and travelers' health. Curr Opin Infect Dis. 2009;22:423–9.
6. Linge JP, Steinberger R, Weber TP, Yangarber R, van der Goot E, Al Khudhairy DH, et al. Internet surveillance systems for early alerting of health threats. Euro Surveill. 2009;14.pii:19162.
7. Brownstein JS, Clark C, Freifield BS, Madoff LC. Digital disease detection—harnessing the web for public health surveillance. N Engl J Med. 2009;360:2153–5. DOI: 10.1056/NEJMp0900702
8. World Health Organization. Outbreak communication: best practice for communication with the public during an outbreak. Report of the WHO Expert Consultation on Outbreak Communications held in Singapore, 21–23 September 2004 [cited 2010 Sep 3]. http://www.who.int/csr/resources/publications/WHO_CDS_2005_32web.pdf
9. Centers for Disease Control and Prevention. Novel H1N1 flu: CDC response. 2009 Aug 19 [cited 2010 Sep 3]. http://www.cdc.gov/h1n1flu/cdcresponse.htm
10. European Centre for Disease Prevention and Control. 2009 Pandemic influenza A(H1N1) [cited 2010 Sep 3]. http://www.ecdc.europa.eu/en/healthtopics/Pages/Influenza_A(H1N1)_Outbreak.aspx

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Zoonotic Cryptosporidiosis from Petting Farms, England and Wales, 1992–2009

To the Editor: Visits to petting farms in England and Wales recently have increased in popularity. Petting farms are commercial operations at which visitors, mainly families and organized groups, are encouraged to have hands-on contact with animals. The ≈1,000 petting farms in the United Kingdom collectively receive >2 million visitors per year, with peak visitor times during school and public holidays. Commercial farms also may host farm visits on single days for group and school visits. The farm attraction business is a substantial part of the rural economy, generating >£12 million annually (*1*).

During 1992–2009, a total of 55 outbreaks of infectious intestinal disease associated with petting farms in England and Wales was reported to the Health Protection Agency. Verocytotoxin-producing *Escherichia coli* O157 (VTEC O157) caused 30 (55%) of these outbreaks (244 persons were affected [range 2–93, mean 8] and 84 were hospitalized); *Salmonella enterica* serovar Typhimurium definitive phage type 104 caused 2 (3%) of the outbreaks. A total of 23 (42%) petting farm outbreaks were caused by *Cryptosporidium* spp. (1,078 persons were affected [range 2–541, mean 45] and 29 were hospitalized). We report on these cryptosporidiosis outbreaks as a reminder of the risk to petting farm visitors.

Contributory factors reported in the cryptosporidiosis outbreaks included direct contact with preweaned lambs, calves, kids, or animal feces (e.g., diarrhea in lambs, a recognized risk factor for cryptosporidiosis; 11/23 [48%]) and inadequate hand washing facilities (7/23 [30%]). Of outbreaks in which hand washing facilities were inadequate, thumb sucking by children was also noted in 1; in another, alcohol-based hand gels and sanitizers, which are ineffective against *Cryptosporidium* spp., were used.

Cryptosporidium spp. are coccidian parasites that infect a wide range of farm livestock, including cattle, sheep, goats, pigs, horses, and deer, but are mainly a veterinary problem in neonatal ruminants. *C. parvum*, for example, is a common agent in the etiology of the neonatal diarrhea syndrome of calves, lambs, and goat kids. Widespread asymptomatic carriage of this parasite exists in livestock in the United Kingdom (*2*). In humans, cryptosporidiosis occurs most commonly in children <5 years of age, can be life threatening in immunocompromised persons, and is caused predominantly by *C. hominis* and *C. parvum* parasites. Fecal–oral transmission can occur directly from animal to person and from person to person or indirectly through contaminated food or water (*2*).

Typing of *Cryptosporidium* spp. has been undertaken by the UK *Cryptosporidium* Reference Unit since 1999. *C. parvum* was identified from

human feces in 12 (75%) of the 16 petting farm outbreaks since 1999 (feces were not submitted for typing in 4). Additionally, *Cryptosporidium* spp. oocysts were detected and confirmed as *C. parvum* from suspected sources (lambs, calves) in 4 (33%) of these 12 outbreaks and linked by GP60 subtype to human cases in 3 outbreaks. Zoonotic risk factors in case–control studies of sporadic cryptosporidiosis cases in England and Wales also have identified an association between *C. parvum* infection and touching farm animals or visiting a farm (*3*).

In petting farm outbreaks, *Cryptosporidium* spp. displayed a seasonal pattern, as did VTEC O157. Cryptosporidiosis outbreaks occurred more often in springtime (18 vs. 5; p = 0.0001) than did VTEC O157 outbreaks, which occurred more frequently during the summer (25 vs. 5; p<0.00001), especially in August (Figure). During spring 2010, two additional *C. parvum* outbreaks associated with contact with lambs at petting farms were reported in England. Control measures included restricting bottle feeding of lambs and enhancing the supervision of hand washing. The associations with outbreaks of cryptosporidiosis in spring and contact with young farm animals also has been reported in Scotland (*4*).

Despite the 2 separate seasonal peaks of infection, care should be exercised throughout the year. The importance of careful attention to hygiene and supervision of children visiting farms and the need for appropriate facilities, such as those for hand washing, are covered in the UK Health and Safety Executive standards; operators of petting farms are expected to meet these standards (*5*). These guidelines also apply to commercial farms hosting open days. A good practice reminder on managing the risks from VTEC O157 in a petting farm context was published by the Health Protection Agency, Health and Safety Executive, and the Local Government Regulation (*6*). Guidance on the control of VTEC O157 infections for farms open to public access applies equally to most gastrointestinal pathogens, including *Cryptosporidium* spp. The need for a sound approach to managing hygiene control measures at petting farms cannot be overemphasized.

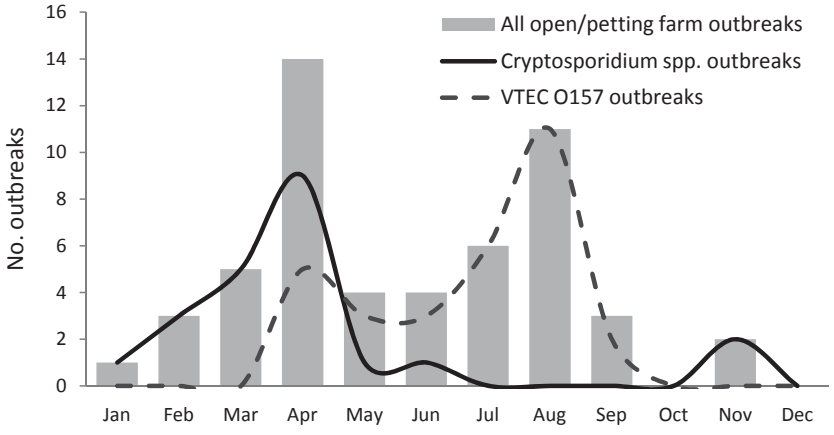

Figure. Outbreaks of cryptosporidiosis and verocytotoxin-producing *Escherichia coli* O157 linked to petting farms, England and Wales, 1992–2009.

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References

1. Restoring confidence in open farms to educate our children. Farmers Guardian 2010, January 29 [cited 2010 May 25]. http://www.farmersguardian.com/home/rural-life/country-view/restoring-confidence-in-open-farms-to-educate-our-children/29936.article
2. Chalmers RM, Giles M. Zoonotic cryptosporidiosis in the UK—challenges for control. J Appl Microbiol. 2010;109:1487–97. DOI: 10.1111/j.1365-2672.2010.04764.x
3. Hunter PR, Thompson RC. The zoonotic transmission of *Giardia* and *Cryptosporidium*. Int J Parasitol. 2005;35:1181–90. DOI: 10.1016/j.ijpara.2005.07.009
4. McGuigan CC, Steven K, Pollock KGJ. Cryptosporidiosis associated with wildlife center, Scotland [letter]. Emerg Infect Dis. 2010;16:895–6.
5. Health and Safety Executive. Avoiding ill health at open farms—advice to farmers (with teachers' supplement) [cited 2010 May 25]. http://www.hse.gov.uk/pubns/ais23.pdf
6. Health and Safety Executive, Local Authorities Coordinators of Regulatory Services, and Health Protection Agency. Understanding and managing the risks from *E. coli* O157 in an open farm context [cited 2010 May 25]. http://www.hpa.org.uk/web/HPAwebFile/HPAweb_C/1267551712693

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Buruli Ulcer Prevalence and Altitude, Benin

To the Editor: Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is one of 13 recently classified neglected tropical diseases (*1*). Little is known about factors influencing its focal distribution. In Benin, altitude may play a role in such distribution of BU.

Incidence, prevalence, and other health-related data are usually reported at national or district levels. These data convey the importance of the disease but do not show the wide variations existing at the village level. Data from the surveillance system (*2*) and surveys (*3*–*6*) in Benin have shown that BU-endemic areas are confined to the southern regions. Substantial variability in endemicity levels have been detected from 1 department to another, at the district and village levels, and from year to year (*2*–*5*).

However, some districts (Lalo in the Mono-Couffo Department, Ouinhi in the Zou Department; Zê in the Atlantique Department; and Adjohoun, Bonou, and Dangbo in the Oueme Department) remain the most persistently BU-endemic from year to year. In addition, these BU-endemic districts are all located at the same latitude. A map of these districts can be superimposed on the Lama depression (a median band, oriented from west to east, that forms a large area at a low elevation, 130 km long with a width from 5 km in the area of Tchi in Lalo to 25 km in the area of Issaba in Pobê) (*7*). This factor prompted us to investigate whether variations in altitude correlate with BU prevalence.

Using a Garmin eTrex global positioning system (Olathe, KS, USA), we collected precise geographic coordinates, including altitude, for each village in 2 persistently BU-endemic districts of the Atlantique Department. We chose districts where BU endemicity was high (Zê) and low (Toffo) (prevalences 52.0 and 7.8/10,000 inhabitants, respectively) (*3*,*5*). On the basis of routine data collected during 2005–2009, we calculated the prevalence of BU in each village of these districts and correlated it with the altitude of the village, first by mapping with Healthmapper 4.3.2 (http://healthmapper.software.informer.com) and then with statistical analyses by using Epi Info 3.5.1 (Centers for Disease Control and Prevention, Atlanta. GA, USA).

We found that highly BU-endemic villages are located most often in lowland areas (online Appendix Figure; www.cdc.gov/EID/content/17/1/153-appF.htm). The mean prevalence of BU was 60.7/10,000 inhabitants in villages with elevations <50 m, which was significantly higher than the prevalence in villages with elevations 50–100 meters (10.2/10,000 inhabitants) and that of villages with elevations >100 meters (5.4/10,000 inhabitants) ($p = 0.0003$; Kruskal-Wallis test).

In addition, we performed a simple linear regression, including all villages (model A) and only BU-endemic villages (prevalence $\neq 0$) (model B). Model A showed that at 0 altitude, the expected prevalence of BU was 26.7/10,000 inhabitants. This prevalence decreased by 0.1/10,000 inhabitants for each meter of increase in altitude (correlation coefficient 0.20; coefficient of determination 4%). Model B demonstrated that at 0 altitude, the expected prevalence was 89.6/10,000 inhabitants. This prevalence decreased by 0.7/10,000 inhabitants for each meter of increase in altitude (correlation coefficient 0.50; coefficient of determination 25%). Therefore, we conclude that a low but significant linear relationship exists between altitude and BU prevalence in disease-endemic villages. Thus, altitude may be 1 factor in determining variations in prevalence (4% for all villages and 25% for BU-endemic villages).

The focal distribution of BU was discussed in 1974 by Meyers et al. in Zaire (*8*). In the Bas-Congo Province, although the concentration of BU in Songololo was high, the nearby broad Bangu plateau, ≈300 meters higher than Songololo, was devoid of BU (D.M. Phanzu, unpub. data). Soil and geologic features (e.g., chemical composition of substrata; vegetation, fauna, and pH of swamps) were raised as environmental factors that might explain this focal distribution (*8*,*9*). The focal distribution of BU was also described by Johnson et al., who found an inverse relationship between the prevalence of the disease in Lalo District villages and distance from the Couffo River (*4*).

Few studies have investigated environmental risk factors (other than water-related) possibly related to the prevalence of BU. In 2008, Wagner et al. suggested that villages with higher prevalence rates were located in areas of low elevation. They associated the high prevalence of BU with farming activities that occurred primarily at low elevations (*10*). Our results are similar, but we have provided additional quantification of the relationship between prevalence and altitude.

One reasonable explanation for the relationship between altitude and BU prevalence is that because lowlands tend to be wetter than higher grounds, they provide more favorable conditions for the proliferation and spread of the etiologic agent. Furthermore, persons are more apt to frequent these wetter lowlands to plant and tend their crops, thus becoming vulnerable to infectious agents in the area.

An extension of this study to all BU-endemic villages is needed to further refine our results. The endemicity of BU is multifactorial; however, our results suggest that altitude should be included in future analytical models of environmental risk factors for this disease.

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References

1. World Health Organization. Buruli ulcer: progress report, 2004–2008. Wkly Epidemiol Rec. 2008;17:145–54.
2. Sopoh GE, Johnson RC, Chauty A, Dossou AD, Aguiar J, Salmon O, et al. Buruli ulcer surveillance, Benin, 2003–2005. Emerg Infect Dis. 2007;13:1374–6.
3. Sopoh GE, Dossou AD, Matilibou G, Jonhson RC. Enquête de prévalence sur l'ulcère de Buruli dans les communes d'Allada, Toffo et Zê: rapport. Geneva: World Health Organization; 2006. p. 53.
4. Johnson RC, Makoutode M, Sopoh GE, Elsen P, Gbovi J, Pouteau LH, et al. Buruli ulcer distribution in Benin. Emerg Infect Dis. 2005;11:500.
5. Sopoh GE, Adinsi V, Johnson RC, Barogui YT, Dossou A, Van der Werf TS, et al. Distribution de l'UB dans la commune de Zê. Med Trop (Mars). 2010;70:379–83.
6. Debacker M, Aguiar J, Steunou C, Zinsou C, Meyers WM, Guédénon A, et al. *Mycobacterium ulcerans* disease (Buruli ulcer) in a rural hospital, southern Benin, 1997–2001. Emerg Infect Dis. 2004;10:1391–8.
7. Kolawolé SA, Boko M. Le Benin [French] Cotonou (Benin) and Paris: EDICEF; 1983. p. 95.
8. Meyers WM, Connor DH, McCullough B, Bourland J, Moris R, Proos L. Distribution of *Mycobacterium ulcerans* infections in Zaire, including the report of new foci. Ann Soc Belg Med Trop. 1974;54:147–57.
9. Portaels F. Epidemiology of mycobacterial diseases. In: Schuster M, editor. Mycobacterial diseases of the skin. Clin Dermatol. 1995;13:207–22.
10. Wagner T, Benbow EM, Brenden OT, Qi J, Johnson RC. Buruli ulcer disease prevalence in Benin, West Africa: associations with land use/cover and identification of disease clusters. Int J Health Geogr. 2008;7:25. DOI: 10.1186/1476-072X-7-25

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Vibrio cholerae O1 in 2 Coastal Villages, Papua New Guinea

To the Editor: Cholera outbreak reports are of international public health interest, especially in areas that were previously cholera free (*1*). Although many recent cholera outbreaks have originated in coastal areas (*2*), identifying the source of cholera introduction has been challenging (*1*). The detection of *Vibrio cholerae* in coastal, brackish and riverine waters in cholera-endemic and cholera-free areas supports the view that autochtonous *V. cholerae* is involved in the introduction of cholera (*3,4*). To our knowledge, cholera has not been reported in Papua New Guinea, despite social and environmental conditions likely to facilitate transmission and the nation's close proximity to cholera-endemic countries (*5,6*).

On August 6, 2009, a physician who visited the coastal village of Lambutina reported an outbreak of acute watery diarrhea that was associated with the death of his father and 4 other persons from this and a neighboring village. The outbreak began in the village of Nambariwa and spread to neighboring Lambutina, Morobe Province. From August 13, multidisciplinary teams worked with the community to reduce the number of deaths through early identification and treatment of case-patients. The teams also worked to limit transmission through improvements to the water and sanitation infrastructure and by encouraging better hygiene practices among the villagers. A suspected case of cholera was defined as acute watery diarrhea or vomiting in a resident of Lambutina or Nambariwa villages since July 22, 2009. In the 2 villages, 77 cases were identified; attack rates were 14% in Lambutina (48/343) and 5.5% in Nambariwa (29/532). The overall case-fatality ratio was 6.5% (5/77); 2 patients died after they were discharged from the referral hospital.

A retrospective frequency-matched case–control study was conducted in Lambutina to identify the risk factors associated with suspected cholera. Neighborhood controls (± 5 years of age) were selected from unaffected households. Univariate and multivariate analyses were conducted with STATA version 10 (StataCorp., College Station, TX, USA).

Of the 48 case-patients in Lambutina, 43 participated in the study with 43 age-matched controls. In addition

to having close contact with patients who had cholera, univariate analysis showed that case-patients were more likely to have had several exposures related to the death of other patients (Table). However, having close contact with a patient was the only independent risk factor (adjusted odds ratio 4.8, 95% confidence interval 1.7–13.4) (Table). Close contact included providing nursing care for patients or carrying patients onto boats for transport to health care facilities.

From the 10 collected samples, 4 isolates were confirmed as *V. cholerae* O1, biotype El Tor, serotype Ogawa, by PCR detection of an O1-specific region of the *rfb* gene using established methods and PCR amplification of the *tcpA* gene polymorphism specific for the El Tor biotype (*7*). The *ctx*AB, *vct* genes (present in toxigenic strains) and the hemolysin gene *hly*A (present in all *V. cholerae* strains) were detected by PCR in all 4 isolates.

Although health authorities promptly identified and responded to the outbreak, they could not determine its origin. The El Niño weather phenomenon generates increased rainfall and elevated sea surface temperatures and is a predictor of cholera outbreaks (*8*), which puts more coastal areas at risk for such outbreaks (*9*). During this outbreak, Papua New Guinea reported above-average rainfall (*10*) and warmer sea surface temperatures. Although cholera may have been introduced to Papua New Guinea through an infectious traveler or by other means, climatic factors may have initiated plankton blooms, the abundance of which have also been associated with increased presence of *V. cholerae* O1. Sea and estuarine waters of these villages are plausible sources of introduction.

In Lambutina, the age-specific attack rates were lowest among young children and increased among persons of middle age and among the elderly. Those providing patient care and lifting during transportation as well as those washing the bodies of the deceased may have been more represented in the >40 years age group; however, this situation may not explain the high attack rates among the elderly.

Generally, after a cholera outbreak is detected, interventions aim to reduce the proportion of deaths to <1%. The overall case-fatality ratio in the outbreak discussed here was 6.5%, which reflects the challenges to accessing adequate health care in remote settings. This difficulty is exacerbated when the disease occurs for the first time because cholera awareness and preparedness will be weak, as can be seen in the early management of cases during this outbreak. Villagers who have close contact with cholera patients are at greater risk for disease and should be a focus of interventions to limit transmission (e.g., eliminating ingestion of contaminated water, improving hygiene and sanitation). Education to increase awareness of the disease and enhanced access to low-osmolarity oral rehydration solution, Hartmann solution, and zinc supplements are essential.

Cholera-endemic and cholera–nonendemic countries with coastal populations are at an increasing risk for cholera outbreaks. Adequate preparation by the health care system is vital to avoid excess deaths.

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Table. Univariate and multivariate analysis of risk factors associated with suspected cholera in Lambutina village, Papua New Guinea, 2009*

	Univariate analysis				Multivariate analysis	
Risk factor	No. cases (%), n = 43	No. controls (%), n = 43	OR (95% CI)	p value	aOR (95% CI)	p value
Attended a funeral	32 (74)	24 (56)	2.3 (0.8–6.4)	0.07	1.8 (0.7–4.9)	0.25
Had death in the family	8 (19)	1 (2)	9.6 (1.1–214.6)	0.02	2.6 (0.2–43.9)	0.51
Consumed food during funeral	38 (88)	34 (79)	2.0 (0.5–7.8)	0.24	NA	NA
Washed the body/clothes of deceased	7 (16)	1 (2)	8.2 (0.9–185.1)	0.03	1.6 (0.1–28.1)	0.74
Had close contact with diarrhea patient	25 (58)	8 (19)	6.1 (2.1–18.3)	0.001	4.8 (1.7–13.4)	0.003
Drank tap water	43 (100)	43 (100)	1.0 (NA)	NA	NA	NA
Boiled water for consumption	1 (2)	0	1.0 (NA)	NA	NA	NA
Washed utensils in the ocean	39 (91)	39 (91)	1.0 (0.2–5.2)	0.64	NA	NA

*OR, odds ratio; CI, confidence interval; aOR, adjusted OR; NA, not applicable.

References

1. Seas C, Miranda J, Gil AI, Leon-Barua R, Patz J, Huq A, et al. New insights on the emergence of cholera in Latin America during 1991: the Peruvian experience. Am J Trop Med Hyg. 2000;62:513–7.
2. Lobitz B, Beck L, Huq A, Wood B, Fuchs G, Faruque S, et al. Climate and infectious disease: use of remote sensing for detection of *Vibrio cholerae* by indirect measurement. Proc Natl Acad Sci U S A. 2000;97:1438–43. DOI: 10.1073/pnas.97.4.1438
3. Lipp EK, Rivera ING, Gil AI, Espeland EM, Choopun N, Louis VR, et al. Direct detection of *Vibrio cholerae* and *ctxA* in Peruvian coastal water and plankton by PCR. Appl Environ Microbiol. 2003;69:3676–80. DOI: 10.1128/AEM.69.6.3676-3680.2003
4. Colwell RR, Seidler RJ, Kaper J, Joseph SW, Garges S, Lockman H, et al. Occurrence of *Vibrio cholerae* serotype O1 in Maryland and Louisiana estuaries. Appl Environ Microbiol. 1981;41:555–8.
5. Simanjuntak CH, Larasati W, Arjoso S, Putri M, Lesmana M, Oyofo BA, et al. Cholera in Indonesia in 1993–1999. Am J Trop Med Hyg. 2001;65:788–97.
6. Agtini MD, Soeharno R, Lesmana M, Punjabi NH, Simanjuntak C, Wangsasaputra F, et al. The burden of diarrhoea, shigellosis, and cholera in North Jakarta, Indonesia: findings from 24 months surveillance. BMC Infect Dis. 2005;5:89. DOI: 10.1186/1471-2334-5-89
7. Keasler SP, Hall RH. Detecting and biotyping *Vibrio cholerae* O1 with multiplex polymerase chain reaction. Lancet. 1993;341:1661. DOI: 10.1016/0140-6736(93)90792-F
8. Colwell RR. Global climate and infectious disease: the cholera paradigm. Science. 1996;274:2025–31. DOI: 10.1126/science.274.5295.2025
9. McMichael A. Planetary overload—global environmental change and the health of the human species. Cambridge (UK): Cambridge University Press; 1993.
10. National Institute of Water and Atmospheric Research. Climate developments in June 2009 [cited 2010 Nov 23]. http://www.niwa.co.nz/our-science/climate/publications/all/icu/2009-07/month

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Clostridium sphenoides Bloodstream Infection in Man

To the Editor: The role of clostridia as intestinal pathogens has been recognized (*1*). However, the full extent of the pathogenicity, clinical spectrum, and optimal therapy of *Clostridium sphenoides* infections remains to be determined. We describe a case of bloodstream infection in a man that was caused by *C. sphenoides*.

A 68-year-old man was admitted to the hospital (Harbor UCLA Medical Center, Los Angeles, CA, USA) after a motor vehicle accident in December 2009. He was afebrile (temperature 37.2°C), was hemodynamically stable, and had generalized abdominal tenderness. Computed tomography scan of the abdomen and pelvis showed laceration of the spleen and focal aortic dissection at the aortic bifurcation. The patient underwent surgical exploration and splenic resection. No signs of bowel ischemia or laceration were identified during surgery. On the second day postoperation, he became hypotensive, and a fever of 39.2°C developed. Blood cultures (anaerobic bottles from 2 sets of blood cultures) grew a gram-positive rod, ultimately identified as *C. sphenoides*.

Bacterial growth was detected in the anaerobic bottles on day 4 of incubation by using the BacT/Alert system (bioMérieux, Marcy l'Etoile, France). The organism was subcultured to Brucella blood agar and was incubated anaerobically. On the basis of Gram staining and analysis of the organism's morphologic features in culture (growth at 37°C, obligate anaerobe with spherical, subterminal spores and hemolytic colonies with irregular edges), its biochemical characteristics (the organism was motile, a citrate fermenter), and 16S rRNA gene sequencing, the organism was identified as *C. sphenoides*. The sequence obtained was 100% identical to a *C. sphenoides* 16S rRNA sequence (GenBank accession no. AB075772). Sensitivity testing with Etest showed that the isolate was susceptible to penicillin (MIC 0.094 μg/mL), ampicillin/sulbactam (MIC 0.125 μg/mL), metronidazole (MIC 1.5 μg/mL), and doripenem (MIC 0.5 μg/mL) and resistant to clindamycin (MIC 12 μg/mL).

The patient was given doripenem, 500 mg intravenously (IV) every 8 hours; metronidazole, 500 mg IV every 8 hours; and vancomycin, 1 gm IV every 12 hours. On the third day postoperation, blood cultures (anaerobic bottle from 1 set of blood cultures) again grew *C. sphenoides*. On the fourth day postoperation, he had persistent fever (38.5°C), became severely hypoxic, and was intubated. Repeat blood cultures were negative for *C. sphenoides*. A computed tomographic scan of the chest showed bilateral pneumonia, and a sputum culture grew *Serratia marcescens*. The patient underwent a 2-week course of doripenem and an 11-day course of metronidazole. He also received vancomycin for 7 days. The patient was eventually discharged to a rehabilitation facility after 2 weeks in the hospital.

C. sphenoides was initially thought not to be pathogenic in humans, but it has been occasionally reported as a human pathogen (Table) (*2–4*). The organism is sometimes acquired from food (*2*). Osteomyelitis (*3*) and peritonitis (*4*) caused by *C. sphenoides* have also been reported. The organism has characteristic biochemical properties, and citrate is a specific substrate for the isolation of *C. sphenoides* (*5*). The pathogenesis of *C. sphenoides* infections in humans remains unclear. *C. sphenoides* may produce small alterations on Vero cells in vitro, such as turning the cells oval without altering their size, and these changes are different from those caused by *C. difficile* (*6*).

An unusual aspect of the infection in our patient was that it repre-

Table. Descriptions of cases of infection with *Clostridium sphenoides**

Authors, year, and reference	Country	Patient age, y/ sex	Underlying conditions/ risk factors	Signs and symptoms	Microbiologic findings	Treatment	Outcome
Sullivan et al., 1980 (*2*)	Canada	39/F	None. Ate Chinese food 8 h before onset of symptoms	Severe abdominal cramps and diarrhea	*C. sphenoides* isolated from stool culture. Susceptibility testing to antimicrobial drugs not reported	No antimicrobial drugs given	Spontaneous recovery within 96 h of onset of illness
Isenberg et al., 1975 (3)	USA	13/M	None. Trauma at the area of osteomyelitis 1 y before diagnosis	Osteomyelitis	*C. sphenoides* isolated from bone culture. Sensitive to penicillins, cephalosporins, chloramphenicol, tetracyclines, macrolides; resistant to aminoglycosides, polymyxins	Phenethicillin 2 g IV daily for 3 d, followed by 1 g IV daily for 3 mo	Recovered. No evidence of disease clinically or radiologically after 3 y of follow up
Felitti 1970 (*4*)	USA	6/F	Chronic neutropenia, lifelong history of recurrent attacks of otitis media, oral ulcers, periodontal abscesses, chronic gingivitis	Fever, abdominal cramps, occasional vomiting, peritonitis	*C. sphenoides* isolated from the peritoneum. Blood cultures negative. Susceptibility testing to antimicrobial drugs not reported	No antimicrobial drugs given	Died

*IV, intravenously.

sents a primary invasion of clostridia in apparently healthy colonic tissue. Ordinarily, the absolute prerequisite for clostridial infection is a focus of necrotic tissue, which this organism then infects (*7*). Reports of invasion without an evident necrotic focus (or a probable focus as in a cancer) are rare (*4*). More studies are needed to clarify the pathogenesis of *C. sphenoides* infections in humans.

Persistent bacteremia over ≥48 hours (second and third days postoperation) indicates that *C. sphenoides* was a true pathogen and the cause of bloodstream infection, rather than an apathogenic member of the colonic flora. MacLennan isolated *C. sphenoides* from war wounds (*7*). In the original report of the discovery of *C. sphenoides* (*8*), medical aspects were referred to unpublished data, and it is not possible to tell whether the 3 patients from whom the organism was isolated in pure culture had gas gangrene or whether simple wound surface colonization was being reported.

We could not identify any report of solitary *C. sphenoides* infection. The organism is extremely uncommon in human feces (*9*) and has been found in only 4% of soil samples (*10*).

In 1 study, *C. sphenoides* was isolated from 2 (6%) of 19 stool samples from children without diarrhea (*6*). These 2 isolates were sensitive to most antimicrobial drugs, including amoxicillin, ampicillin, aztreonam, ceftriaxone, chloramphenicol, and penicillin G (*6*). However, data on susceptibilities of *C. sphenoides* to various antimicrobial agents are lacking. We report susceptibility of *C. sphenoides* to carbapenems and metronidazole and resistance to clindamycin.

The full extent of the pathogenicity, clinical spectrum, and optimal therapy of *C. sphenoides* infections remain to be determined. Clinicians should be aware of the possible pathogenic role of *C. sphenoides* in humans.

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References

1. Clostridia as intestinal pathogens. Lancet. 1977;2:1113–4.
2. Sullivan SN, Darwish RJ, Schieven BC. Severe diarrhea due to *Clostridium sphenoides*: a case report. Can Med Assoc J. 1980;123:398.
3. Isenberg HD, Lavine LS, Painter BG, Rubins WH, Berkman JI. Primary osteomyelitis due to an anaerobic microorganism. Am J Clin Pathol. 1975;64:385–8.
4. Felitti VJ. Primary invasion by *Clostridium sphenoides* in a patient with periodic neutropenia. Calif Med. 1970;113:76–8.
5. Walther R, Hippe H, Gottschalk G. Citrate, a specific substrate for the isolation of *Clostridium sphenoides*. Appl Environ Microbiol. 1977;33:955–62.
6. Ferreira CE, Nakano V, Avila-Campos MJ. Cytotoxicity and antimicrobial susceptibility of *Clostridium difficile* isolated from hospitalized children with acute diarrhea. Anaerobe. 2004;10:171–7. DOI: 10.1016/j.anaerobe.2004.02.003
7. MacLennan JD. Anaerobic infections of war wounds in the Middle East. Lancet. 1943;2:94. DOI: 10.1016/S0140-6736-(00)87071-3
8. Report of the Committee upon Anaerobic Bacteria and Infections. Medical Research Council Special Report Series, Great Britain, #39, 1919 [cited 2010 Dec 1]. http://www.archive.org/stream/cu31924000323687/cu31924000323687_djvu.txt

9. Kahn MC. Anaerobic spore bearing bacteria of the human intestine in health and in certain diseases. J Infect Dis. 1924;35:423.

10. Lindberg RB. The bacterial flora of battle wounds at the time of the primary debridement. Ann Surg. 1955;141:369. DOI: 10.1097/00000658-195503000-00012

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Cholera: The Biography

Christopher Hamlin

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Cholera: The Biography, by Christopher Hamlin, joins Obesity, Asthma, Hysteria, Diabetes, Thalassaemia, and Down's Syndrome as part of the Oxford University Press series Biographies of Diseases. As great a read as Tuberculosis (Biographies of Disease) (*1*), Hamlin's book does an excellent job of treating a complex subject with scientific rigor while also being completely accessible to a lay audience. It includes a glossary of terms and suggestions for further reading. In short, I would highly recommend this book to anyone interested in learning more about the origins and history of a "disease" about which the more we know the less certain we become.

The word disease appears in quotation marks above because, as the book makes eminently clear, the concept and case definition of cholera are defined differently in different contexts. As is stated in Chapter 6, Cholera's Last Laugh:

> [Cholera] may not be (or may not always have been) a South Asian export; is caused by a group of genetically unstable organisms, whose main ecological niche is warm seas not the human intestine; is, as a clinical matter, indistinct from other severe diarrheas (including the old cholera nostras) and is often defined in clinical rather than bacteriological terms; is subject to seasonal and environmental factors, not merely to the presence of infected persons; is not exclusively, or often even predominantly, water-borne, and may not always be a fecal-oral disease; [and] is as much a political problem as ever.

Hamlin makes a clearly articulated argument that politics plays a large part in how the lay public and public health professionals perceive and respond to cholera. As this biography elucidates, cholera has historically not only driven individual destiny (Mary Lennox would never have left India for Mistlethwaite Manor had not her parents, her ayah, and most of the servants died of cholera in the first pages of Frances Hodgson Burnett's book The Secret Garden), but it has also defined and reflected prevailing attitudes about the politics, economy, race relations, and social hierarchies of entire nations to an extent not matched until the advent of AIDS. In Chapter 3, Citizen Cholera, the reasons given for the Italian prime minister's absolute denial of the 1911 cholera epidemic bear a chilling resemblance to the motivations behind South Africa's President Mbeki's 20th century denial of the AIDS epidemic.

The book ends on a cautionary note, stating that even in the face of modern public health interventions, climate change could trigger ever larger outbreaks as rising, warming seas expand the niche of *Vibrio cholerae*. However, Hamlin's final statement sums up both the central conundrum of cholera and the central satisfaction of cholera's treatment in this book: "Cholera will not disappear nor cease to mean. A great challenge will be to respond to the meanings it is given."

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Reference

1. Albalak R. Tuberculosis (biographies of disease) [book review]. Emerg Infect Dis. 2010;9:1505–6.

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Peter Paul Rubens (1577–1640) ***The Gathering of the Manna*** **(c. 1625) Oil on canvas (487.68 cm × 411.48 cm)** Bequest of John Ringling, 1936, Collection of The John and Mable Ringling Museum of Art, the State Art Museum of Florida, a division of Florida State University

Manna to Gall

Polyxeni Potter

"No undertaking, however vast, has ever surpassed my courage," professed Peter Paul Rubens, whose work comprised cathedral domes and altarpieces, portraits, landscapes, and designs for sculpture and architecture. Courage also drove his exuberant style of flamboyant color and movement. "That Homer of painting, the father of warmth and enthusiasm in art," said of him Eugene Delacroix, eclipsed most other artists, "Not because of his perfection in any one direction, but because of that hidden force—that life and spirit—which he put into everything he did." Delacroix, who borrowed and absorbed much from him, wrote in his personal journal that his admiration was so great, he "cared to be Rubens."

He was described as of "tall stature, a stately bearing … rosy cheeks, chestnut brown hair, sparkling eyes but with passion restrained, a laughing air, gentle and courteous." Known as "the Apelles of his age" and "the most learned painter in the world," Rubens was hardworking; multilingual; a prodigious collector; and a fine diplomat, negotiating treaties all over Europe for more than 25 years. His friend the theologian Gaspar Scioppius wrote, "I know not what to praise most, his ability in painting, in which he attains the most exalted rank attained by any man of his century, or his knowledge of literature, his enlightened taste, and the all too rare agreement between his words and his deeds."

Rubens' career spanned a tumultuous age in Europe, filled with political, religious, and cultural developments, many of which he was able to influence. Shifting geographic borders and denominational allegiances often worked to his advantage as his diplomatic connections brought him royal commissions and generous court assignments. An age of contradictions, the 17th century saw in the sciences a move toward specific methods of inquiry, while the arts turned to an imaginative style. Bourgeois capitalism was on the rise, and the powerful of Europe were building exquisite palaces. Italian art was spreading everywhere, while Italy was losing its edge in international trade. This, known as the age of the Baroque, produced many great masters, among them Caravaggio; Bernini; and Rubens, who epitomized and defined it.

A native of Siegen, Germany, Rubens lived and went to school in Antwerp, where he became a master. According to his nephew, he was soon "seized with a desire to see Italy and to view at first hand the most celebrated works of art, ancient and modern, in that country, and to form his art after these models." In Venice, he studied Titian, Veronese, Bassano, and Tintoretto, whose influences guided

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"the great speed and furor of his brush." His following so grew that soon he would call himself "the busiest and most harassed man in the world."

When he returned to Antwerp, his large studio employed some of the greatest Flemish masters of the day, among them Anthony van Dyck and Frans Snyders. He relied on their work to meet demand, something he openly acknowledged. Danish physician Otto Sperling visited his studio in 1621 and observed "a good number of young men each occupied on a different work, for which Rubens had provided a chalk drawing with touches of color added here and there." Yet, Sperling added, "Kings and princes have heaped gifts and jewels upon him."

In his mature years, he painted detailed models for large projects. These models stood alone as major works and along with draft sketches, hundreds of which have survived, give a glimpse into Rubens' understanding of subjects, genius for composition and spontaneity of execution, the creative process itself. Among the painted models were designs made for a thriving tapestry market to which Rubens had ties through his family.

The Gathering of the Manna, on this month's cover, was a painted model, part of a series, for tapestries commissioned by Archduchess Isabella of Spain, early employer and long-time patron. In this series, tapestries were shown within tapestries. *The Gathering of the Manna* was hung with cord and tassels from three lions' heads attached to an architrave inside a framework of spiraling columns within an elaborate border.

The Old Testament story unfolded according to tradition. After leaving Egypt, the Israelites wandered in the desert for many days, starving. They appealed to God through Moses for food, "And in the morning … behold, upon the face of the wilderness there lay a small round thing, as small as the hoar frost on the ground." The prophet, light emanating from his head, acknowledged the miracle, along with the figure on the right, possibly his brother Aaron. On the left, a woman with a child in tow held a basket filled with manna "like wafers made with honey." Baskets high to collect the nourishment, the biblical figures engaged in a spirited dance, their gestures broad and theatrical, positions centered and balanced. They gathered and moved in perfect harmony. Moses lifted gaze and hand upward as in wonder and thanks. Mother and child headed gracefully toward the back.

Throughout history, as in biblical times, availability of food was a primary concern, one so great, it merited divine intervention. Safety of food also goes all the way back. The Israelites knew they were not to hoard manna for it would spoil and become poisonous. We have, for the most part, resolved availability. Although not optimally distributed, food is plentiful, and refrigeration and other means have eased spoilage. Yet, known foodborne pathogens and unspecified agents cause havoc in the United States and around the world, even if we have not been able to fully estimate the precise numbers of illnesses and deaths.

During a period of illness in 1623, English poet John Donne wrote about his disease and recovery, during which, like Moses in crisis, he "debated" with God. The book, Devotions upon Emergent Occasions, examined the stages of illness and commented on the human condition. "How little of man is the heart! And yet it is all by which he is; and this continually subject, not only to foreign poisons conveyed by others, but to intestine poisons, bred in ourselves by pestilential sicknesses." These sicknesses, and others of a more sentimental nature, preoccupied Donne. In "Twicknam Garden" (1633), he wrote about love, a condition he likened to a spider because of its ability to infuse poison into everything, "The spider love, which transubstantiates all, / And can convert manna to gall."

The poet's graphic conversion of the sacred to the poisonous made for good reading, and the account of his illness was extremely popular in his day, even though he did not identify the illness, quantify, or provide any information that might help readers avoid pestilential sicknesses. For, then as now, reducing "intestine poisons" required information about the many factors along the farm-to-table pathway that can introduce or amplify microbial contamination and verify the feasibility and effectiveness of interventions. When it comes to food and illness caused by it, Donne's biblical reference fits. The food by which we live can kill us and does, and unless we can choreograph the gathering and distribution of manna as well as Rubens did, the total picture with all its functioning and useful parts will continue to elude us.

Bibliography

1. Freedberg D. The hand of Rubens. New York: Gagosian Gallery Exhibition Catalogue; 1995.
2. John Donne. From devotions upon emergent occasions [cited 2010 Nov 1]. http://www.wwnorton.com/college/english/nael/noa/pdf/27636_17th_u01_donne-1-19.pdf
3. Lamster M. Master of shadows: the secret diplomatic career of the painter Peter Paul Rubens. New York: Anchor Books; 2010.
4. Morris JG Jr. How safe is our food? Emerg Infect Dis. 2011;17:126–8.
5. Scallan E, Hoeskra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011;17:7–15.
6. Scallan E, Griffin PM, Angulo FJ, Tauxe RV, Hoekstra RM. Foodborne illness acquired in the United States—unspecified agents. Emerg Infect Dis. 2011;17:16–22.
7. Singleton E. Holland. Chicago: AC McClurg and Co.; 1908. The gathering of the manna [cited 2010 Nov 1]. http://www.willemswebs.com/ringlingdocents/manna.htm

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EMERGING INFECTIOUS DISEASES

Upcoming Issue

Zoonoses in the Bedroom

Neurologic Manifestations of Hepatitis E Infection

Arboviral Prevalence in Mosquitoes during Rift Valley Fever Outbreak, Kenya

Risk Factors for *Cryptococcus gattii* Infection, British Columbia, Canada

Leptospirosis in Hawaii, 1999–2008

Phocine Distemper Virus in Seals, US East Coast, 2006

Investigation of *Coccidioides immitis* Infections among Organ Transplant Recipients

Penicillium marneffei in Humans and Rodents, China

Possible Increased Pathogenicity of Reassorted Pandemic (H1N1) 2009 Virus

Human Infection with Non-O157 Shiga Toxin–producing *Escherichia coli*, Switzerland, 2000–2009

Severe Cases of Pandemic (H1N1) 2009 in Children, Germany

Surveillance for West Nile Virus in Dead Wild Birds, South Korea, 2005–2008

Novel HIV-1 Recombinant Forms in Antenatal Cohort, Montreal, Quebec, Canada

Primary Amebic Meningoencephalitis Caused by *Naegleria fowleri*, Karachi, Pakistan

Usefulness of Published PCR Primers in Detecting Human Rhinovirus Infection

Characteristics of Patients with Oseltamivir-Resistant Pandemic (H1N1) 2009 Infection, United States

Clinical Aspects of Pandemic (H1N1) 2009–associated Pneumonia in Children, Japan

Lower Viral Loads in Pandemic versus Seasonal Influenza Infections, Singapore

Surveillance for Oseltamivir Resistance in Pandemic (H1N1) 2009 Virus, Mexico

Complete list of articles in the February issue at http://www.cdc.gov/eid/upcoming.htm

Upcoming Infectious Disease Activities

February 4–7, 2011
International Meeting on Emerging Diseases and Surveillance (IMED 2011)
Hotel Hilton, Vienna, Austria
http://imed.isid.org

February 27–March 2, 2011
CROI 2011: 18th Conference on Retroviruses and Opportunistic Infections
Boston, Massachusetts, USA
http://www.retroconference.org/2011

April 1–4, 2011
Annual Scientific Meeting of The Society for Healthcare Epidemiology of America
(SHEA) 2011
Dallas, Texas, USA
http://www.shea2011.com

July 8–10, 2011
International Society for Infectious Diseases Neglected Tropical Diseases Meeting (ISID-NTD)
Boston, MA, USA
http://ntd.isid.org

November 16–19, 2011
7th World Congress of the World Society for Pediatric Infectious Diseases (WSPID 2011)
Melbourne, Australia
http://www.kenes.com/wspid2011/mailshot/ms3.htm

2012

June 13–16, 2012
15th International Congress on Infectious Diseases (ICID)
Bangkok, Thailand
http://www.isid.org/15th_icid

Announcements

To submit an announcement, send an email message to EIDEditor (eideditor@cdc.gov). In 50–150 words, describe timely events of interest to our readers. 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 and earn continuing medical education (CME) credit, please go to **http://www.medscapecme.com/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.com. If you are not registered on Medscape.com, 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 is acceptable as evidence of participation in CME activities. If you are not licensed in the US and want to obtain an AMA PRA CME credit, please complete the questions online, print the certificate and present it to your national medical association.

Article Title

Public Health Implications of Cysticercosis Acquired in the United States

CME Questions

1. Which of the following statements about the presentation and prevalence of cysticercosis acquired within the United States is correct?

A. Cysticercosis primarily presents as pulmonary disease
B. In this report, cysticercosis was detected in all 50 US states
C. Cysticercosis was primarily reported in immigrants from Asia
D. Cysticercosis is a severe, potentially fatal disease posing a considerable economic burden

2. Your patient is a 27-year-old woman whose parents are immigrants from Latin America. She presents with seizures and is thought to have neurocysticercosis.

Which of the following statements about transmission of this illness is most likely correct?

A. Droplet inhalation is the presumed route of infection
B. The responsible pathogen is a hookworm
C. A presumptive source of infection may be identified among household members or close personal contacts in about 20% of cases
D. There is no good serologic test to confirm the diagnosis

3. The patient has positive serology for *Taenia solium* and is diagnosed with neurocysticercosis, as is her brother. Which of the following public health strategies would most likely be recommended?

A. Examination of urine samples from close contacts
B. Only persons with recent travel to endemic areas should be examined
C. A source of infection should be looked for
D. Carriers do not need to be treated

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 and earn continuing medical education (CME) credit, please go to **http://www.medscapecme.com/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.com. If you are not registered on Medscape.com, 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 is acceptable as evidence of participation in CME activities. If you are not licensed in the US and want to obtain an AMA PRA CME credit, please complete the questions online, print the certificate and present it to your national medical association.

Article Title

Hepatitis E Virus Infection without Reactivation in Solid-Organ Transplant Recipients, France

CME Questions

1. Based on results from this study, which of the following patients would be anticipated to be at highest risk for acquisition of hepatitis E virus (HEV) infection?

A. A 65-year-old man undergoing kidney transplantation
B. A 45-year-old woman undergoing liver transplantation
C. A 76-year-old man 1 year post liver transplant.
D. A 36-year-old woman 6 months post kidney transplant

2. A 49-year-old female patient who is HIV positive undergoes a liver transplant. Six months later she is found to be HEV positive. Which of the following factors would increase her risk to evolve to a chronic infection?

A. Alcohol use
B. Female sex
C. Immunocompromise associated with HIV infection
D. Comorbid hepatitis C virus or hepatitis B virus infection

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

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.

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The journal is intended for professionals in infectious diseases and related sciences. We welcome contributions from infectious disease specialists in academia, industry, clinical practice, and public health, as well as from specialists in economics, social sciences, and other disciplines. Manuscripts in all categories should explain the contents in public health terms. For information on manuscript categories and suitability of proposed articles, see below and visit www.cdc.gov/eid/ncidod/ EID/instruct.htm.

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Title Page. Give complete information about each author (i.e., full name, graduate degree(s), affiliation, and the name of the institution in which the work was done). Clearly identify the corresponding author and provide that author's mailing address (include phone number, fax number, and email address). Include separate word counts for abstract and text.

Keywords. Include up to 10 keywords; use terms listed in Medical Subject Headings Index Medicus.

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Biographical Sketch. Include a short biographical sketch of the first author—both authors if only two. Include affiliations and the author's primary research interests.

References. Follow Uniform Requirements (www.icmje.org/index.html). Do not use endnotes for references. Place reference numbers in parentheses, not superscripts. Number citations in order of appearance (including in text, figures, and tables). Cite personal communications, unpublished data, and manuscripts in preparation or submitted for publication in parentheses in text. Consult List of Journals Indexed in Index Medicus for accepted journal abbreviations; if a journal is not listed, spell out the journal title. List the first six authors followed by "et al." Do not cite references in the abstract.

Tables. Provide tables within the manuscript file, not as separate files. Use the MS Word table tool, no columns, tabs, spaces, or other programs. Footnote any use of boldface. Tables should be no wider than 17 cm. Condense or divide larger tables. Extensive tables may be made available online only.

Figures. Submit figures as separate files, in the native format when possible (e.g., Microsoft Excel, PowerPoint). Photographs should be submitted as high-resolution (600 dpi) .jpg or .tif files. Other file formats may be acceptable; contact fue7@cdc.gov for guidance. Figures should not be embedded in the manuscript file. Use color only as needed. Use Arial font for figure lettering. Figures, symbols, lettering, and numbering should be clear and large enough to remain legible when reduced to print size. Large figures may be made available online only. Place figure keys within the figure; figure legends should be provided at the end of the manuscript file.

Videos. High-quality video files are accepted in the following formats: AVI, MOV, MPG, MPEG, and WMV. The files should be no longer than 5 minutes in length.

Types of Articles

Perspectives. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles should provide insightful analysis and commentary about new and reemerging infectious diseases and related issues. Perspectives may address factors known to influence the emergence of diseases, including microbial adaptation and change, human demographics and behavior, technology and industry, economic development and land use, international travel and commerce, and the breakdown of public health measures.

Synopses. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. This section comprises concise reviews of infectious diseases or closely related topics. Preference is given to reviews of new and emerging diseases; however, timely updates of other diseases or topics are also welcome.

Research. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Report laboratory and epidemiologic results within a public health perspective. Explain the value of the research in public health terms and place the findings in a larger perspective (i.e., "Here is what we found, and here is what the findings mean").

Policy and Historical Reviews. Articles should not exceed 3,500 words and 40 references. Use of subheadings in the main body of the text is recommended. Photographs and illustrations are encouraged. Provide a short abstract (150 words), 1-sentence summary, and biographical sketch. Articles in this section include public health policy or historical reports that are based on research and analysis of emerging disease issues.

Dispatches. Articles should be no more than 1,200 words and need not be divided into sections. If subheadings are used, they should be general, e.g., "The Study" and "Conclusions." Provide a brief abstract (50 words); references (not to exceed 15); figures or illustrations (not to exceed 2); tables (not to exceed 2); and biographical sketch. Dispatches are updates on infectious disease trends and research that include descriptions of new methods for detecting, characterizing, or subtyping new or reemerging pathogens. Developments in antimicrobial drugs, vaccines, or infectious disease prevention or elimination programs are appropriate. Case reports are also welcome.

Commentaries. Thoughtful discussions (500–1,000 words) of current topics. Commentaries may contain references but no abstract, figures, or tables. Include biographical sketch.

Another Dimension. Thoughtful essays, short stories, or poems on philosophical issues related to science, medical practice, and human health. Topics may include science and the human condition, the unanticipated side of epidemic investigations, or how people perceive and cope with infection and illness. This section is intended to evoke compassion for human suffering and to expand the science reader's literary scope. Manuscripts are selected for publication as much for their content (the experiences they describe) as for their literary merit. Include biographical sketch.

Letters. Letters commenting on recent articles as well as letters reporting cases, outbreaks, or original research, are welcome. Letters commenting on articles should contain no more than 300 words and 5 references; they are more likely to be published if submitted within 4 weeks of the original article's publication. Letters reporting cases, outbreaks, or original research should contain no more than 800 words and 10 references. They may have 1 figure or table and should not be divided into sections. No biographical sketch is needed.

Books, Other Media. Reviews (250–500 words) of new books or other media on emerging disease issues are welcome. Title, author(s), publisher, number of pages, and other pertinent details should be included.

Conference Summaries. Summaries of emerging infectious disease conference activities are published online only. Summaries, which should contain 500–1,000 words, should focus on content rather than process and may provide illustrations, references, and links to full reports of conference activities.

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Online Reports. Reports on consensus group meetings, workshops, and other activities in which suggestions for diagnostic, treatment, or reporting methods related to infectious disease topics are formulated may be published online only. These should not exceed 3,500 words and should be authored by the group. We do not publish official guidelines or policy recommendations.

Announcements. We welcome brief announcements (50–150 words) of timely events of interest to our readers. Announcements may be posted online only, depending on the event date. Email to eideditor@cdc.gov.